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invited review

Beyond insulin-like growth factor-1 (IGF-1) normalization: portal insulin and hepatic growth hormone (GH) sensitivity across metabolic disease
Aart J. van der Lely, Sebastian J. Neggers, John J. Kopchick, Cesar L. Boguszewski

consensus

Latin American Thyroid Society expert panel consensus on anaplastic thyroid cancer
Inés Califano, Fabiano Mesquita Callegari, Janete Maria Cerutti, Aline Lauda Freitas Chaves, Gilberto de Castro Junior, Jose Miguel Dominguez Ruiz-Tagle, Agustin Falco, Ana Amélia Oliveira Hoff, Luiz Paulo Kowalski, Ana Luiza Maia, Marcos David Pereira, Alvaro Sanabria, Fabian Pitoia

originals

GLP-1 receptor agonists in kidney transplant recipients with post-transplant diabetes: efficacy and safety outcomes from a retrospective cohort study
Ricardo E. T. Navarrete, Joana C. Freitas, Isabel Fonseca, Ana Cunha, La Salete Martins, Joao Roberto Sa

Mild and non-persistent fasting plasma glucose elevation in the first trimester of pregnancy is not associated with increased risk of gestational diabetes mellitus and adverse pregnancy outcomes
Natalia Gattass-Ferreira, Carlos Antonio Negrato, Ana Luiza de Mattos Telles, Gabriela Rogonsky da Costa, Claudia Saunders, Marcus Miranda de Oliveira, Melanie Rodacki, Lenita Zajdenverg

Hypercholesterolemia induced by hypothyroidism is ameliorated by taurine supplementation through the hepatic AMPK/ACC pathway
Marina Souza Matos, Georgia Correa Atella, Vânia Maria Corrêa da Costa,

Association between triglyceride to high-density lipoprotein cholesterol ratio and new-onset diabetes mellitus
Chunhui Yin, Qi Qi, Xinyu Wu, Lei Li, Yue Jiang, Jing Yu, Yun Zhang, Quanle Han, Shouling Wu, Kangbo Li,

Prognostic factors and survival in parathyroid carcinoma: a 50-year single-center cohort study
Luiza Helena Crispim da Silva, Letícia Almeida Pontes, Marília D'Elboux Guimarães Brescia, Regina Matsunaga Martin, Felipe Lourenço Ledesma, Hudson Sá Sodré, Venâncio Avancini Ferreira Alves, Fábio Luiz de Menezes Montenegro, Felipe Ferraz Magnabosco

Timing of 17-hydroxyprogesterone measurement during the standard-dose Synacthen test in pediatric patients evaluated for non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency
Nurdan Çiftci, Emine Çamtosun, İsmail Dündar, Edip Ünal, Nurullah Çelik, Ayşehan Akıncı

Comprehensive insights into bone health, hormonal patterns, and fracture risks among men with drug resistant epilepsy: a crucial evaluation
Cristian Pieper, Leonardo Vieira Neto, Eduardo Ferreira da Gama, Francisco P. Paranhos Neto, Miguel Madeira, Laura M. Carvalho Mendonça, Isabella D'Andrea Meira, Maria Lucia Fleiuss Farias

LncRNA *GSEC* impedes the reprogramming of glucose metabolism in papillary thyroid carcinoma by inhibiting the IGF2BP2/GLUT1 axis
Long Ren, Kai Zhang, Xiaotian Yu, Yong Jiang

Association between vitamin D deficiency and type 2 diabetes in an Indigenous Brazilian population
Hully Cantão dos Santos, Rafael de Oliveira Alvim, Gabriela Callo Quinte, Carolina Perim de Faria, Adriana Santos da Silva, José Geraldo Mill,

Vitamin D status and its association with metabolic syndrome and cardiometabolic markers in postmenopausal women: a population-based study
Laura Alves Cota e Souza, Thaís Teixeira Silva Diniz, Angélica Alves Lima,

Characteristics of transgender health-related provider-to-provider consultations in Brazil's public primary health care system: A cross-sectional study from a Southern Brazilian telehealth service
Geferson Pelegrini, Fabiana Carvalho, Dimitris Rucks Varvaki Rados, Tayane Muniz Fighera, Natan Katz, Roberto Nunes Umpierre, Marcelo Rodrigues Gonçalves

Impact of premature mortality attributable to obesity on years of life lost in Brazil
Luís Jesuino de Oliveira Andrade, Gabriela Correia Matos de Oliveira, Osmario Jorge de Mattos Salles, Alcina Maria Vinhaes Bittencourt, Luís Matos de Oliveira

Extrathyroidal extension is independently associated with cervical lymph node metastasis in pediatric papillary thyroid carcinoma
Mario Sergio Rocha Macedo, Rodrigo Schuler Honorio, Willer Feitosa Menezes, Paula Assunção Medeiros, Claudio Pinheiro Dias, Clovis Pinheiro Macedo, Luis Alberto Albano Ferreira, Gdayllon Cavalcante Meneses, Wellington Alves Filho

Predictive factors for remission in pediatric Graves' disease treated with antithyroid drugs: a retrospective study
Ana João Fernandes, João Pedro Gomes, Rita Aldeia da Silva, Mariana Portela, Sofia Martins, Olinda Marques, Ana Antunes, Maria Miguel Gomes

Association between autoimmune thyroiditis and clinicopathological features of papillary thyroid carcinoma: a prospective study
Amira Bouchenna, Abdelghani Tibouk, Brahim Ghennam, Khawla Boumaraf, Samia Ould Kablia, Meriem Bensalah

Neurodevelopmental outcomes in children with transient hypothyroxinemia of prematurity assessed using the Denver Developmental Screening Test II (DDST-II)
Yavuz Özer, Aslan Yılmaz, Gizem Yılmaz, Aysun Ayaz Sarı, Hande Turan, Gürkan Tarçın, Dilek Bingöl Aydın, Ersin Ulu, Yıldız Perk, Mehmet Vural, Icay Evliyaoğlu

Malignancy risk score in thyroid nodules with atypia of undetermined significance cytology: an approach based on cytological subgrouping and ultrasonographic data
Yusuf Öztürk, Naile Kökbudak, Muhammet Kocabaş, Melia Karaköse, Mustafa Kulaksizoğlu, Feridun Karakurt

Evolution of thyroid fine-needle aspiration biopsy performance over 15 years: implications for risk stratification in clinical practice
Hakan Doğruel, Elif Nur Karaoğlu, Nusret Yılmaz, Mustafa Aydemir, Hasan Çalış, Cumhur Arıcı, Güzide Ayşe Ocak, İnanç Elif Gürer, Ramazan Sarı

Association of physical activity with arterial stiffness: the mediating role of the visceral fat metabolism score
Huihua Jiang, Xiaoli Shao, Liqin Jiang, Teng Zhang

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Comparison of clinical characteristics and predictive performance of severity scoring systems in hypertriglyceridemic versus non-hypertriglyceridemic acute pancreatitis: a retrospective cohort study
Zhen Chen, Min-xing Liu, Zhen-yu Chu, Li-min Jia

Glycemic responses to low-intensity, short-duration aerobic exercise sessions in hospitalized women with gestational diabetes: a pilot study
Edilson Vitória Nunes, Marcelo Zanusso Costa, Lidiane Pozza Costa, Gabriela de Lemos Uliano, Cristine Lima Alberton, Jorge Luiz de Brito Gomes, Juliano Boufleur Farinha

Quality of life outcomes in Brazilian adults with childhood-onset growth hormone deficiency: impact of transition-age growth hormone therapy
Kettyuscia Coelho e Oliveira, Mariana Peduti Halah, Luís Eduardo Cruvinel Pinto, Thaís Milioni Luciano, Ryann Luccas de M. Souza, Renata D. Sicchieri Pugliesi, Davi C. Aragon, Paula C. L. Elias, Margaret de Castro, Sonir R. Antonini

Analysis of differences in serological indicators between Graves' disease and transient thyrotoxicosis during pregnancy
Yuxin Jie, Gengxue Jin, Bo Sun, HuanHuan Zhang, Hui Chen

Assessment of dehydroepiandrosterone sulfate (DHEA-S) suppression following the 1 mg overnight dexamethasone suppression test in women with hirsutism and elevated DHEA-S
Hamide Piskinpasa, Didem Acarer Bugun, Mehmet Emin Piskinpasa, Sema Ciftci, Ilkay Cakir

Quantitative magnetic resonance imaging (MRI) evaluation of visceral and hepatic fat in Cushing's syndrome: insights into cortisol-driven metabolic changes
Rania Naguib, Marwa Ahmed Salah Ahmed, Sherif Mohamed Elsharkawi, Hany A. Elkattawy, Mohamed Ahmed Hassan, Hend Naguib

brief communications

Obesity, diabetes, and other metabolic disorders among the Bororo Indigenous population
Luiz F. Viola, Amaury Lelis Dal Fabbro, João Paulo Botelho Vieira-Filho, Laércio Joel Franco, Regina S. Moises

Clinical and functional evidence supporting pathogenicity of a novel APOA5 variant in familial chylomicronemia syndrome
Johnayro Gutiérrez, Pablo Castaño, Claudia Monsalve, Nestor López, Fernando Rivera Toquica, Edwin Mora, Jessica Cristina Armijos, Juliana Lores, Henry Mauricio Arenas, Germán Camilo Giraldo, Diana Sánchez, Natalia Agudelo, Gregorio Fariña, Gabriela Berg, Juan Patricio Nogueira

case reports

Rare types of congenital adrenal hyperplasia: report of five children with 11β-hydroxylase deficiency including pathogenic and novel *CYP11B1* variants
Anju Bala, Sayan Banerjee, Arun George, Priyanka Srivastava, Mohit Kumar, Neha KC, Jaivinder Yadav, Rakesh Kumar, Devi Dayal

Long-term follow-up of neonatal severe hyperparathyroidism: redefining calcium management
Inês Meira, João Menino, Patrícia Ferreira, Joana Queirós, Diana Silva

Pregnancy in a patient with metastatic functioning midgut neuroendocrine tumor receiving lanreotide after peptide receptor radionuclide therapy
Beatriz Tavares da Silva, Ariana Maia, Maria Teresa Pereira, Sara Santos, Susana Garrido, Joana Vilaverde, Marta Sales Moreira, Clara Pinto, Ana Paula Santos, Jorge Dores

in memoriam

A tribute to Doris Rosenthal, "La grande Dame de la thyroidologie brésilienne" (1932-2026)
Denise P. de Carvalho, Rui M. B. Maciel

letters to the editor

Methodological considerations in long-term diabetes mortality studies
Chris Munayco Carhuamaca

Response to the letter to the editor: Methodological considerations in long-term diabetes mortality studies
Lucas Casagrande Passoni Lopes, Marina dos Santos de Carvalho Pinto, Mauro Wieczorek, Carlos Antonio Negrato

Sociodemographic considerations regarding the European Portuguese Validation of the Diabetes Distress Scale (DDS-17)
Rodrigo Jesus Ccoto-Apolinario

Response to the letter to the editor: Sociodemographic considerations regarding the European Portuguese Validation of the Diabetes Distress Scale (DDS-17)
Maria Inês Serpa Paz Teixeira de Melo, Diana Falcão, Dina Isabel Campos, Mariana M. dos Santos, Jorge Hernâni-Eusébio, Anabela Barreto Silva, Francielle Lopes dos Reis, Maria Carlota Borba Brum, Júlio César Ferreira Bertoloto, Maria da Graça Rocha Penha, Sheila de Castro Cardoso Toniasso, Rodrigo Fernandes Soares, Elen Gineste Baccin, Ticiana da Costa Rodrigues, Francielle Lopes dos Reis, Maria Carlota Borba Brum, Júlio César Ferreira Bertoloto, Maria da Graça Rocha Penha, Sheila de Castro Cardoso Toniasso, Rodrigo Fernandes Soares, Elen Gineste Baccin, Ticiana da Costa Rodrigues, Camila Pereira Baldin, Robson Martins Pereira

erratum

Dietary pattern and night work: metabolic syndrome in healthcare workers
Francielle Lopes dos Reis, Maria Carlota Borba Brum, Júlio César Ferreira Bertoloto, Maria da Graça Rocha Penha, Sheila de Castro Cardoso Toniasso, Rodrigo Fernandes Soares, Elen Gineste Baccin, Ticiana da Costa Rodrigues, Camila Pereira Baldin, Robson Martins Pereira

Role of insulin-regulated aminopeptidase as potential biomarker in insulin resistant polycystic ovary syndrome patients
Osman Köse, Koray Gök, Elif Köse, Sezen Irmak Gözükara, Abdullah Tüten, Mehmet Sühha Bostancı

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invited review

Beyond insulin-like growth factor-1 (IGF-1) normalization: portal insulin and hepatic growth hormone (GH) sensitivity across metabolic disease

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ABSTRACT

Clinical management of growth hormone (GH) deficiency, acromegaly, and diabetes mellitus is dominated by single-analyte targets: serum insulin-like growth factor-1 (IGF-1) in the GH disorders and glycated hemoglobin in diabetes. This reductive framework obscures a central regulatory fact: GH does not autonomously dictate hepatic IGF-1 output. Whether GH is translated into IGF-1 by the hepatocyte is gated by the concentration of insulin reaching the liver through the portal vein. We propose liver sensitivity to GH (LSG), operationally defined as portal-insulin-determined hepatic GH receptor (GHR) availability, as a unifying variable that places type 1 and type 2 diabetes, obesity, liver failure, and acromegaly on a single continuum rather than treating them as separate phenomena. We develop the molecular basis of insulin-dependent GHR regulation, distinguish the divergent roles of portal versus systemic insulin, and argue that this distinction explains why subcutaneous insulin therapy in type 1 diabetes corrects glycemia while leaving the hepatic GH-resistant state intact. We then re-examine the weight-gain liability of insulinotropic antidiabetic agents, the discordant GH/IGF-1 signatures produced by GLP-1 receptor agonists, the prognostic and potentially therapeutic role of IGF-1 in cirrhosis, and the metabolic limitations of current acromegaly treatment targets when viewed through the LSG framework. The aim is to give the treating physician a mechanistic, rather than purely biochemical, account of how metabolism behaves in these states.

Keywords: Growth hormone; insulin-like growth factor-1; insulin; portal vein; acromegaly; diabetes mellitus; liver cirrhosis; GLP-1 receptor agonists

INTRODUCTION

Three of very familiar endocrine conditions, growth hormone deficiency (GHD), acromegaly, and diabetes mellitus, are managed as though each were a single-dial problem. Growth hormone (GH) deficiency is dosed to normalize serum IGF-1; acromegaly is treated to lower GH or block GH action until insulin-like growth factor-1 (IGF-1) falls within the age- and sex-adjusted reference range; type 1 diabetes is dosed with insulin until glycated hemoglobin normalizes (1-4). The clinical appeal is obvious: each disease acquires a clean efficacy metric, and competing therapies can be ranked by the proportion of patients reaching target.

The cost of this convenience is conceptual. GH, IGF-1, and insulin do not act in parallel; they continuously rewrite one another's production and action (5-7). Treating IGF-1 as a faithful readout of GH status, or glycated hemoglobin as a complete description of metabolic control, ignores the variable that couples the two systems together. That variable is the amount of insulin reaching the liver through the portal circulation. In this review we make this coupling explicit and have named it liver

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sensitivity to GH (LSG), and use it to connect disorders that are conventionally taught in isolation. We focus on type 1 and type 2 diabetes, obesity, liver failure, and acromegaly as the settings in which a clinician who reasons only from IGF-1 or glucose levels will be misled.

DEFINING LIVER SENSITIVITY TO GROWTH HORMONE

LSG is best understood through an electrical analogy. Picture each hepatocyte as a light bulb whose filament is the JAK2/STAT5 cascade that drives IGF-1 transcription. GH is the current flowing into the bulb. Portal insulin is the dimmer switch on the wall. The same current produces a bright or a dim light depending entirely on where the dimmer is set. Crank the dimmer up (high portal insulin) and a small current yields a bright output; turn it down (low portal insulin) and even a large current barely registers.

This is not metaphorical handwaving. Insulin controls the expression of human hepatic GH receptors (GHRs) (8). In the HuH7 hepatoma model, Leung and cols. showed that insulin upregulates total and intracellular GHRs concentration-dependently and increases cell-surface GHRs expression in a biphasic fashion, with a peak at approximately 10 nmol/L, modulating GH-induced JAK2 phosphorylation in parallel with surface receptor abundance (8). Surface GHR availability is the net result of divergent insulin actions on biosynthesis and translocation, mediated respectively by the MAPK and PI3K pathways (8). The dimmer, in other words, dictates the number of GHRs that reach the hepatocyte surface to catch the GH signal.

Defining LSG as portal-insulin-determined hepatic GHR availability converts four disease vignettes into four positions on one axis. Low-LSG states (type 1 diabetes, end-stage liver disease, prolonged fasting) feature scarce portal insulin, relatively low GHR expression by the hepatocytes, thus GH-resistant livers, low IGF-1, and a compensatory rise in GH. High-LSG states (type 2 diabetes, obesity, acromegaly) feature abundant portal insulin, relatively high GHR expression by the hepatocytes, thus GH-hypersensitive livers, and IGF-1 output that outruns what the prevailing GH concentration would otherwise produce. The clinical signatures that follow are not a list of unrelated facts but predictable consequences of the dimmer setting.

MOLECULAR GATEKEEPING: HOW INSULIN SETS THE DIMMER

Insulin and IGF-1 both suppress GH at the somatotroph, but by separate mechanisms. Using Cre/loxP knockdown of the insulin receptor and the IGF-1 receptor in primary mouse pituitary cultures, Gahete and colleagues showed that insulin-mediated suppression of GH is independent of the IGF-1 receptor, and pharmacological dissection in mouse and baboon cultures confirmed that insulin and IGF-1 recruit different intracellular routes to inhibit pituitary GH secretion (5). The two signals converge on the same output but travel different roads.

At the hepatocyte, the decisive event is GHR surface translocation. Insulin's biphasic effect on surface GHRs, with divergent MAPK-driven biosynthesis and PI3K-driven translocation, means that the hepatic GH sensitivity is set post-transcriptionally and dynamically, on a timescale fast enough to respond to feeding and fasting (8). Superimposed on this is SIRT1, which negatively regulates GH-induced IGF-1 production by inhibiting STAT5 activation, providing a nutrient-sensing brake that produces GH resistance during fasting, starvation, and malnutrition (6).

A second, faster amplifier operates through IGF-binding protein-1 (IGFBP-1). Insulin acutely suppresses hepatic IGFBP-1 secretion; because IGFBP-1 restrains IGF-1 action, high portal insulin raises not only total IGF-1 generation but also the free, bioactive fraction (9,10) The dimmer therefore has two ganged controls: GHR availability (how much IGF-1 is made) and IGFBP-1 production (how much of it is active).

The buffering architecture of the circulation matters for interpretation. Six high-affinity IGFBPs regulate IGF-1; in serum, IGFBP-3 and IGFBP-5 are held in a ternary complex with the acid-labile subunit, slowing clearance such that total circulating IGF-1 in adults sits near 100 nM, roughly a thousandfold higher than free insulin (9). Both ligands can cross-activate each other's receptors at reduced affinity (11). Consequently, when portal insulin is low, the liver must limit IGF-1 production to prevent IGF-1 from

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inducing hypoglycemia through the insulin receptor. It accomplishes this precisely by reducing hepatic GHR expression (8).

THE PORTAL-SYSTEMIC INSULIN GRADIENT: THE CENTRAL ARGUMENT

The single most clinically consequential point in this review is that portal insulin and systemic insulin are not interchangeable. The hepatocyte is bathed by portal blood; it never directly samples the systemic concentration that subcutaneous injection or peripheral assay reports. A recent update by the same conceptual lineage has formalized this, arguing that intra-portal insulin is the proximate regulator of hepatic GH sensitivity and that discordant GH/IGF-1 profiles across disease states are best read as a "secondary association" driven by portal insulinization rather than by GH secretion itself (12).

This distinction dissolves several apparent paradoxes. In type 1 diabetes, subcutaneous insulin restores systemic concentrations but leaves the portal vein relatively insulin-poor; the liver stays GH-resistant even when glucose is controlled. During peritoneal dialysis, insulin delivered in the dialysate or intraperitoneally reaches the portal circulation more directly, partially restoring hepatic GHR expression, which is one reason intraperitoneal dosing requirements differ from subcutaneous (13). In acromegaly, the effect of the systemic hyperinsulinemia is more than adequate to counter GH's peripheral lipolysis, yet the high portal insulin still fails to neutralize hepatic GH drive as the liver behaves as supersensitive for GH, so gluconeogenesis persists (14). The recurring therapeutic error, treating a portal-compartment problem with a systemic-compartment intervention, becomes visible only once the gradient is made explicit.

SYSTEMS-LEVEL FEEDBACK AND MODIFIERS

Closing the loop, IGF-1 feeds back on GH secretion through GHRH-dependent hypothalamic mechanisms, demonstrated by pharmacologic and genetic-mouse interventions (15). The net physiological circuit: insulin raises hepatic GHR expression, making the liver GH-sensitive, increasing IGF-1 and thereby suppressing pituitary GH; low portal insulin during fasting reduces GHR expression, lowering IGF-1 and releasing pituitary GH from feedback restraint.

Two modifiers deserve mention because they alter the dimmer's calibration. Sex steroids and the metabolic partitioning of GH itself. In GHD, women require higher GH replacement doses than men because androgens enhance and estrogens attenuate GH action, an effect that is route-dependent, with oral estrogen blunting liver-mediated GH action by directly inhibiting GHR signaling (16). Second, the metabolic partitioning of GH itself: GH is anabolic in most tissues but catabolic in adipose tissue, where it promotes lipolysis, splitting triglycerides into free fatty acids. Chronic GH excess suppresses insulin's anti-lipolytic action, increasing free fatty acid flux and promoting lipotoxic insulin resistance (10). The dimmer analogy still applies, but the bulb is wired to different fixtures in different tissues (**Figure 1**).

LOW-LSG STATES I: TYPE 1 DIABETES AND THE MISDIRECTED COMPARTMENT

Type 1 diabetes is the cleanest low-LSG state. Because endogenous insulin production fails, portal insulin is low even under subcutaneous therapy, which raises only systemic levels. The classic intraportal-infusion work of Shishko and colleagues in recent-onset type I diabetes patients showed that IGF-1 normalization depends on both glycemic control and the route of insulin delivery: only when insulin was returned to the portal vein did serum IGF-1, IGFBP-1, and GH normalize (17).

The functional consequence is striking. The type 1 diabetic liver behaves as if fasting even while the patient eats: it encounters portal-borne nutrients but no portal insulin, and the absence of insulin overrides the presence of nutrients as a signal, so the hepatocyte runs gluconeogenesis and lipolysis postprandially. Systemic insulin lowers glucose but does not relieve hepatic GH resistance; only portal delivery addresses the underlying derangement (17,18). In end-stage renal disease requiring peritoneal dialysis, providing insulin via dialysate restores hepatic GHR expression, permits GH-driven IGF-1 recovery, restores feedback on GH, and thereby improves whole-body insulin sensitivity, lowering the insulin dose needed for control (13) (**Figure 2**).

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Wurzburger and cols. analyzed the IGF-I concentrations before and after 7 days treatment with recombinant human GH in diabetes subjects (19). In case of absence of C-peptide (CpN), patients were considered to have no endogenous pancreatic beta-cell activity. Patients who were C-peptide positive (CpP) still had endogenous insulin secretion. The net increase in IGF-I concentrations after rhGH treatment was significantly lower in CpN patients than in CpP. These results are in keeping with an important role of portal insulin in GH-induced hepatic IGF-I secretion (19).

LOW-LSG STATES II: LIVER FAILURE AND IGF-1 DEFICIENCY

Cirrhosis combines two hits: too few functioning hepatocytes to make IGF-1, and, as glucose and insulin fall, declining portal insulin that renders the surviving hepatocytes even more GH-resistant (8,20). The result is severe IGF-1 deficiency with GH driven far into the acromegaly range, producing a catabolic state of proteolysis and lipolysis comparable to extended starvation and contributing directly to sarcopenia and progressive malnutrition (21,22).

The clinical relevance of IGF-1 in this context has become increasingly clear. In advanced chronic liver disease, IGF-1 signaling tracks hepatic dysfunction and fibrogenesis and independently predicts liver-related mortality, supporting IGF-1 as a prognostic biomarker rather than a bystander (23). Replacement strategies remain investigational: a pilot randomized trial reported improved albumin with low-dose IGF-1 (24), and rodent gene-therapy models show antifibrotic and pro-regenerative effects (25). However, large-scale human efficacy data are absent and the safety profile of rhIGF-1 constrains where it could reasonably be used. In severe primary IGF-1 deficiency, the population with the longest exposure, mecasermin causes hypoglycemia in a quarter to two-fifths of treated patients, together with injection-site lipohypertrophy, lymphoid and tonsillar hypertrophy, and rarely intracranial hypertension (26,27). Two features of cirrhosis amplify the first of these: hepatic glycogen reserve and gluconeogenic capacity are already reduced, so hypoglycemia is common at baseline (20), and low IGFBP-3 and acid-labile subunit concentrations mean that any given dose delivers a disproportionately large free IGF-1 fraction (9). Efficacy expectations should equally be tempered by evidence of target-tissue resistance: normalizing serum IGF-1 for one week in patients with alcoholic cirrhosis produced no change in functional hepatic nitrogen clearance, although it also produced no nitrogen accumulation and therefore no signal of encephalopathy risk (28). The theoretical oncological objection is less straightforward than it first appears, since low rather than high serum IGF-1 predicts the development of hepatocellular carcinoma in hepatitis C-related cirrhosis (29). The plausible niche is consequently narrow and should be defined prospectively: Child-Pugh B disease with documented sarcopenia, stable glycemia, and no hepatocellular carcinoma under standard surveillance, with muscle mass, frailty index, and decompensation events rather than albumin as endpoints, and with a bridge-to-transplant indication as the most defensible first target. Until such a trial exists, IGF-1 in cirrhosis is best used as a prognostic stratifier (23) rather than as a treatment. Read through the LSG lens, IGF-1 replacement is an attempt to bypass a broken dimmer-and-bulb assembly by supplying the output directly, which is defensible only where the assembly itself cannot be repaired.

HIGH-LSG STATES: TYPE 2 DIABETES, OBESITY, AND THE WEIGHT-GAIN TRAP

In type 2 diabetes and obesity, portal insulin is high to compensate for insulin resistance, so the liver is GH-hypersensitive: it needs little GH to produce adequate IGF-1, and the GH/insulin ratio falls (30,31). Low GH status in obesity is largely functional and reverses with sustained weight loss (32). The therapeutically important corollary is that any agent which further raises endogenous portal insulin signaling will deepen the low-GH/high-insulin imbalance, and adipocytes facing that ratio can only store, not mobilize, lipid, the ideal physiology for weight gain.

This predicts the differential weight effect of antidiabetic drug classes. Sulfonylureas and PPAR-γ agonists, which raise endogenous insulin or insulin signaling, promote weight gain even without increased caloric intake (33), whereas insulin-sensitizing

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approaches and agents that do not push portal insulin are the rational metabolic choice. The incretin classes complicate and enrich this picture. GLP-1 receptor agonists, now first-line where atherosclerotic cardiovascular disease or heart failure coexists with type 2 diabetes (34), produce a discordant GH/IGF-1 signature: chronic treatment increases GH secretion while total IGF-1 falls modestly, plausibly via reduced IGFBP-3 and shifts in hepatic GH sensitivity (35,36). Mechanistically this is what the LSG framework predicts when a therapy improves insulin sensitivity and reduces the portal insulin demand: the dimmer is turned down, GH secretion rises to compensate, and the resulting GH-IGF-1 discordance reflects altered hepatic responsiveness rather than a biological paradox. The next-generation multi-agonists, including molecules that deliberately engage the IGF-1 receptor alongside incretin receptors, places the GH/IGF-1 axis an intentional therapeutic target rather than an incidental consequence of treatment (37) (**Figure 3**).

THE INTEGRATIVE CASE: ACROMEGALY AS MAXIMAL LSG DYSREGULATION

Acromegaly is where every mechanism in this review converges. The somatotroph adenoma drives GH excess, which induces insulin resistance and systemic and portal hyperinsulinemia. The high portal insulin makes an already GH-flooded liver even more GH-sensitive, thereby amplifying IGF-1 production. In parallel, hyperinsulinemia-driven suppression of IGFBP-1 raises the free, active IGF-1 fraction on top of that (14,38,39). The dimmer is jammed wide open while the generator continues to overproduce current.

The power of the insulin contribution is illustrated by an old observation with a modern echo: hypoinsulinemia induced by prolonged fasting can completely normalize serum IGF-1 in acromegaly (40), and a eucaloric very-low-carbohydrate ketogenic diet, which lowers insulin, produces the same directional effect (41). GH also has concentration-dependent tissue effects, requiring less GH for gluconeogenesis than for IGF-1 generation. Thus, systemic hyperinsulinemia can blunt peripheral lipolysis while the portal compartment stays under GH control and keeps driving hyperglycemia (14,42).

This is why IGF-1 normalization alone is an inadequate treatment target. Therapies differ sharply in their metabolic footprint even at equivalent IGF-1 control. The GHR antagonist pegvisomant blocks GH action without suppressing insulin secretion and consistently improves insulin sensitivity, fasting glucose, and glycosylated hemoglobin (43,44). First-generation somatostatin receptor ligands are broadly glucose-neutral, whereas the second-generation ligand pasireotide frequently worsens glycemic control by suppressing insulin secretion, an effect reversible on discontinuation. In contrast, dopamine agonists tend to have more favorable effects (45,46). A patient brought to identical serum IGF-1 level targets on pasireotide versus pegvisomant can therefore occupy opposite ends of the glucose-metabolism spectrum. Choosing therapy on serum IGF-1 alone, blind to where each drug sets portal insulin and hepatic GH sensitivity, leaves half the clinical picture unmanaged (**Figure 4**).

ESTIMATING LSG IN CLINICAL PRACTICE

Portal insulin is not measurable outside the research setting, so clinical use of the LSG concept depends on proxies, and three are already available. Fasting IGFBP-1 is the closest single circulating readout of hepatic insulin exposure: serum IGFBP-1 and sex hormone-binding globulin follow estimated portal insulin concentrations rather than whole-body insulin sensitivity, and are appropriate markers of sensitivity only where endogenous insulin secretion is intact (47), while fasting IGFBP-1 correlates with clamp-derived hepatic insulin sensitivity more closely than insulin or C-peptide, and does so independently of adiposity (48). High IGFBP-1 therefore indicates a low-LSG liver and suppressed IGFBP-1 a high-LSG liver, with the added advantage that IGFBP-1 simultaneously reports the free IGF-1 fraction. Second, C-peptide indexes endogenous and therefore portal insulin delivery, which peripheral insulin cannot do, since the liver extracts up to 85% of insulin at first pass (12). The functional confirmation is already contained in this review: the IGF-1 increment after rhGH was significantly smaller in C-peptide-negative than in C-peptide-positive patients (19). Third, IGF-1

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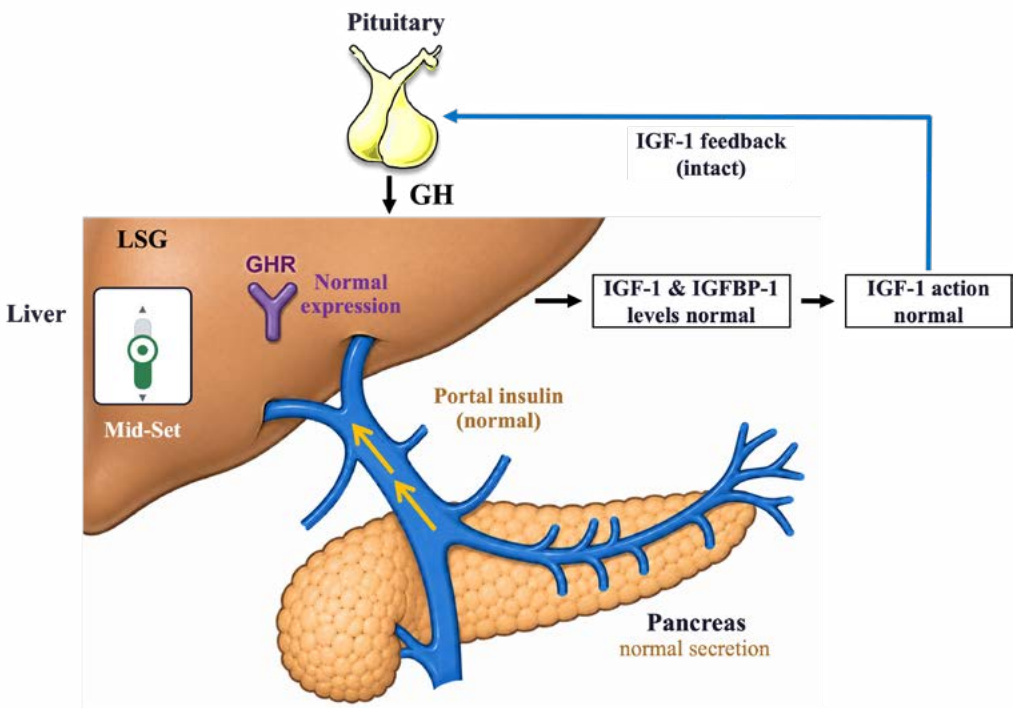


Figure 1. Normal physiology. Portal insulin holds hepatic GHR availability (LSG) at a physiological midpoint, so pituitary GH is translated into hepatic IGF-1 in proportionate fashion and circulating IGF-1 is normal. IGF-1 exerts GHRH-dependent negative feedback on the pituitary, restraining GH secretion; the loop is self-correcting and no arm dominates. GH: growth hormone; GHR: GH receptor; IGF-1: insulin-like growth factor-1; IGFBP-1: IGF-binding protein-1; LSG: liver sensitivity to GH (portal-insulin-set hepatic GHR availability). Arrow thickness denotes the magnitude of each flux; the vertical LSG slider denotes hepatic GH-receptor availability; the orange arrows are the portal insulin setting the slider; the blue line denotes the intact IGF-1 negative feedback on the pituitary.

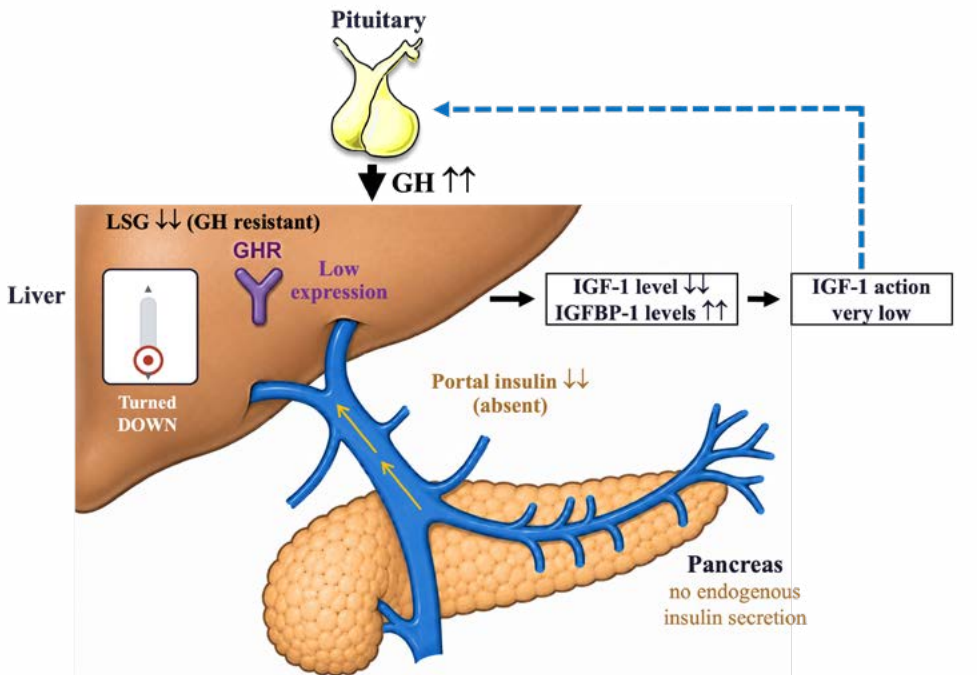


Figure 2. Type 1 diabetes mellitus. Absent endogenous insulin leaves the portal vein insulin-poor even when subcutaneous therapy normalizes systemic insulin, so the LSG slider is turned down and the liver is GH-resistant. Hepatic IGF-1 output collapses; loss of IGF-1 negative feedback releases pituitary GH into the acromegaly range. Correcting glycemia does not relieve hepatic GH resistance—only portal (e.g., intraperitoneal) insulin delivery restores the slider. GH: growth hormone; GHR: GH receptor; IGF-1: insulin-like growth factor-1; IGFBP-1: IGF-binding protein-1; LSG: liver sensitivity to GH (portal-insulin-set hepatic GHR availability). Arrow thickness denotes the magnitude of each flux; the vertical LSG slider denotes hepatic GH-receptor availability; the orange arrows are the portal insulin setting the slider; the dotted blue arrow denotes the impaired IGF-1 negative feedback on the pituitary.

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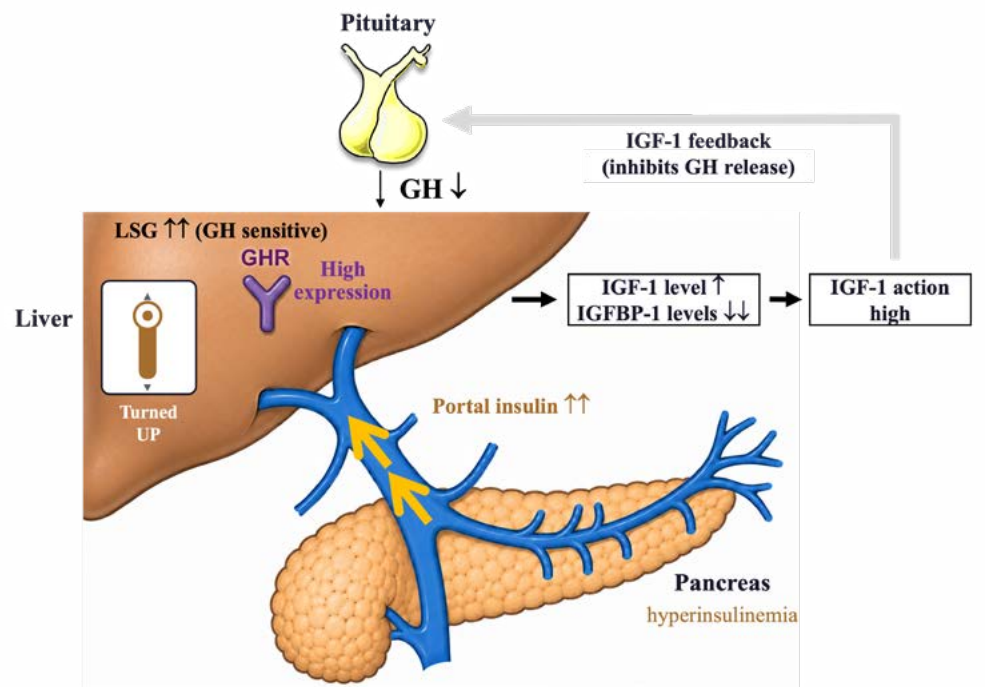


Figure 3. Early type 2 diabetes mellitus and obesity. Insulin resistance drives portal hyperinsulinemia, turning the LSG slider up: a GH-hypersensitive liver produces ample IGF-1 from little GH, and strong IGF-1 feedback suppresses GH secretion. The resulting low GH/high insulin ratio disables adipocyte lipolysis and favors fat storage, which is why agents that further raise endogenous portal insulin promote weight gain and insulin-sensitizing agents are preferable. GH: growth hormone; GHR: GH receptor; IGF-1: insulin-like growth factor-1; IGFBP-1: IGF-binding protein-1; LSG: liver sensitivity to GH (portal-insulin-set hepatic GHR availability). Arrow thickness denotes the magnitude of each flux; the vertical LSG slider denotes hepatic GH-receptor availability; the orange arrows are the portal insulin setting the slider; the gray arrow denotes IGF-1 negative feedback inhibiting GH release from the pituitary.

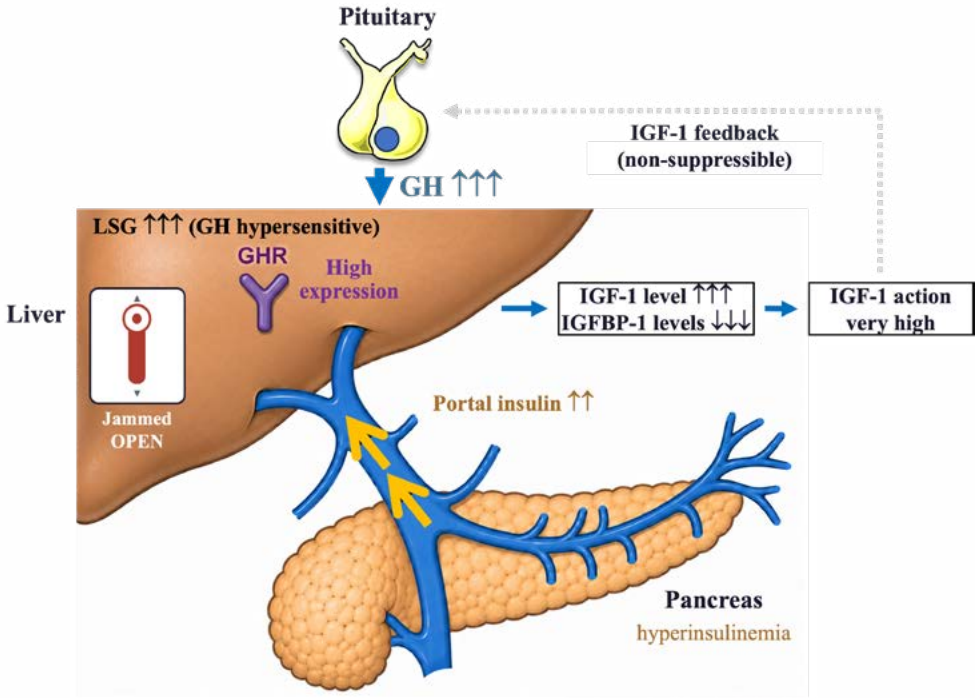


Figure 4. Acromegaly. The somatotroph adenoma floods the liver with autonomous GH that is not restrained by feedback. GH-induced insulin resistance raises portal insulin, turning the LSG slider up and rendering the GH-flooded liver even more GH-sensitive; concurrent IGFBP-1 suppression raises the free, bioactive IGF-1 fraction. Every arm points the same way, so IGF-1 normalization alone is an incomplete treatment target. That lowering insulin by prolonged fasting or a very-low-carbohydrate diet can normalize IGF-1 confirms the strength of the insulin arm. GH: growth hormone; GHR: GH receptor; IGF-1: insulin-like growth factor-1; IGFBP-1: IGF-binding protein-1; LSG: liver sensitivity to GH (portal-insulin-set hepatic GHR availability). Arrow thickness denotes the magnitude of each flux; the vertical LSG slider denotes hepatic GH-receptor availability; the orange arrows are the portal insulin setting the slider; the dotted gray arrow denotes nos-suppressible IGF-1 negative feedback on the pituitary.

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should be read against a concurrent GH rather than alone. Low IGF-1 with elevated GH identifies the GH-resistant, low-LSG liver; normal or high IGF-1 with suppressed GH identifies the GH-hypersensitive, high-LSG liver of obesity and type 2 diabetes. Where a dynamic measure is preferred, the IGF-1 generation test serves as a functional LSG probe, since hyperinsulinemic obese subjects generate a larger IGF-1 response to exogenous GH than lean subjects (12). We propose the combination of fasting IGFBP-1, C-peptide, and the IGF-1/GH pair as a practical bedside estimate of LSG, while emphasizing that none of these proxies has been validated against directly measured portal insulin in humans. Prospective validation against clamp-derived measures of hepatic insulin action is required before such an estimate is allowed to guide treatment.

THERAPEUTIC IMPLICATIONS AND FUTURE DIRECTIONS

Three practical conclusions follow. First, in acromegaly, treatment selection should weigh the metabolic phenotype alongside IGF-1: pegvisomant or a dopamine agonist for the patient with glucose intolerance and caution should be exerted with pasireotide treatment where diabetes is present (43,45,46). Second, in type 2 diabetes the GH/insulin ratio reframes drug choice: preferred agents are those that lower rather than raise portal insulin demand, while noting the GH/IGF-1 discordance seen with GLP-1 receptor agonists as expected rather than alarming.(35-37) Third, in cirrhosis, IGF-1 deserves attention both as a prognostic marker and as well as a therapeutic candidate, awaiting adequately powered trials (23,24).

More broadly, the field would benefit from routinely interpreting the GH/IGF-1 axis in light of portal insulin status rather than treating circulating IGF-1 as a context-free readout of GH activity. The emergence of multi-agonist metabolic drugs that intentionally engage the IGF-1 receptor (37), together with the development of GHR-directed and IGF-axis-directed agents in oncology and metabolism, makes a portal-insulin-aware framework increasingly important for predicting both on- and off-target metabolic effects.

CONCLUSIONS

GH is not simply a hormone that raises circulating IGF-1 levels. Whether GH is permitted to generate hepatic IGF-1 is determined by the insulin that reaches the liver through the portal vein, a variable capture by what we have called liver sensitivity to GH (LSG). Placing type 1 and type 2 diabetes, obesity, liver failure, and acromegaly along a single LSG continuum explains their otherwise disconnected GH/IGF-1/insulin signatures, exposes the portal-systemic compartment error that limits conventional insulin therapy in type 1 diabetes, and reframes treatment selection in both diabetes and acromegaly around metabolic phenotype rather than glycosylated hemoglobin and IGF-1 alone. For the treating physician, reasoning from the dimmer rather than from the bulb is the difference between describing metabolism and understanding its regulation.

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consensus

Latin American Thyroid Society expert panel consensus on anaplastic thyroid cancer

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ABSTRACT

Anaplastic thyroid cancer (ATC) is a highly aggressive histologic subtype of thyroid malignancy. Its rarity, rapidly progressive clinical course, along with limitations in health-care resources make management particularly challenging in Latin America. In recent years, substantial advances in the understanding of ATC biology have led to modifications in therapeutic strategies. This consensus aims to raise awareness and provide practical, evidence-based guidance for the diagnosis and management of ATC, summarizing current diagnostic and therapeutic advances while offering an accessible framework tailored to the Latin American context. A multidisciplinary panel of experts in endocrinology, surgery, oncology, pathology, and genetics from Latin American countries developed a series of recommendations. Each recommendation is accompanied by its rationale and supporting evidence, adapted to the regional context to facilitate implementation. All recommendations were reviewed and voted on through a non-anonymous process. Recommendation wording was pre-specified and linked to voting thresholds: "should" indicated unanimous agreement (100%); "is recommended" indicated ≥70% agreement; and "may be considered" was used when agreement was <70% and/or when applicability depended on local availability, feasibility, or patient-specific clinical context. The consensus includes 37 recommendations covering the initial approach, diagnosis, and management of locoregional and advanced disease. By integrating current evidence with expert consensus and accounting for regional resource variability, this document aims to harmonize clinical practice, support multidisciplinary care, and facilitate timely diagnosis and treatment. These recommendations may also serve as a foundation for future research initiatives and the development and updating of regional health policies for this highly lethal disease.

Keywords: Thyroid cancer; anaplastic thyroid cancer; Latin America

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INTRODUCTION

Anaplastic thyroid cancer (ATC) is a rare and rapidly progressive neoplasia composed of undifferentiated thyroid cells (1). Historically, its prognosis was almost uniformly lethal, with a 12-month overall survival (OS) of 21% (2). Over recent years, a growing knowledge of its molecular landscape, the wider use of molecular testing, and the introduction of targeted therapies and immunotherapy have significantly transformed the

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outcomes of this disease for patients receiving optimal care (3,4). Nevertheless, a large proportion of patients continue to face poor outcomes due to unequal access to a timely diagnosis and advanced treatments. Limited expertise, a scarcity of specialized centers, substantial regional variability in access to molecular testing, and restricted access to state-of-the-art therapies are common challenges in the management of rare diseases. For ATC, effective treatment also requires a swift diagnosis and timely access to appropriate diagnostic and therapeutic interventions. These challenges are further intensified in resource-constrained countries, where patients often face multiple overlapping barriers, including socio-cultural factors and fragile healthcare systems (5–7).

Key challenges in the management of ATC in Latin America

- **Geographic barriers:** Most specialized centers are predominantly located in capital and major cities, limiting access for patients in remote or underserved regions.
- **Health care systems disparities:** Marked variability in access to public healthcare, social security and private insurance sectors.
- **Infrastructure gaps:** Diagnostic and therapeutic technologies are not uniformly or readily accessible.
- **Specialists:** Limited availability of dedicated training programs resulting in a reduced number of highly skilled specialists.
- **Limited access to molecular testing:** Constrained by high costs, inconsistent insurance coverage, and uneven regional availability.
- **Limited access to novel therapies:** Driven by high cost, limited reimbursement and, in some cases, lack of regulatory approval.
- **Limited availability of clinical trials:** Most regions have minimal or no participation opportunities.

Addressing such inequities underscores the importance of developing context-sensitive recommendations that target both clinical and systemic gaps. Therefore, this consensus aimed to 1) raise awareness and offer guidance on the current management of ATC, 2) summarize the latest evidence on diagnostic and therapeutic approaches, and 3) provide an accessible framework for diagnosis and treatment that is adapted to the Latin American context and aligned with the need to inform and update health policies.

MATERIALS AND METHODS

This document presents expert recommendations developed under the auspices of the Latin American Thyroid Society to provide pragmatic, regionally applicable guidelines for the evaluation and management of ATC. Clinical questions were identified by the coordinating group and assigned to 13 panelists according to their areas of expertise. The panel consisted of clinicians and clinician-scientists (five endocrinologists, two surgeons, four oncologists, one pathologist, and one geneticist) who are actively involved in the clinical management and research of advanced thyroid cancers, including ATC. Members represented multiple tertiary referral centers across Latin America, reflecting diverse practice patterns. At the outset, a table outlining six key topics in this field (**Table 1**) was circulated to all panel members, who then prioritized areas of focus. The panel held its inaugural virtual meeting in January 2025.

Following this meeting, an initial draft was prepared. Each section was authored by one panel member and independently reviewed by two additional members. Recommendations were formulated by interpreting the available evidence within the Latin American healthcare context, including resource availability and regional practice patterns. The draft underwent a full-group review to collect feedback and guide subsequent refinements. All recommendations were discussed and revised through structured virtual meetings and electronic communications. Given that the limited and heterogeneous evidence base for many clinical questions in ATC is predominantly derived from observational studies, small case series, or extrapolation from other settings, we did not apply a formal framework to grade the quality of evidence or the strength of recommendations.

Recommendation phrasing was pre-specified and linked to voting thresholds: “should” indicated unanimous agreement (100%); “is recommended” indicated ≥70% agreement; and “may be considered”

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Table 1. Key priority areas addressed in the recommendations

Priority area	Description
1. Initial evaluation	1.a Emergency evaluation of rapidly growing thyroid masses
	1.b Diagnostic approach and differential diagnosis
	1.c Acquisition of diagnostic material
	1.d Molecular profiling in anaplastic thyroid cancer
2. Staging and pre-therapy assessment	2.a Multidisciplinary team-based care, therapeutic planning and integration of palliative care
	2.b. Diagnostic imaging, endoscopic procedures, and thyroid neck morbidity scores
3. Surgical management	3.a The role of surgery for localized disease
	3.b Technical surgical considerations and the role of neoadjuvant therapy
	3.c Elective tracheostomy
4. External beam radiotherapy (EBRT)	4.a EBRT following R0/R1 resection
	4.b EBRT in unresectable disease
5. Systemic therapy	5.a "Bridging" therapy
	5.b Dabrafenib plus trametinib for *BRAF*-V600E-mutated anaplastic thyroid cancer
	5.c Neoadjuvant strategies in *BRAF*-V600E-mutated anaplastic thyroid cancer
	5.d Other selective inhibitors (or targeted therapies)
	5.e Cytotoxic chemotherapy and multikinase inhibitors
	5.f Immunotherapy
6. Other aspects	6.a. Follow-up strategies

was used when agreement was <70% and/or when applicability was expected to depend heavily on local availability, feasibility, or the patient-specific clinical context. All recommendations were voted on in a single round using an open (non-anonymous) voting process. Abstentions were not permitted; therefore, all panelists voted on every recommendation. Recommendations were finalized after incorporating edits aimed at improving wording consistency and operational clarity. The document was then shared with selected Latin American Thyroid Society members for additional input and discussion.

INITIAL EVALUATION

Emergency evaluation of rapidly growing thyroid masses

- **Recommendation 1:** A rapidly enlarging thyroid mass should be considered a medical emergency and managed accordingly.
- **Recommendation 2:** In patients with an enlarging neck mass, a rapid and coordinated multidisciplinary approach should be implemented to confirm the diagnosis, evaluate airway safety, and assess disease extent.
- **Recommendation 3:** Sudden changes in differentiated thyroid cancer (DTC) behavior should raise suspicion of transformation to ATC and prompt further evaluation.
- **Recommendation 4:** When ATC coexists with a more differentiated follicular-derived thyroid carcinoma, management should be guided by the anaplastic component.

Rapidly growing thyroid masses, defined as those that enlarge over the course of weeks, should be considered a clinical emergency and managed with a fast-track diagnostic plan. The most likely causes of a rapidly enlarging thyroid mass include ATC and thyroid lymphoma, two aggressive malignancies that require prompt recognition and timely treatment (8). For ATC, reducing diagnostic delays has resulted in improved outcomes, allowing patients to participate in clinical trials and improving OS (9,10). For rapidly growing thyroid masses, the initial approach differs from the routine evaluation of thyroid nodules. Confirmation of the diagnosis, staging, and, in the case of ATC, assessment of the *BRAF* mutational status should be conducted simultaneously and swiftly, ideally within days.

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Cross-sectional imaging is essential for accurately assessing the extent of disease. While fine-needle aspiration (FNA) with immediate cytopathologic assessment can support a rapid diagnosis, performing a concurrent core-needle biopsy (CNB) is usually advised to improve diagnostic accuracy and enable molecular profiling (8,11). Airway compromise is the most urgent concern and must be evaluated at every clinical encounter. Every effort should be made to ensure an expedited diagnosis and management, including hospital admission and early involvement of a multidisciplinary team (12,13). Prompt referral to experienced centers or consultation with a tumor board should be considered when specialized expertise or technical resources are limited.

A very small subset of DTC undergoes anaplastic transformation, a finding that supports the concept that pre-existing DTC is a risk factor for ATC (reported at around 2% after reoperation for recurrent disease). Risk factors for anaplastic transformation include older age ≥55 years, multiple surgical procedures for recurrent disease (14), and, particularly, the co-occurrence of *TERT* promoter mutations with the *BRAF* V600E mutation (15). Early recognition of this possibility is critical, despite the risk of overdiagnosis, because timely evaluation and intervention may influence management options and patient outcomes.

The coexistence of both DTC and ATC is a recognized phenomenon. ATC can arise de novo or from DTC and poorly DTC through the progressive accumulation of genomic alterations (16). Co-occurring DTC is frequently observed in patients with ATC; this is consistent with a branching evolution during disease development, which may hypothetically occur at any stage of progression (16). The clinicopathological characteristics of ATC that develops from DTC remain poorly understood, and the distinct treatment modalities for DTC and ATC further add to this complexity (17).

Management of concomitant ATC and DTC remains poorly defined, given the scarcity of evidence and the limited guidance provided in major published guidelines (13,18–20). Because differentiated features characteristic of follicular cells and DTC, including expression of the thyroid-stimulating hormone receptor and the sodium-iodide symporter, are absent in ATC, TSH suppression is not indicated, and radioactive iodine is ineffective and thus not recommended (13). There is broad consensus that when an ATC component is present, treatment should primarily target the ATC due to its markedly worse prognosis.

Diagnostic approach and differential diagnosis of ATC

- **Recommendation 5:** The definitive diagnosis of ATC should be established promptly using FNA cytology and/or CNB, supported by a comprehensive immunohistochemical (IHC) panel to exclude alternative diagnoses.

ATCs are characterized by marked aggressiveness, including frequent extrathyroidal extension, extensive necrosis, high mitotic activity (often atypical), pronounced nuclear pleomorphism, and angiolymphatic invasion (1). Morphologically, they are highly heterogeneous and may exhibit epithelioid, spindle cell, giant cell, pleomorphic, paucicellular, rhabdoid, small cell, osteoclast-like giant cell-rich, angiomatoid, or squamous patterns (1,21). Recognition of these morphological patterns is important because of the broad range of differential diagnoses, although the patterns themselves do not carry prognostic or therapeutic significance. The differential diagnosis includes other primary thyroid malignancies, high-grade lymphomas, melanomas, sarcomas, and, less commonly, metastases (22,23).

A broad IHC panel is crucial to distinguish ATC from its mimickers (1,22–28) (**Table 2**). In many cases, the most compelling evidence for ATC comes from the lack of expression of markers that would otherwise support other diagnostic possibilities. Cytokeratins (e.g., AE1/AE3, CK7, and CK8/18) are positive in approximately 75% of cases. However, the absence of cytokeratin expression does not exclude ATC (23). The thyroid lineage marker PAX8 is positive, at least focally, in 40–60% of cases (23). Its expression can support a diagnosis of follicular thyroid cell origin. Thyroglobulin (a thyroid-specific protein) and thyroid transcription factor-1 (TTF-1) are usually negative in ATC, although the latter may show focal or weak positivity in 10–30% of cases (22).

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Table 2. Differential diagnosis of anaplastic thyroid carcinoma

Tumor type	Key distinguishing features	Helpful IHC markers
Poorly differentiated thyroid carcinoma	Intermediate morphology and prognosis between DTC and ATC. The Turin criteria* are useful for diagnosis	Positive: thyroglobulin (focal), TTF-1 (focal) Negative: calcitonin
Medullary thyroid carcinoma	May show focal anaplastic areas mimicking ATC	Positive: Calcitonin, CEA, Chromogranin A, Synaptophysin, INSM-1 Negative: thyroglobulin
High-grade lymphoma	May resemble ATC morphologically. Positive for lymphoid markers, which are absent in ATC	Positive: CD45, CD20 Negative: cytokeratin, thyroglobulin
Intrathyroidal thymic carcinoma (CASTLE)	Composed of squamoid or undifferentiated cells; less pleomorphism than ATC; thymic differentiation	Positive: CD5, CD117 Negative: thyroglobulin
Melanoma	May mimic ATC with pleomorphic epithelioid cells; melanin pigment may be minimal or absent	Positive: S100, SOX10, Melan-A, HMB-45 Negative: thyroglobulin
Sarcomas (primary thyroid or metastatic)	Rare; may resemble ATC morphologically; lack epithelial markers; consider if no pre-existing or co-existing DTC component is identified and/or negative for MAPK mutation (e.g., *BRAF* or *RAS*)	Subtype-specific markers: Angiosarcoma: CD31, ERG-1: Leiomyosarcoma: SMA Rhabdomyosarcoma: Desmin, MyoD1, Myogenin Favor ATC: MAPK mutations (*BRAF*, *RAS*)
Metastases to the thyroid	Usually in patients with known primary malignancy, morphology may overlap with ATC; lack of thyroid differentiation	Histological correlation with the known primary tumor and use of site-specific immunohistochemical markers are essential to accurately establish the diagnosis Negative: thyroid lineage markers such as thyroglobulin, TTF-1, PAX8 or only focally positive in metastases
Riedel's thyroiditis	Dense fibrosis replacing normal thyroid parenchyma, often extending beyond the thyroid, chronic inflammatory infiltrate, obliterative phlebitis. Absence of granulomatous inflammation and neoplastic features	Increased number of IgG4-positive plasma cells (≥10 IgG4+ plasma cells per high-power field and an IgG4+/IgG ratio ≥20%) are considered supportive

ATC: anaplastic thyroid cancer; CEA: carcinoembryonic antigen; DTC: differentiated thyroid cancer; IHC: immunohistochemistry; INSM-1: insulinoma-associated protein 1; MAPK: mitogen-activated protein kinase; PAX8: Paired-box gene 8; TTF-1: Thyroid Transcription Factor-1. *Turin criteria include: 1) the existence of a solid/insular/trabecular pattern; 2) the absence of the usual nuclear characteristics of papillary thyroid cancer; and 3) the presence of at least one of the following features: convoluted nuclei, ≥3 mitosis per 2 mm^2, and tumor necrosis (29).

In certain cases, the assessment of proliferative activity based on mitotic figures may be challenging. In these situations, Ki-67 IHC offers a more reliable alternative. ATC typically displays a high Ki-67 proliferation index, often ≥30% (23). In addition, p53 often shows an aberrant IHC pattern in approximately 50–60% of ATCs, suggesting a somatic *TP53* mutation; this presents as either a complete loss of p53 protein expression or markedly increased p53 protein abundance due to an extended protein half-life (1,20).

True squamous differentiation, along with immunoexpression of p63, p40, and CK5/6, can be seen in ATCs. In the current World Health Organization Classification (5th Edition), squamous cell carcinoma of the thyroid is considered a subtype of ATC (1,20). Because the pooled incidence of the *BRAF* V600E mutation is 33% (range, 0–93%) in ATCs, IHC for the *BRAF* V600E mutation using the VE1 antibody may serve as a surrogate for molecular testing in the preoperative setting or immediate post-diagnostic period, particularly for guiding targeted BRAF inhibitor therapy (20). However, IHC is an imperfect predictor of mutation status; therefore, positive results should be confirmed by molecular testing whenever possible (23).

Acquisition of diagnostic material

The first-line method for obtaining material from a neck mass is ultrasound-guided FNA (23). FNA performs well as a screening test, demonstrating a sensitivity of 100% and a positive predictive value of 89% (29). This is a rapid, safe, and cost-effective procedure capable of providing a preliminary morphological diagnosis within minutes (30). Multiple needle passes should be performed in different areas of the lesion to ensure the sampling of viable tumor cells and to avoid sampling only necrotic areas. Recent advances in

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molecular techniques allow for the reliable detection of common driver mutations, such as *BRAF* V600E, *RAS*, and *TERT* promoter mutations, even in small cytologic samples (31).

For each pass, part of the material is used to prepare smears, while the remainder can be allocated to cell block preparation. To optimize the quality of the cell block for IHC, some dedicated passes should be performed specifically for this purpose. Whenever feasible, a CNB should be performed concurrently with the FNA. This approach provides complementary tissue for histological diagnosis and ancillary studies, including comprehensive genomic testing (e.g., DNA- or RNA-based next-generation sequencing [NGS] panels) (11).

As with FNA, CNB samples may be nondiagnostic if only fibrotic or necrotic tissue is obtained (32); ultrasound (US) plays a critical role in identifying solid, viable areas for targeted sampling (11). When available, rapid on-site evaluation by either a cytotechnologist or a pathologist is highly beneficial for confirming sample adequacy during the procedure (33). In emergency settings, especially for patients presenting with respiratory compromise, an open biopsy may be performed during a tracheostomy.

Molecular profiling in ATC

- **Recommendation 6:** Rapid *BRAF* V600E testing should be performed as soon as possible after diagnosis (ideally within 24 hours) when targeted therapies are available and timely testing is feasible. While molecular testing is preferred when accessible, IHC may be used as an initial rapid screening method, followed by molecular confirmation whenever feasible.
- **Recommendation 7:** More comprehensive molecular profiling should be performed whenever targeted therapies or clinical trials are available. In settings where available, targeted NGS panels are recommended for both targeted and broad detection of actionable alterations.
- **Recommendation 8:** Consider obtaining blood for a liquid biopsy when adequate tumor tissue is unavailable, as it represents a less sensitive but minimally invasive alternative for molecular testing.

Because ATC often exhibits marked genomic heterogeneity, broad NGS panels are preferred, as they enable the detection of coexisting driver mutations, rare fusions, and biomarkers relevant to targeted therapy or immunotherapy decisions, such as tumor mutational burden and microsatellite instability. Alternatively, when NGS is not feasible, selected actionable alterations may be assessed using targeted assays, provided that corresponding therapies and/or clinical trials are available. The main genetic alterations in ATC are summarized in **Table 3**.

When primary tumor tissue is unavailable or inadequate, and the sampling of recurrent or metastatic disease is unfeasible or insufficient, liquid biopsy using circulating tumor DNA and/or circulating tumor RNA may serve as a minimally invasive alternative for the rapid detection of actionable point mutations or fusions, respectively (34). While less sensitive than tissue-based assays, this approach may better capture tumor heterogeneity and the broader spectrum of genomic alterations.

ATC is characterized by a complex genomic landscape with a broad spectrum of somatic mutations and a generally moderate mutational burden. These alterations can be grouped into early mutations, driver events activating the mitogen-activated protein kinase (MAPK) and PI3K/AKT pathways, which are also commonly found in DTC, and late mutations, such as *TERT* promoter and *TP53* mutations, which play a critical role in anaplastic transformation (20,35–37).

Although the *BRAF* V600E mutation is one of the most frequent early alterations identified in ATC (20,38), mutations in *RAS* genes (*HRAS*, *KRAS*, and *NRAS*) are also frequently observed (20,38). Multiple studies have shown that *RAS* mutations are associated with poorer survival outcomes (36). While the *BRAF* V600E mutation represents the most prevalent and clinically actionable MAPK driver, other MAPK-related fusions, including *RET*, *ALK*, and *NTRK*, are also clinically actionable when detected (20,23,35,39–41).

Alterations in tumor suppressor genes and components of the PI3K/AKT/mTOR signaling pathway are more frequently observed in ATC than in PTC, contributing significantly to its aggressive phenotype. Overall, genetic alterations in the PI3K/AKT/mTOR

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Table 3. Genetic alterations in ATC: prevalence and testing methods

Genetic alteration	Prevalence	Testing method	Notes
BRAF V600E	0–93%	IHC, Sanger Sequencing, PCR*, DNA-based NGS panel	Most common actionable driver: Urgent reflex testing for *BRAF* V600E is critical Associated with rapid and durable treatment responses
RAS (*HRAS/KRAS/NRAS*)	9–50%	Sanger Sequencing, PCR, DNA-based NGS panel	No approved direct inhibitors; prognosis and trial-eligibility implications
TERT promoter (C228T/C250T)	30–75%	PCR*, Sanger sequencing, DNA-based NGS panel	Strong predictor of poor outcomes; genetic duet with *BRAF* or *RAS* further worsens prognosis
ROS1 fusions	Rare	PCR*, Sanger sequencing, RNA-based NGS panel	Case reports show responses to selective inhibitors
ALK fusions	<5%	FISH, RT-PCR, RNA-based NGS panel	Case reports show responses to selective inhibitors
NTRK fusions	<5%	FISH, RT-PCR, RNA-based NGS panel	FDA-approved therapies for any tumor with *NTRK* fusion
RET fusions	<5%	FISH, RT-PCR, RNA-based NGS panel	FDA-approved therapies for *RET*-driven thyroid tumors
PTEN mutation	0–18%	DNA-based NGS panel	Off-label therapies, limited efficacy
TP53	40–80%	IHC, Sanger sequencing, NGS panel	Not targetable
PIK3CA mutation	0–25%	Sanger sequencing, DNA-based NGS panel	No approved targeted therapies; potential eligibility for clinical trial
High tumor mutation burden	10–20%	Broad NGS	Predicts response to immunotherapy; important when driver mutations are absent; tumor-agnostic approval
Microsatellite stability-high (MSI-H)	<5%	Broad NGS	Predicts response to immunotherapy; important when driver mutations are absent; tumor-agnostic approval

IHC: immunohistochemistry; *Single gene PCR (allele-specific, digital PCR); NGS: next generation sequencing.

pathway can be found in 40–57% of ATC patients (20,35,38). Mutations in *PIK3CA* are found in up to 25% of ATC cases, while *PTEN* mutations or loss of expression are reported in up to 18% of cases. These alterations represent potential therapeutic targets; consequently, agents inhibiting various components of this pathway, such as mTOR inhibitors, have been explored in several small clinical trials (39).

These mutations also hold significant prognostic importance, as they are linked to adverse outcomes such as metastatic dissemination and poorer OS in ATC patients. Furthermore, *BRAF* V600E mutations frequently cooperate with alterations in the PI3K/AKT/mTOR pathway, including *PIK3CA* mutations. This co-occurrence drives aggressive tumor phenotypes, decreases survival, and contributes to resistance to BRAF inhibitors (20).

Loss-of-function mutations in the *TP53* gene are exceptionally common in ATC, making them one of the most prevalent genetic alterations. With reported frequencies ranging from 42.6% to 75%, these mutations are strongly associated with genomic instability and a poor prognosis (35). Copy number loss in the tumor suppressor genes *CDKN2A*/2B suggests that these alterations may the underlying drivers of disease progression and dedifferentiation (37).

TERT promoter mutations (C228T and C250T) are highly prevalent in aggressive thyroid cancers, particularly in ATC, where frequencies can reach up to 75% (20,38,40). These mutations are strong independent prognostic markers for poor outcomes, including distant metastasis and increased disease-specific mortality. The coexistence of *TERT* promoter mutations with *BRAF* or *RAS* mutations is associated with a worse prognosis, highlighting a "genetic duet" that confers heightened aggressiveness (20,40).

STAGING AND PRE-THERAPY ASSESSMENT

Multidisciplinary team-based care, therapeutic planning and integration of palliative care

- **Recommendation 9:** ATC management should be delivered by a multidisciplinary team, with attention to both curative-intent strategies and palliative goals, alongside the active engagement of patients and their families or caregivers to ensure holistic care.

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- **Recommendation 10:** Due to the poor prognosis and limited evidence guiding many management decisions in ATC, special consideration should be given to patient preferences after thorough discussion.

A coordinated multidisciplinary approach is essential in ATC, given the disease's aggressiveness, historically poor prognosis, and recent therapeutic advances that can meaningfully extend survival. Multidisciplinary tumor board evaluation within days of diagnosis is vital to ensure timely and coordinated care. Teams should rapidly clarify the goals of care, whether therapeutic or palliative (4,13). Treatment planning constitutes a medical emergency that necessitates individualized approaches. One model is MD Anderson's Facilitating Anaplastic Thyroid Cancer Specialized Treatment program, a rapid-access pathway designed to accelerate diagnosis, molecular profiling, and treatment through the integration of a group of endocrinologists, oncologists, head and neck surgeons, pathologists, radiologists, radiation oncologists, and palliative care specialists (3,4). Most patients require combined treatment modalities tailored to their disease stage and performance status, while supportive care and airway evaluation must remain priorities. Continuous multidisciplinary oversight is necessary to monitor treatment response, manage toxicity, and adapt strategies as the disease evolves.

Patients and their families should receive clear and timely prognostic information, along with support to weigh the risks and benefits of available options (12,13). Realistic expectations should be established, and patient preferences regarding the extent of aggressive interventions must be discussed and respected. In the context of advanced disease, certain therapies (e.g., chemotherapy) may reduce quality of life and increase healthcare costs without improving survival.

Early discussions are therefore essential, covering topics such as airway management (and the potential need for a tracheostomy), nutritional support, hospice care, and end-of-life preferences, including symptom control for pain and dyspnea (13,42). Palliative care should be integrated from the time of diagnosis to address symptoms, psychosocial needs, and family support. Depending on the disease trajectory and patient values, care may combine active therapy with supportive measures or transition to exclusively palliative approaches. Failure to discuss end-of-life considerations may lead to a poor prognostic understanding, consequently impairing shared decision-making and delaying referral to palliative care services (43).

Diagnostic imaging, endoscopic procedures, and thyroid neck morbidity scores

- **Recommendation 11:** Cross-sectional imaging of the neck (contrast-enhanced computed tomography [CT] or magnetic resonance imaging [MRI]) should be performed in all patients diagnosed with ATC to comprehensively assess the extent of the tumor and its invasion into adjacent structures.
- **Recommendation 12:** To thoroughly assess disease extension, an ^{18}F-FDG PET/CT is recommended for all patients diagnosed with ATC. If it is not readily available, a contrast-enhanced CT of the neck, thorax, abdomen, and pelvis should be conducted to avoid delaying treatment.
- **Recommendation 13:** A contrast-enhanced brain MRI is recommended at the diagnosis of ATC to ascertain the presence of brain metastases. Additionally, it should be performed in the presence of suggestive clinical findings.
- **Recommendation 14:** An assessment of vocal cord mobility, ideally via laryngoscopy, should be performed in all patients diagnosed with ATC to assess vocal cord function and potential tumor involvement of the larynx and upper trachea.
- **Recommendation 15:** Upper gastrointestinal endoscopy is recommended at the diagnosis of ATC in patients with clinical or radiographic evidence suggesting upper gastrointestinal tract involvement.
- **Recommendation 16:** Bronchoscopy is recommended at the diagnosis of ATC in patients with clinical or radiographic evidence suggesting tracheal involvement.

Given the aggressive nature of ATC, comprehensive imaging is essential for accurately defining

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disease extent and guiding management. Several imaging modalities may be used depending on availability. Local invasion is almost universal and must be carefully characterized for treatment planning (44,45). Distant metastases are present in up to 50% of patients, most commonly involving the lungs, bones, and brain (44–48).

Cross-sectional imaging of the neck, using CT or MRI, assesses the local extent of the tumor and its invasion into adjacent structures, which is essential for surgical planning and evaluating resectability (4,13,49–51). An ^{18}F-FDG PET/CT scan provides a detailed assessment of both local and distant disease involvement. It is particularly effective in detecting metastases that might be missed by other imaging techniques, significantly influencing management strategies by identifying previously unknown metastatic sites (4,44,52,53). Although an ^{18}F-FDG PET/CT is considered the test of choice for staging, its availability in most Latin American centers is limited or nonexistent. Even in centers where it is available, its use is frequently delayed due to health system–related barriers. In settings where an ^{18}F-FDG PET/CT is inaccessible, a contrast-enhanced CT or MRI of the neck, chest, abdomen, and pelvis represents a practical alternative, given its broader availability (4,13). Additionally, if bone metastases are detected, further evaluation (e.g., a spine MRI in the case of vertebral metastases) is advised to characterize the lesions more thoroughly (4,13).

With brain metastases present in up to 5% of cases, the utility of universal brain imaging remains debated. Nevertheless, given its potential to influence management, brain imaging is advised at diagnosis for all patients and is mandatory in the presence of symptoms suggestive of neurological involvement (e.g., headaches or neurological deficits). MRI is preferred over CT due to its greater sensitivity for detecting brain metastases (4,12,13).

In ATC, the risk of laryngeal nerve involvement and subsequent vocal cord paralysis necessitates meticulous surgical planning, underpinned by a thorough assessment of vocal cord function (4,13). The preferred evaluation method is a laryngeal assessment via fiberoptic laryngoscopy, which provides a comprehensive examination of vocal cord mobility and can detect direct tumor involvement of the larynx and upper trachea (4,13,54). In scenarios where fiberoptic laryngoscopy is unavailable, direct and indirect laryngoscopy or a transcutaneous laryngeal US may serve as alternative diagnostic tools. While a transcutaneous laryngeal US is typically employed in the postoperative evaluation of thyroidectomy patients, it represents a viable option for assessing vocal cord function in this context. Nevertheless, a limitation of this modality is that most patients present with a high tumor volume, which obscures the vocal cords and prevents the use of alternative viewing windows, such as a lateral approach (13,55,56).

Upper gastrointestinal endoscopy and bronchoscopy are not typically included in the initial staging protocol for ATC. Nevertheless, they become pertinent investigations in instances of clinical or radiographic evidence suggesting airway or upper gastrointestinal tract involvement or bleeding. In such cases, endoscopic findings can significantly influence the management strategy, guiding therapeutic decisions appropriate to the patient's condition (4,13,57).

SURGICAL MANAGEMENT OF ATC

- **Recommendation 17:** Surgery should be offered exclusively when R0 or R1 resection is feasible, and it should be followed by comprehensive adjuvant multimodal therapy.
- **Recommendation 18:** For stage IVA and IVB disease, upfront surgical intervention should remain standard practice when anatomically and functionally appropriate.
- **Recommendation 19:** R2 (debulking) surgery should be strictly avoided due to a lack of clinical benefit and increased patient morbidity.
- **Recommendation 20:** Neoadjuvant targeted therapy should be strongly considered for patients with *BRAF* V600E mutations presenting with initially unresectable disease.
- **Recommendation 21:** For patients with *BRAF* V600E-negative ATC and initially unresectable diseases, alternative neoadjuvant therapies should be considered when actionable alterations are identified by molecular profiling.

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- **Recommendation 22:** In patients with stage IVC ATC, surgery is generally not recommended; however, it may be considered in exceptional cases presenting with a limited systemic disease burden, or for symptom management.
- **Recommendation 23:** Elective tracheostomy should not be routinely performed.

The role of surgery for localized disease

Most patients are not candidates for upfront surgery due to local disease extension. For individuals presenting with resectable stage IVA or IVB disease, surgical intervention represents a cornerstone of potentially curative treatment. The recommended approach involves a total thyroidectomy accompanied by central compartment lymph node dissection, with selective lateral neck dissection based on the extent of nodal involvement. However, surgery alone is insufficient, and all patients require adjuvant postoperative therapy, including intensity-modulated radiation therapy and systemic treatment (13,58).

Technical surgical considerations and the role of neoadjuvant therapy

When evaluating a patient with ATC, operability should be assessed first, followed by resectability. Operable patients are those without extensive metastatic disease and are fit for major surgery. Surgery should be performed only when complete macroscopic tumor removal is feasible and an R0 or, at most, R1 resection is realistically achievable. Debulking (R2) resections provide no survival benefit, significantly increase morbidity, and may delay the initiation of systemic therapy (13,58–61).

Tumors invading the prevertebral fascia, brachial plexus, neck or mediastinal vessels, or requiring non-reconstructable tracheal or esophageal resection are generally considered unresectable (62). Surgical management of ATC requires highly specialized expertise and multidisciplinary planning frequent invasion of critical cervical structures. Extension to the trachea, esophagus, or major vessels may demand complex *en bloc* resections performed in collaboration with thoracic, vascular, or reconstructive surgery teams (13,63). However, radical procedures are usually discouraged and reserved for selected cases. Many patients are better candidates for molecularly guided neoadjuvant therapy, with regular reassessment to determine potential conversion to resectability or, failing this, transition to palliative care (64,65). Ongoing controversy about upfront surgery versus neoadjuvant approaches has prompted attempts to standardize operability criteria, utilizing tools such as the Thyroid Neck Morbidity Complexity and Surgical Morbidity Complexity Scores (66,67).

The identification of actionable genetic alterations, particularly the *BRAF* V600E mutation, has led to a paradigm shift in the management of unresectable ATC. In tumors harboring targetable mutations, neoadjuvant targeted therapy may downstage previously inoperable disease, enabling surgical resection and potentially improving both local control and survival. This strategy has produced what is likely the most important and transformative change in clinical practice to date (see the next sections on BRAF inhibitors used alone or in combination with immunotherapy) (66,68). During neoadjuvant treatment, the patient should be regularly re-evaluated using contemporary imaging to assess tumor response and the possibility of conversion to a less aggressive surgical procedure. When tumor resectability remains uncertain, the surgeon may perform intraoperative staging to assess invasion of major vessels or the upper aerodigestive tract.

Patients with stage IVC disease and distant metastases present unique challenges. In this setting, surgical intervention is generally reserved for palliative purposes, aimed at addressing life-threatening complications (e.g., airway obstruction, uncontrolled bleeding, or severe dysphagia). While curative resection remains uncommon in metastatic disease, carefully selected patients with limited metastatic burden and technically feasible primary tumor resection may experience modest survival benefits when surgery is incorporated into a comprehensive multimodal treatment plan (59–61,69).

Elective tracheostomy

Given that nearly all patients with ATC are at risk for airway obstruction, elective tracheostomy was historically considered a palliative option. Nevertheless,

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tracheostomy significantly decreases quality of life, and its impact on survival prolongation is limited. Bleeding and tumor growth through the incision are frequent complications. Consequently, a paradigm shift has occurred over the past decade, and currently, prophylactic tracheostomy is not recommended (42). However, the potential need for a procedure in the event of acute airway distress must be discussed. Emergency tracheostomy is technically demanding and should be performed in an operating room by experienced head and neck surgeons under general anesthesia. Emergency tracheostomy conducted for symptomatic airway obstruction carries a 30-day mortality rate of 17.6% (70).

EXTERNAL BEAM RADIOTHERAPY (EBRT)

- **Recommendation 24:** Postoperative EBRT should be indicated for all patients with ATC after R0/R1 resections.
- **Recommendation 25:** Radiosensitizing chemotherapy is recommended for all patients receiving EBRT after R0/R1 resections.
- **Recommendation 26:** EBRT should be a part of the therapeutic approach of unresectable ATC as it enhances regional control and palliates symptoms.

EBRT following R0/R1 resections

Local complications are a leading cause of morbidity and mortality in ATC (71,72). Improving locoregional control remains a critical component of ATC management, even after satisfactory surgical resection. The role of adjuvant therapy (radiation or chemoradiation) has been evaluated predominantly in retrospective series, which included heterogeneous populations, varying radiation doses, and combinations with different radiosensitizers.

Despite these limitations, multiple studies and a 2016 meta-analysis demonstrate that adding postoperative radiotherapy or chemoradiation to surgery for stage IVA/IVB ATC improves local control and approximately doubles OS compared to surgery alone, especially in patients who underwent R1/R2 resection. These findings were confirmed in National Cancer Database analyses, where multimodal treatment (surgery plus chemoradiation) increased 1-year OS from approximately 13–22%, with the greatest benefit observed in patients who had R0/R1 resections (73–75).

Very low-dose doxorubicin is the most studied radiosensitizer in ATC, with combinations with EBRT showing improved response rates and survival (76,77) but raising concerns regarding toxicity. Weekly taxanes (docetaxel, paclitaxel) are currently the most frequently used radiosensitizers (78). However, OS remains poor. The addition of immunotherapy or tyrosine kinase inhibitors to chemoradiation has shown no clear benefit and is not recommended outside clinical trials (79,80).

EBRT for unresectable disease

For patients with locoregionally confined but unresectable ATC, EBRT, alone or in combination with systemic therapy, remains a cornerstone of treatment, particularly when neoadjuvant systemic strategies are not feasible. EBRT can provide durable locoregional control and palliate symptoms such as pain, dysphagia, or airway compromise (13,58).

Given the rapid progression of ATC, hypofractionated EBRT regimens, which deliver higher doses per fraction over a shorter overall treatment duration, have been investigated. Hypofractionated EBRT has demonstrated non-inferior OS compared to conventional fractionation, with manageable toxicity. Dose escalation to ≥50 Gy has been associated with improved OS, and higher radiation doses (≥60 Gy) have been correlated with better outcomes, even in the absence of surgery (81–83). These findings support the integration of hypofractionated EBRT into multimodal treatment approaches for select patients, particularly those with good performance status and without significant metastatic burden (84–86).

Concurrent chemoradiotherapy can further enhance locoregional control and may, in rare cases, enable surgical resection of initially unresectable tumors. Patients who have undergone R2 resection or present with unresectable but non-metastatic disease and are suitable candidates for aggressive therapy should be considered for intensity-modulated radiation therapy with concurrent systemic therapy (13,87).

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For patients with advanced, symptomatic, or metastatic disease not amenable to curative treatment, palliative EBRT is a key component of supportive care. Administered at lower doses over shorter periods, palliative EBRT can effectively alleviate airway obstruction, bleeding, or pain, thereby improving quality of life. Treatment decisions should be individualized, taking into account performance status, care objectives, and anticipated toxicity.

Overall, EBRT, whether with curative or palliative intent, plays a critical role in the multidisciplinary management of ATC, with hypofractionated approaches offering a promising balance between efficacy, treatment duration, and tolerability.

The technical aspects of radiotherapy for ATC are summarized below.

Principles of external beam radiotherapy in ATC

Dose

- Doses <50 Gy are not recommended.
- Doses >60 Gy are associated with improved OS.

Fractionation

- 60 Gy in 2 Gy fractions is the most standard regimen.
- Hyperfractionated regimens are not recommended.
- Hypofractionated regimens may be considered in patients with poor performance status.

Target volume

- The tumor bed and cervical lymph nodes, including cervical and upper mediastinal areas, should be included.
- Level I, retropharyngeal, and retrostyloid regions are not routinely included.

Technique

- Intensity-modulated radiation therapy is the preferred technique.
- Lack of advanced planning techniques should not delay treatment.

Radiosensitizing chemotherapy

- Taxanes are the most frequently used agents.
- Other agents may also be considered.

SYSTEMIC THERAPIES IN UNRESECTABLE NON-METASTATIC/METASTATIC DISEASE

"Bridging" therapies

- **Recommendation 27:** In patients who wish to pursue an intensive therapeutic approach, bridging therapies (cytotoxic chemotherapy or lenvatinib) are recommended when delays in initiating more effective targeted treatments are anticipated.

Due to the imminent threat of ATC progression, starting treatment without delay is a critical discussion point for patients desiring a more intensive approach. While awaiting molecular results or access to targeted therapies, the use of cytotoxic agents (such as taxanes, platinum compounds, and/or doxorubicin) (**Table 4**) as an initial "bridging" therapy represents a reasonable approach.

Objective response rates can be achieved in up to 21% of cases, with disease control rates of up to 64% and a median OS of approximately 6 months. Although these outcomes are modest, they may stabilize the disease and prevent further progression while awaiting the initiation of more effective targeted therapies (78). Lenvatinib may also be used as an alternative bridging therapy (88). Partial responses, or stable disease with minor responses to a rapid improvement in respiratory symptoms, have been reported in a small number of patients. The risk of fistula formation in patients with ATC should be carefully considered (89).

Dabrafenib/trametinib for *BRAF*-mutated ATC

- **Recommendation 28:** Dabrafenib/trametinib (DT) should be promptly initiated as the first-line systemic therapy for unresectable or metastatic *BRAF* V600E-mutated ATC, either as a single strategy or in combination with immunotherapy.
- **Recommendation 29:** Restaging with contrast-enhanced CT is recommended after 1–3 months of DT therapy to reassess the feasibility of surgical resection.
- **Recommendation 30:** When DT is unavailable, other BRAF inhibitors may be considered as an alternative.

The *BRAF* V600E mutation represents the most common actionable genetic alteration in ATC (90). Clinical evidence supports the efficacy of dabrafenib

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Table 4. Systemic pharmacological agents commonly employed in the management of anaplastic thyroid cancer (ATC)

Selective inhibitor	Description
BRAF V600E mutated ATC	Dabrafenib 150 mg PO twice daily and trametinib 2 mg PO daily
NTRK fusion-positive ATC	• Larotrectinib 100 mg PO twice daily • Entrectinib 600 mg PO daily
RET fusion-positive ATC	Selpercatinib 160 mg PO twice daily
ALK fusion-positive ATC	Crizotinib 250 mg PO twice daily
Systemic chemotherapy (combined regimes)	• Paclitaxel/carboplatin: • Paclitaxel 60–100 mg/m^2 IV, carboplatin AUC 2 IV (weekly) Paclitaxel 135–175 mg/m^2 IV, carboplatin AUC 5–6 IV (every 3–4 weeks) • Docetaxel/doxorubicin: • Docetaxel 20 mg/m^2 IV, doxorubicin 20 mg/m^2 IV (weekly) • Docetaxel 60 mg/m^2 IV, doxorubicin 60 mg/m^2 IV (with G-CSF) (every 3–4 weeks) (4) • Doxorubicin/cisplatin: • Doxorubicin 60 mg/m^2 IV, cisplatin 40 mg/m^2 IV (every 3 weeks)
Systemic chemotherapy (monotherapy)	Paclitaxel 60–90 mg/m^2 IV (weekly) or 135–200 mg/m^2 (every 3–4 weeks) Doxorubicin 20 mg/m^2 IV (weekly) or 60–75 mg/m^2 IV (every 3 weeks)
Radiosensitizing chemotherapy	• Paclitaxel 30–60 mg/m^2 IV weekly • Docetaxel 20 mg/m^2 IV weekly
Immunotherapy	Pembrolizumab 200 mg IV every 3 weeks
Other possible agents	
Lenvatinib/pembrolizumab	Lenvatinib 20–24 mg PO daily and pembrolizumab 200 mg IV every 3 weeks
Pemetrexed/carboplatin	Carboplatin AUC 5-6 plus pemetrexed 500 mg/m^2 IV every 3 weeks

AUC: area under the curve; G-CSF: granulocyte colony-stimulating factor; IV: intravenous; PO: per os (oral administration).

(a BRAF inhibitor) and trametinib (a MEK inhibitor), collectively termed DT, in treating *BRAF* V600E-mutated ATC (**Table 3**). The 2017 Rare Oncology Agnostic Research (ROAR) basket trial demonstrated an initial objective response rate (ORR) of 69% in 16 patients, including one complete response, and a one-year OS rate of 80% (91). The US Food and Drug Administration (FDA) approved DT in 2018 for locally advanced or metastatic ATC with a *BRAF* V600E mutation when there are no satisfactory locoregional options (92). A six-year follow-up revised the ORR to 56%, with three complete responses, a median progression-free survival (PFS) of 6.7 months, and a median OS of 14.5 months (93).

Real-world data on ATC are scarce. Small UK and Argentinian series suggest modest survival and variable responses to DT. A large Spanish/Portuguese registry (214 pts) reported a median OS of 4.5 months, with access to NGS and *BRAF* mutation status as key prognostic factors (94–96).

Neoadjuvant approach to *BRAF*-mutated ATC

R0/R1 resection is a key prognostic determinant of OS in ATC and should remain the therapeutic goal, either at initial presentation or after neoadjuvant treatment. Responses to DT are typically rapid and robust, yet short-lived, prompting the use of a neoadjuvant approach. In unresectable *BRAF*-V600E-mutated ATC, DT has yielded response rates that enabled surgery in approximately 70% of patients (66). Responses should be assessed early and every 1–3 months using cross-sectional imaging; surgery, performed by expert head and neck surgeons, should be planned as soon as resection is feasible (4) or earlier, based on a suspicion of progressive disease. The addition of immunotherapy to DT is discussed below (see the **Immunotherapy section**).

Evidence regarding the benefit of other BRAF/MEK inhibitors is limited. Their use may be considered when DT is unavailable (97).

Other selective inhibitors

- **Recommendation 31:** If actionable fusions (*RET*, *NTRK*, *ALK*) are detected, treatment with selective inhibitors is recommended under tumor-agnostic indications, despite limited evidence and generally lower observed response rates.

Approximately half of ATCs are non-*BRAF*-V600E-mutated ATC tumors and are associated with

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mutations in the *RAS*, *NF1*, *PIK3CA*, and *PTEN* genes (**Table 3**) (35). Currently, these mutations are considered non-targetable. In contrast, targetable gene fusions involving *RET*, *NTRK*, *ALK*, and *BRAF* are rare, with individual frequencies below 5% (35). Whether these tumors can be effectively treated with selective inhibitors remains an open question. The small number of ATC patients with gene fusions has limited proper investigation, and current data are scarce.

The best-studied fusion-specific inhibitor is the NTRK inhibitor larotrectinib. Reported pooled data from three phase I/II larotrectinib clinical trials (NCT02576431, NCT02122913, and NCT02637687) included 27 patients with *NTRK* fusion-positive thyroid cancer, 7 of whom had ATC (98). In the ATC cohort, the ORR was 27% (95% confidence interval: 4–71), the median PFS was 2.2 months (95% confidence interval: 0.9–NE), and the 12- and 24-month PFS rates were both 17%; these findings were driven by a single patient with poorly differentiated thyroid carcinoma who achieved a durable response (98).

The MD Anderson group analyzed *NTRK* fusion-positive ATC and showed that responses to first-line NTRK inhibitors are often shorter than those observed in DTC. Newer NTRK/ROS1 inhibitors (e.g., repotrectinib and taletrectinib) are currently under investigation, and available data suggest that first-line NTRK inhibitor monotherapy in ATC typically yields non-durable responses (99). Regarding ALK inhibitors, at least two case reports describe significant tumor responses to crizotinib (100,101). In one case, a remarkable response was subsequently achieved with ceritinib and brigatinib after crizotinib failure (101).

The phase I/II LIBRETTO-001 trial of the RET inhibitor selpercatinib included a small number of patients with *RET* fusion-positive ATC, with at least one achieving a durable partial response (≥18 months) and another achieving stable disease. In the broader cohort of previously treated *RET* fusion-positive thyroid cancers (which included these ATC cases), the overall response rate was 85.4% and the median duration of response was 26.7 months; however, ATC-specific efficacy data were not reported separately (102,103).

Overall, the available data are too limited to draw firm conclusions. Based on reported experiences, ATC with targetable fusions is extremely rare, and responses to fusion inhibitors, although possible, are variable and generally short-lived. Neoadjuvant therapy with selective inhibitors may be considered for unresectable ATC tumors harboring actionable gene fusions (104). Further research exploring other specific inhibitors and combination therapies is necessary before these treatments can be recommended as first-line options.

Cytotoxic chemotherapy and multikinase inhibitors

- **Recommendation 32:** For patients with unresectable or metastatic ATC without targetable mutations, or when molecular testing or targeted therapies are unavailable, enrollment in clinical trials is recommended. If clinical trials are unavailable, recommended options include lenvatinib/pembrolizumab, cytotoxic chemotherapy, or best supportive care.
- **Recommendation 33:** Lenvatinib-based systemic therapy (preferably lenvatinib plus pembrolizumab) is recommended for patients with metastatic or unresectable, non-*BRAF*-mutated ATC in centers with multidisciplinary expertise. This is particularly relevant when clinical trials or molecularly targeted options are not available, provided that contraindications to antiangiogenic agents are carefully excluded and close toxicity monitoring is ensured.

Given the limited efficacy of conventional therapies in patients with ATC without targetable mutations, participation in clinical trials is strongly encouraged (13). Unfortunately, access to clinical trials in Latin America remains highly limited, representing a major barrier to the implementation of innovative therapies for ATC in the region.

Therefore, in the absence of clinical trials, the combination of RT and cytotoxic chemotherapy is generally recommended for patients with good performance status who wish to pursue aggressive therapy (13,105). However, treatment responses are infrequent and, when achieved, are typically short-lived. Given the complexity of management decisions in ATC, a careful discussion weighing the potential

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benefits and drawbacks of aggressive treatment is necessary.

Various cytotoxic regimens, most commonly incorporating doxorubicin, cisplatin, or taxanes (paclitaxel or docetaxel), have been employed in ATC (106,107) (**Table 4**). Doxorubicin (administered weekly or every three weeks) remains the only cytotoxic agent specifically approved by the FDA for this indication. Pemetrexed plus carboplatin has shown promise in patients with refractory thyroid cancer, including one patient with ATC who was successfully treated following third-line therapy (108).

Tyrosine kinase inhibition has emerged as a potential option for non-*BRAF*-V600E mutated ATC, although its activity as monotherapy remains modest. Among multikinase/antiangiogenic agents, lenvatinib is the best characterized. Prospective and real-world series show objective responses in only 11–15% of patients, with disease control rates ranging from roughly one-third to two-thirds of cases and a median OS generally below 12 months. These limited benefits led to its regulatory approval in Japan; however, other guidelines maintain a more cautious stance due to associated toxicity and the frequent aerodigestive tract invasion observed in ATC, which typically contraindicate its use (13,88,109–111).

In clinical practice, lenvatinib is increasingly combined with immune checkpoint inhibition (most often pembrolizumab) to potentiate antitumor immunity and improve the depth and duration of response. This strategy has already been incorporated into the NCCN algorithms for metastatic, *BRAF*-negative disease when clinical trials or molecularly matched therapies are unavailable (58,112,113). Nevertheless, current evidence is derived from small, predominantly single-arm cohorts, and responses remain notably less robust than those obtained with *BRAF/MEK* inhibition in *BRAF*-mutated ATC. Antiangiogenic toxicity is a major limiting factor.

In settings where pembrolizumab is unavailable or unaffordable, lenvatinib monotherapy remains an option, with the understanding that responses are typically less durable. Other tyrosine kinase inhibitors, including sorafenib, imatinib, gefitinib, and pazopanib, have not demonstrated consistent or clinically meaningful activity as single agents and are therefore not routinely recommended (114–117).

For patients with an Eastern Cooperative Oncology Group performance status of 3–4, end-of-life care discussions are paramount and generally preferred over systemic therapy. For patients with an Eastern Cooperative Oncology Group performance status of 0–2, end-of-life discussions remain a reasonable option; however, systemic therapy (initiated after multidisciplinary review) may offer superior symptom relief and an improved quality of life.

Immunotherapy

- **Recommendation 34:** The addition of pembrolizumab, when available, is recommended in *BRAF*-V600E-mutated ATC, since responses to DT are often robust but typically short-lived. This strategy may help delay or overcome resistance and improve the likelihood of durable response.
- **Recommendation 35:** In patients with unresectable or metastatic ATC who have high PD-L1 expression, high tumor mutation burden or microsatellite instability and lack other actionable mutations, checkpoint inhibitors may be considered, preferably in the context of a clinical trial.
- **Recommendation 36:** In patients with non-*BRAF* V600E-mutated ATC harboring other targetable alterations, immunotherapy may be considered in combination with other selective inhibitors, if available.

Immunotherapy, particularly immune checkpoint inhibitors targeting the PD-1/PD-L1 axis, has emerged as a promising therapeutic option for ATC. ATC tumors frequently express immune markers such as PD-L1, and immune infiltration is a hallmark of the disease, which justifies the immunotherapeutic approach. PD-L1 expression is observed in approximately 11–28% of cases, and mismatch repair deficiency occurs in over 10% in some series, supporting a rationale for immunotherapeutic approaches. Tumor PD-L1 expression, tumor mutational burden, and mismatch repair status may help identify likely responders (118).

For patients lacking actionable alterations, single-agent spartalizumab demonstrated an ORR of 19% and median OS of 5.9 months, with higher PD-L1 expression

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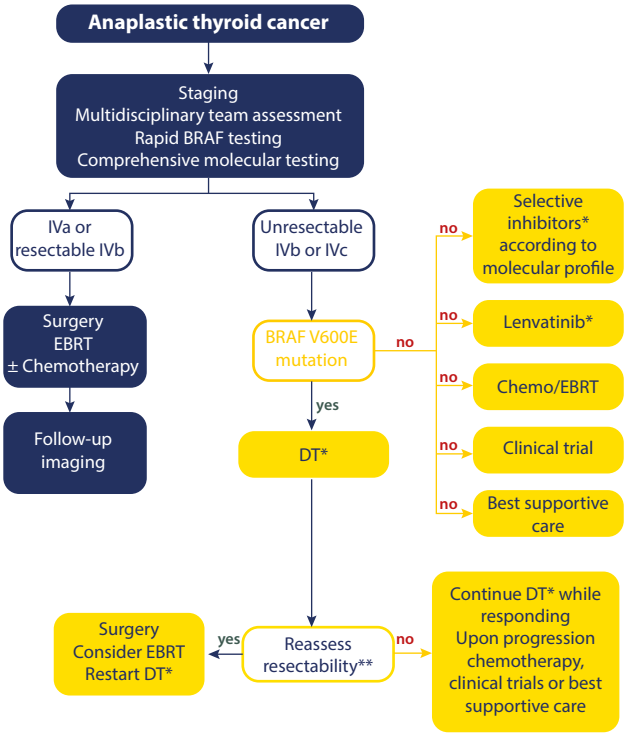
correlating with better responses (118). Pembrolizumab has shown benefit in high tumor mutational burden or high microsatellite instability tumors (ORR 29%) (119). Dual inhibition with nivolumab plus ipilimumab achieved an ORR of 30% in a small ATC cohort (120).

Combination strategies integrating immunotherapy with targeted agents have shown encouraging results. In a phase II trial, atezolizumab with genomically matched targeted therapy (*BRAF* V600E variant: vemurafenib plus cobimetinib; *RAS*- or *NF1/2*- mutant: cobimetinib; non-*BRAF/RAS/NF1/2* mutant: bevacizumab) achieved a median OS of 19 months, with the *BRAF* V600E cohort reaching 43 months (121). The combination of selective inhibitors and immunotherapy has been more *commonly studied* in *BRAF*-mutated ATC. Although data remain limited, in patients with *BRAF* V600E mutation disease, multidisciplinary tumor boards should evaluate the feasibility of integrating anti–PD-1 agents, especially in those with high tumor burden or suboptimal response to DT alone.

Resistance to BRAF inhibitors, remains a significant clinical challenge (122). To overcome this risk, the MD Anderson group proposes an induction therapy following a dual-step approach: DT is initiated to achieve swift cytoreduction and relieve present or imminent compressive symptoms. Within approximately four weeks, an immune checkpoint inhibitor targeting the PD-1/PD-L1 axis is added to counteract or delay development of therapeutic resistance (3). The addition of pembrolizumab to DT (DTP) improved outcomes in a retrospective study of 94 patients with ATC (123). DTP increased median OS from 9 to 17 months (p = 0.037) and PFS from 4 to 11 months (p = 0.049). The neoadjuvant cohort achieved the most favorable results, with a median OS of 63 months and a 50% pathological complete response rate, which was significantly associated with longer survival (p = 0.018). In a multicenter phase II trial (NCT04675710), neoadjuvant DTP enabled R0/R1 resection in 97% of patient with *BRAF* V600E-mutated ATC, compared to a historical rate of 5%. DTP demonstrated improved resectability, PFS (13.9 months), and OS, supporting its adoption as standard-of-care in *BRAF*-mutated ATC when feasible (124).

Regarding other selective inhibitors, the combination of larotrectinib with immunotherapy was attempted in one patient with *NTRK* fusion-positive ATC but resulted in significant hepatotoxicity (99). Moreover, the MD Anderson experience with combining immunotherapy and other fusion-targeted inhibitors, such as selpercatinib (*RET* fusion) and alectinib (*ALK* fusion), has also been associated with hepatotoxicity (3)

An algorithm for the therapeutic management of ATC is shown in **Figure 1**.



*Immunotherapy; **Every 2-4 weeks.
DT: Dabrafenib/Trametinib; EBRT: external beam radiotherapy.

Figure 1. Management algorithm for anaplastic thyroid cancer.

OTHER ASPECTS

Follow-up strategies

- **Recommendation 37:** ^{18}F-FDG PET-TC (if available) or cross-sectional imaging should be periodically performed after initial therapy to assess disease status.

Due to its higher sensitivity, ^{18}F-FDG PET/CT is the preferred modality for follow-up in patients with ATC. Nevertheless, in settings where PET/CT is not available,

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cross-sectional imaging (CT or MRI) of the neck, chest, abdomen, and pelvis—with measurement of target lesions to objectively assess disease status—may be used as an alternative. Additional imaging studies (e.g. brain MRI) should be obtained based on clinical indications. It is recommended that imaging follow-up be performed every 4–8 weeks after initial therapy (12), while PET/CT may be performed every 3–6 months (58). Patients receiving targeted therapies with neoadjuvant intent should undergo more frequent evaluations (every 4–6 weeks) to identify those with tumor responses who may become eligible for surgical resection (12). Symptomatic metastases (e.g., bone, brain) should be considered for local therapies, including EBRT, thermal ablation procedures, and/or treatment with bone antiresorptive agents such as intravenous bisphosphonates or denosumab (13,58).

FINAL CONSIDERATIONS

Despite significant advances in the molecular characterization and treatment of ATC, translating these gains into real-world benefit remains particularly challenging in Latin America. In many countries across the region, access to comprehensive molecular testing, multidisciplinary care, and high-cost systemic therapies is inconsistent and frequently limited to a small number of referral centers. The availability of DTs in Latin America may be constrained by cost, regulatory hurdles, or supply issues, necessitating alternative approaches essential to ensure equitable care.

In a multicenter cohort study from Argentina, 67% of patients underwent molecular testing for *BRAF* V600E; however, fewer than 50% of mutation-positive cases received DT therapy (125). This gap between diagnostic capability and access to matched targeted treatment reflects a broader structural issue and underscores the urgent need to align guideline-based recommendations with local feasibility.

To address these disparities, region-specific strategies, including political and health policy initiatives are necessary. These strategies should involve advocating for the incorporation of DT into national formularies, implementing fast-track approval pathways for drugs with proven benefit in ATC, and promoting regional price negotiations, patient-access programs, and public–private partnerships. In parallel, strengthening referral networks, concentrating complex ATC care in experienced centers, and ensuring timely access to palliative radiotherapy, surgery, and optimal supportive care are critical steps. Lastly, establishing collaborative regional registries and pragmatic clinical studies is essential to generate real-world evidence regarding outcomes, toxicities, and patterns of care, thereby informing treatment algorithms and policy decisions. Implementing these strategies is essential to bridging the gap between what is clinically possible and what is realistically available to patients with ATC in the region.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

GLP-1 receptor agonists in kidney transplant recipients with post-transplant diabetes: efficacy and safety outcomes from a retrospective cohort study

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ABSTRACT

Objective: To assess the efficacy and safety of glucagon-like peptide-1 receptor agonists (GLP-1 RAs) in kidney transplant recipients (KTRs) with post-transplant diabetes mellitus (PTDM). **Subjects and methods:** This retrospective, single-center cohort study included 24 KTRs with PTDM who initiated GLP-1 RA therapy as an add-on to existing treatments between August 2013 and April 2024. Outcomes assessed included fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), body weight, body mass index (BMI), blood pressure, lipid profile, renal function, and adverse events, with data collected at baseline and last follow-up. **Results:** Over a mean follow-up of 3.2 ± 2.1 years, GLP-1 RA therapy was associated with trends toward weight loss (−3.6 ± 8.5 kg; $p = 0.051$) and BMI reduction (−1.4 ± 3.3 kg/m^2; $p = 0.056$). HbA1c decreased by −0.2% ($p = 0.362$), while FPG increased by +12.1 mg/dL ($p = 0.232$). Significant improvements were observed in total cholesterol (−41.5 mg/dL; $p < 0.001$) and systolic blood pressure (−8.5 mmHg; $p = 0.041$). Serum creatinine and estimated glomerular filtration rate remained stable (−0.09 mg/dL, $p = 0.305$; +3.2 mL/min/1.73 m^2, $p = 0.206$, respectively). Nausea occurred in 21% (n = 5), and no other severe adverse events were reported. **Conclusion:** In KTRs with PTDM, GLP-1 RA therapy was associated with improvements in lipid profile and blood pressure, accompanied by non-significant trends toward weight reduction and stable renal function, and demonstrated an acceptable safety profile. Prospective studies are warranted to confirm long-term cardiorenal benefits.

Keywords: kidney transplantation; diabetes mellitus; GLP-1 receptor agonists; glycemic control; renal outcomes; safety

INTRODUCTION

Post-transplant diabetes mellitus (PTDM) is a common and clinically significant metabolic complication following kidney transplantation. Its pathogenesis is complex and multifactorial, frequently representing the progression of a dysmetabolic continuum that may begin prior to transplantation (1). This condition compromises both patient survival and long-term graft function. Key contributing factors include pre-transplant overweight or obesity and excessive post-transplant weight gain, both of which exacerbate insulin resistance and accelerate pancreatic β-cell dysfunction (2–4). Moreover, the combination of immunosuppressive therapy—particularly glucocorticoids and calcineurin inhibitors—alongside surgical stress and shifting metabolic demands creates a physiologically unstable environment that predisposes patients to early post-transplant hyperglycemia (5).

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Despite the high prevalence and clinical burden of PTDM, therapeutic options for its management remain limited, especially in consideration of graft function and the complexities of immunosuppressive regimens (6). Traditional agents such as sulfonylureas are commonly used in this setting but carry an increased risk of hypoglycemia, particularly in patients with impaired renal function. Repaglinide has shown modest efficacy in transplant recipients with PTDM, while dipeptidyl peptidase-4 inhibitors have demonstrated improvements in insulin resistance and β-cell function without increasing the risk of hypoglycemia. However, these inhibitors have neutral cardiovascular effects, and their pharmacokinetic interactions with immunosuppressants may require closer monitoring of immunosuppressive drug levels (7,8).

In this context, glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have emerged as promising therapeutic agents. These agents are structurally analogous to endogenous GLP-1, underpinning their metabolic efficacy. In type 2 diabetes, GLP-1 RAs are well established as effective therapies, achieving significant HbA1c reductions, sustained weight loss, and a low risk of hypoglycemia; several agents are also approved for obesity due to their favorable effects on body weight and glycemic control. Their pleiotropic actions extend beyond glycemic control and include appetite suppression, delayed gastric emptying, enhanced glucose-dependent insulin secretion, and improved insulin sensitivity. In the kidney transplantation, where post-transplant weight gain is frequent and metabolic risk factors are highly prevalent, GLP-1 RAs offer the advantage of addressing both hyperglycemia and excess weight, while minimizing hypoglycemia risk. This is a critical consideration in transplant recipients exposed to complex immunosuppressive regimens and multiple comorbidities. This dual benefit supports their potential role in individualized metabolic management for this high cardiometabolic risk (9,10).

Large cardiovascular outcomes trials, such as LEADER (11), SUSTAIN-6 (12), PIONEER 6 (13), and REWIND (14), have demonstrated reductions in major adverse cardiovascular events (MACEs) and renoprotective effects, establishing GLP-1 RAs as recommended treatment options for individuals with diabetes and elevated cardiorenal risk. These benefits have led to their prioritization in contemporary clinical practice guidelines, including for patients with chronic kidney disease or heart failure, irrespective of glycemic targets (15).

Nevertheless, robust data on the use of GLP-1 RAs in KTRs are still lacking, despite the clinical need for effective and safe therapeutic strategies in this setting. These patients often exhibit overlapping metabolic, cardiovascular, and immunosuppressive challenges, necessitating interventions that address not only glycemic control but also long-term risk modification. In this retrospective study, we examined the metabolic impact, safety, and treatment patterns of GLP-1 RA therapy in kidney transplant recipients with PTDM.

SUBJECTS AND METHODS

This retrospective, single-center cohort study was conducted at a tertiary university hospital kidney transplant center. Eligible participants were KTRs aged > 18 years with a diagnosis of PTDM who initiated a GLP-1 RA as an add-on to existing antidiabetic regimens. General exclusion criteria were multi-organ transplantation, pre-existing diabetes (type 2 or other forms), and missing baseline or follow-up laboratory data. The specific exclusion criterion was concomitant sodium-glucose cotransporter 2 inhibitors (SGLT2i) therapy, in order to avoid confounding.

The study (reference no. 2022.264 [209-DEFI/224-CE]) was approved by the institutional Human Research Ethics Committee, with informed consent waived due to the retrospective and anonymized data collection. Patients initiating GLP-1 RA therapy between August 2013 and April 2024 were eligible for inclusion. Follow-up continued until treatment discontinuation, switch to another GLP-1 RA, or the study cutoff date. Inclusion in outcome analyses required at least one follow-up visit and a minimum of three months of continuous therapy.

Data collection

Data collected included patient demographics (e.g., age, sex, weight, body mass index [BMI]), pre-transplant comorbidities, immunosuppressive regimens, and PTDM-related information. Treatment data

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comprised the specific GLP-1 RA agent, initial and maintenance doses, and concomitant medications (antihypertensives, lipid-lowering agents, and antidiabetic therapies), which were recorded at GLP-1 RA initiation and at the last follow-up. Clinical and laboratory variables assessed at baseline and follow-up included hemodynamic measures (systolic blood pressure [SBP] and diastolic blood pressure [DBP]), glycemic indices (fasting plasma glucose [FPG], HbA1c), lipid parameters (total cholesterol, low-density lipoprotein cholesterol [LDL-c], high-density lipoprotein cholesterol [HDL-c], triglycerides), and renal function markers (urea, serum creatinine, estimated glomerular filtration rate [eGFR]). Safety evaluation included adverse events potentially related to GLP-1 RA therapy: gastrointestinal symptoms, hypoglycemia, diabetic ketoacidosis, volume depletion, hospitalizations, and treatment discontinuation. **Table 1** summarizes the diagnostic criteria for PTDM, eGFR, BMI, and relevant comorbidities, with all parameters standardized according to established guidelines to ensure analytical consistency (1,16–19).

Outcome measures

Efficacy outcomes included changes in FPG, HbA1c, total cholesterol, LDL-c, HDL-c, triglycerides, BMI, SBP, and DBP. Safety outcomes focused on renal parameters (eGFR and serum creatinine) and therapy-related adverse events, specifically hypoglycemia, gastrointestinal intolerance, and other drug-related events. All laboratory tests were conducted using standardized methods at the institution's central laboratory.

Statistical analysis

Data management was performed in Microsoft Excel (v. 2108), and analyses were conducted using SAS (v. 9.4; SAS Institute Inc., USA). Continuous variables are reported as mean ± standard deviation or median with interquartile range (Q1–Q3), depending on distribution as assessed by the Shapiro-Wilk test. Categorical variables are presented as absolute frequencies and percentages. Within-group comparisons between baseline and follow-up were assessed using paired Student's t-tests for normally distributed variables and Wilcoxon signed-rank tests for non-normally distributed data. For subgroup analyses, independent t-tests or Mann-Whitney U tests were used as appropriate. A two-sided p-value < 0.05 was considered statistically significant.

RESULTS

Derivation of the cohort

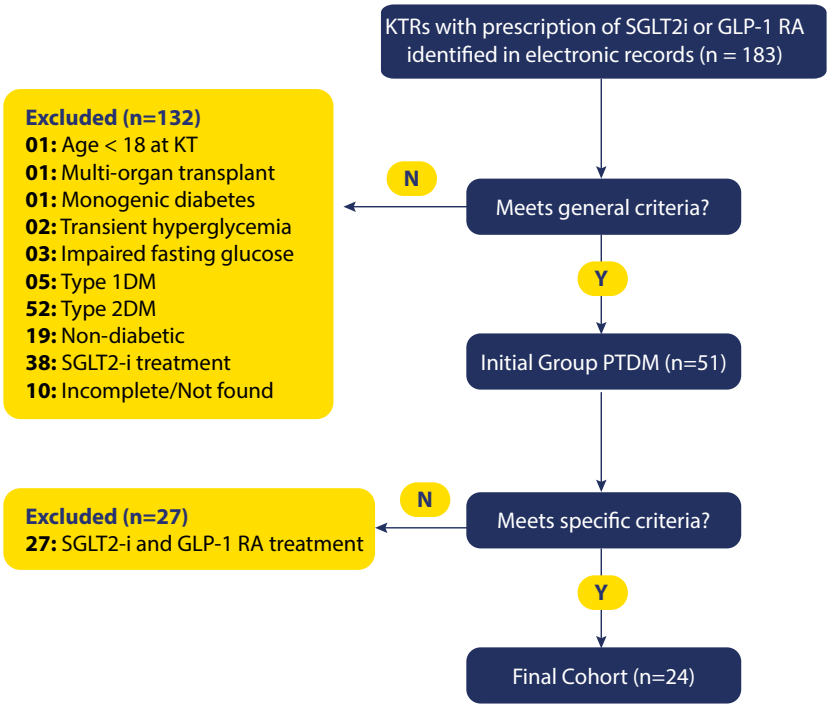
Of 183 KTRs initially screened, 132 were excluded for the following criteria: age <18 years (n = 1), multi-organ transplantation (n = 1), monogenic diabetes (n = 1), transient hyperglycemia (n = 2), impaired fasting glucose (n = 3), pre-existing type 1 (n = 5) or type 2 diabetes (n = 52), non-diabetic status (n = 19), incomplete data (n = 10), or initiation of SGLT2i as add-on therapy (n = 38). The remaining 51 patients had confirmed PTDM and were treated with either GLP-1 RA or combined GLP-1 RA and SGLT2 inhibitors. Of these, 27 patients receiving combination therapy were excluded to avoid treatment confounding. The final cohort consisted of 24 patients who initiated GLP-1 RA as add-on therapy to their existing antidiabetic regimen (**Figure 1**).

Table 1. Clinical definitions and diagnostic criteria used in this study

Parameter	Definition	Source
PTDM	HbA1c ≥6.5% or FPG ≥126 mg/dL ≥ 3 months post-transplant	ADA (16) Sharif et al. (1)
eGFR	2021 CKD-EPI equation	NKF-K/DOQI (17)
BMI	Weight (kg)/height (m^2)	WHO (18)
Dyslipidemia	Abnormal lipid levels or use of lipid-lowering therapy	KDIGO-CKD (20)
Obesity	BMI ≥ 30 kg/m^2	WHO (18)
Hypertension	BP ≥ 130/80 mmHg or antihypertensive use	NKF-K/DOQI (17)
Anemia	Hemoglobin < 11 g/dL (men/women)	KDIGO-CKD (19)

ADA: American Diabetes Association; BMI: body mass index; BP: blood pressure; CKD-EPI: chronic kidney disease epidemiology collaboration; eGFR: estimated glomerular filtration rate; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; NKF-K/DOQI: National Kidney Foundation Kidney Disease Outcomes Quality Initiative; PTDM: post-transplant diabetes mellitus; WHO: World Health Organization.

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GLP-1 RA: Glucagon-like peptide-1 receptor agonists; SGLT2i: sodium-glucose cotransporter-2 inhibitors; PTDM: post-transplant diabetes mellitus; KT: kidney transplantation.

Figure 1. Flowchart depicting the cohort selection.

Baseline characteristics

The cohort included 24 KTRs with PTDM, predominantly male (67%), with a mean age of 61.5 ± 10.3 years. All patients were White. Hypertension and dyslipidemia were universal, whereas obesity (BMI ≥ 30 kg/m^2) was present in 29%. Heart failure and ischemic heart disease were documented in 46% and 21% of patients, respectively. Most grafts were from deceased donors (75%), and the majority were first transplants (92%). Immunosuppressive therapy primarily comprised corticosteroids (96%), mycophenolate mofetil (92%), and tacrolimus (83%). The mean duration of PTDM was 5.7 ± 3.5 years, and the mean time from transplantation to GLP-1 RA initiation was 7.6 ± 4.9 years. At baseline, mean HbA1c was 7.4 ± 1.7%, BMI was 27.3 ± 5.4 kg/m^2, and eGFR was 46.1 ± 15.7 mL/min/1.73 m^2. The lipid profile showed elevated triglycerides (200.5 ± 126.9 mg/dL) and LDL-c (94.9 ± 51.7 mg/dL). GLP-1 RAs were initiated as add-on therapy in all patients: dulaglutide (50%), liraglutide (25%), and semaglutide (25%), with a mean treatment duration of 3.2 ± 2.1 years. Detailed baseline characteristics are presented in **Table 2.**

Changes in antidiabetic therapy

Following GLP-1 RA initiation, adjustments in background antidiabetic therapy were observed. Sulfonylurea use decreased from 17% to 8%, and DPP-4 inhibitor use declined from 29% to 17%, while metformin use decreased from 29% to 25%. Among GLP-1 RAs, dosing varied as follows: dulaglutide (0.75 mg [33%] or 1.5 mg [67%] weekly), semaglutide (0.25 mg [17%] or 1.0 mg [83%] weekly), and liraglutide (1.2 mg [50%] or 1.8 mg [50%] daily). Treatment discontinuation occurred in four patients (17%), all due to gastrointestinal intolerance, predominantly nausea. Basal insulin use increased from 46% at baseline to 66% at final evaluation. Details regarding GLP-1 RA type, dosing, and discontinuation are listed in **Table 3.**

Efficacy outcomes

Glycemic control remained stable, with HbA1c showing a non-significant reduction from 7.4 ± 1.7% to 7.2 ± 1.2% (p = 0.362), and FPG increasing from 114.5 ± 29.8 to 126.5 ± 37.9 mg/dL (p = 0.232). Significant improvements were observed in total cholesterol (182.9 ± 61.9 to 141.4 ± 32.6 mg/dL; p < 0.001) and LDL-c (94.9 ± 51.7 to 72.4 ± 28.6 mg/dL; p = 0.014). Triglycerides

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Table 2. Baseline characteristics of KTRs with PTDM treated with GLP-1 RAs

Variables	Value
	mean ± SD (range) or n (%)
Demographic characteristics	
Age (years)	61.5 ± 10.3 (46.0–81.0)
Sex (male/female)	16 (67)/8 (33)
Ethnicity (white)	24 (100)
Family history of diabetes	11 (46)
Comorbidities	
Hypertension	24 (100)
Dyslipidemia	24 (100)
Hyperuricemia	9 (38)
Obesity (based on BMI ≥ 30 kg/m^2)	7 (29)
Heart failure	11 (46)
Ischemic heart disease	5 (21)
Pre- and post-transplant clinical history	
Time from KT to study inclusion (years)	7.6 ± 4.9 (1.6–18.0)
Primary kidney disease	
Glomerular diseases	6 (25)
Tubulointerstitial diseases	4 (17)
ADPKD	4 (17)
Unknown	8 (33)
Other causes	2 (8)
Duration of renal replacement therapy (years)	5.6 ± 5.7 (1.0–21.3)
Hemodialysis/peritoneal dialysis/none	18 (75) / 6 (25)/NR
Hepatitis C status	NR
Cytomegalovirus serostatus	14 (58)
Donor type (deceased/living)	18 (75)/6 (25)
Transplant number (first/retransplant)	22 (92)/2 (8)
Immunosuppressive agents	
Corticosteroids	23 (96)
Mycophenolate mofetil	22 (92)
mTOR inhibitors	20 (83)
Tacrolimus	5 (21)
Cyclosporine	3 (12)
Azathioprine	2 (8)
PTDM-Related data	
Duration of PTDM (years)	5.7 ± 3.5 (0.3–10.6)
Duration of GLP-1 RA therapy (years)	3.2 ± 2.1 (1.4–6.3)
Laboratory parameters	
Hemoglobin (g/L)	12.5 ± 1.7 (9.9–16.1)
HbA1c (%)	7.4 ± 1.7 (5.7–11.5)
Triglycerides (mg/dL)	200.5 ± 126.9 (51.0–586.0)
Serum creatinine (mg/dL)	1.7 ± 0.6 (1.0–2.7)
eGFR (mL/min/1.73 m^2)	46.1 ± 15.8 (27.0–85.6)
Antihypertensive and lipid-lowering therapies	
Statins	24 (100)
Ezetimibe (all in combination with statins)	10 (42)
Angiotensin-converting enzyme inhibitors	14 (58)
Angiotensin II receptor blockers	5 (21)
Calcium channel blockers	16 (67)
Beta-blockers	15 (62)
Diuretics	7 (29)

ADPKD: autosomal dominant polycystic kidney disease; BMI: body mass index; eGFR: estimated glomerular filtration rate; GLP-1 RA: glucagon-like peptide-1 receptor agonists; HbA1c: glycated hemoglobin; KT: kidney transplantation; NR: not reported; PTDM: post-transplant diabetes mellitus.

and HDL-c remained unchanged (p = 0.149 and p = 0.399, respectively). No adjustments to statin therapy were made during follow-up. Anthropometric parameters showed trends toward improvement that did not reach statistical significance: body weight decreased from 73.2 ± 14.7 to 69.6 ± 14.0 kg (p = 0.051) and BMI from 27.3 ± 5.4 to 25.9 ± 5.2 kg/m^2 (p = 0.056). The proportion of normal-weight patients increased from 29% to 50%, while obesity prevalence decreased from 29% to 25% (Figure 2). The SBP decreased significantly from 143.0 ± 15.7 to 134.5 ± 15.6 mmHg (p = 0.041), while DBP remained stable (p = 0.665). These changes occurred without the introduction of new antihypertensive drug classes or escalation to higher-potency agents. Detailed results are reported in Table 3.

Safety outcomes

Renal function remained stable throughout the follow-up period, with no significant changes in serum creatinine (1.7 ± 0.6 to 1.6 ± 0.6 mg/dL; p = 0.305) or eGFR (46.1 ± 15.8 to 49.3 ± 14.7 mL/min/1.73 m^2; p = 0.206) (Table 4). Among 20 patients on tacrolimus-based immunosuppression with available data, mean tacrolimus trough levels remained stable from baseline to final follow-up (6.4 ± 2.1 vs. 6.2 ± 2.3 ng/mL; p = 0.68). Gastrointestinal intolerance was the most frequent adverse event, with nausea reported in five patients (21%), leading to treatment discontinuation in four cases (two on dulaglutide, two on semaglutide). Hypoglycemic episodes occurred in three patients (12.5%), all of whom were receiving concomitant basal insulin. Mild urinary tract infections were observed in four patients (17%), with no cases requiring discontinuation of therapy. No cases of pancreatitis, gallbladder disease, or other serious adverse events were reported.

DISCUSSION

In this retrospective study of 24 KTRs with PTDM, add-on therapy with GLP-1 RAs over a mean follow-up of 3.2 years was associated with significant improvements in total and LDL cholesterol and SBP, as well as non-significant trends toward weight and BMI reduction. Glycemic control, assessed by HbA1c and FPG, remained stable throughout the follow-up, and renal

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Table 3. GLP-1 RA Regimens, treatment duration, and adverse events

GLP-1 RA	n (%)	Dose: n (%)	Mean duration (years)	Discontinuation, n (%)	Main adverse events
Dulaglutide	12 (50)	0.75 mg: 4 (33); 1.5 mg: 8 (67)	3.4 ± 2.1	2/12 (17)	Nausea
Liraglutide	6 (25)	1.2 mg: 3 (50); 1.8 mg: 3 (50)	3.1 ± 1.9	0 (0)	None reported
Semaglutide	6 (25)	0.25 mg: 1 (17); 1.0 mg: 5 (83)	3.0 ± 2.2	2/6 (33)	Nausea
Total	24 (100)		3.2 ± 2.1	4/24 (17)	

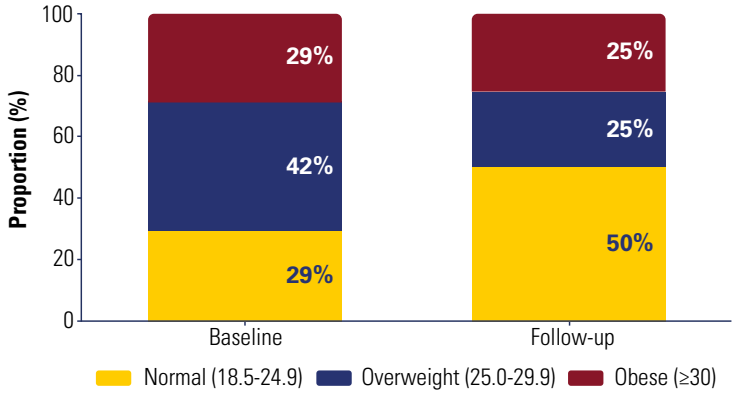


Figure 2. Change in BMI category distribution from baseline to follow-up during GLP-1 RA treatment.

Table 4. Changes in clinical parameters from baseline to follow-up during GLP-1 RA therapy in KTRs with PTDM

Variable	Baseline	Follow-up	Δ	p-Value
	mean ± SD	mean ± SD	mean ± SD	
Glycemic control				
HbA1c (%)	7.4 ± 1.7	7.2 ± 1.2	0.2 ± 1.4	0.362
FPG (mg/dL)	114.5 ± 29.8	126.5 ± 37.9	+12.0 ± 48.3	0.232
Lipid panel				
Total cholesterol (mg/dL)	182.9 ± 61.9	141.4 ± 32.6	-41.5 ± 47.9	**0.0003**
LDL-c (mg/dL)	94.9 ± 51.7	72.4 ± 28.6	-22.5 ± 41.3	**0.014**
HDL-c (mg/dL)	49.5 ± 11.9	47.9 ± 10.6	-1.6 ± 9.3	0.399
Triglyceride (mg/dL)	200.5 ± 126.9	166.9 ± 70.8	-33.6 ± 110.3	0.149
Anthropometric measures				
Body weight (kg)	73.2 ± 14.7	69.6 ± 14.0	--3.6 ± 8.5	0.051
BMI (Kg/m^2)	27.3 ± 5.4	25.9 ± 5.2	-1.4 ± 3.3	0.056
Hemodynamic data				
Systolic BP (mmHg)	143.0 ± 15.7	134.5 ± 15.6	-8.5 ± 19.3	**0.041**
Diastolic BP (mmHg)	71.5 ± 18.3	75.0 ± 13.0	+3.5 ± 14.9	0.665
Renal function				
Creatinine (mg/dL)	1.7 ± 0.6	1.6 ± 0.6	-0.1 ± 0.4	0.305
eGFR (mL/min/1.73 m^2)	46.1 ± 15.8	49.3 ± 14.7	+3.2 ± 11.9	0.206

BP: blood pressure; BMI: body mass index; eGFR: estimated glomerular filtration rate; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; KT: kidney transplantation; PTDM: post-Transplant diabetes mellitus; SD: standard deviation.

function was preserved. These findings contribute to the growing body of evidence regarding the potential role of GLP-1 RAs in the post-transplant setting.

In our cohort, the modest HbA1c reduction of 0.2% is consistent with the variable glycemic responses reported in the literature. Kukla and cols. (20) reported a mean reduction of 0.6% in a heterogeneous cohort consisting of both kidney and multi-organ transplant recipients with either pre-existing diabetes or PTDM. Singh and cols. (21) observed a mean reduction of 0.7% after six months in a mixed transplant population, while Mallik and cols. documented a 1.3% decrease in KTRs (22). A more pronounced reduction of 1.9% was described by Liou and cols. (23) over 19.4 months

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in a small retrospective series. In contrast, Kim and cols. (24) found no significant change when switching from prandial insulin to dulaglutide. This variation underscores the heterogeneity of treatment response across different study populations and settings.

Unlike the REWIND trial (14), in which dulaglutide was associated with modest reductions in LDL-c and total cholesterol, our predominantly dulaglutide-treated cohort exhibited more pronounced declines in both parameters. We also observed non-significant improvements in HDL-c and triglycerides. These lipid outcomes are consistent with the diverse effects reported in other cardiovascular outcome trials: LEADER (11) and SUSTAIN-6 (12) demonstrated reductions in LDL-c and triglycerides without significant changes in HDL-c, while PIONEER 6 reported minimal lipid alterations (13).

Similar benefits were noted in blood pressure, with a clinically meaningful reduction in SBP (8.5 mmHg), exceeding the modest declines reported in those trials, where systolic reductions varied by 1.2–2.6 mmHg (11,12,14), whereas DBP remained stable. These findings reinforce the potential hemodynamic advantages of GLP-1 RAs, particularly in high-risk transplant recipients. Beyond lipid and blood pressure improvements, GLP-1 RA therapy was associated with a trend toward weight reduction (mean loss of 3.6 kg; $p = 0.051$) and a decrease in BMI (1.4 kg/m^2; $p = 0.056$). Although these changes did not reach statistical significance, they are in line with earlier studies among transplant populations. Mallik and cols. reported a mean weight reduction of 2.7 kg and a BMI decrease of 0.9 kg/m^2 after one year of therapy (22), while Kukla and cols. (20) observed a greater reduction of 9.4 kg and 1.9 kg/m^2. Collectively, these findings suggest a possible role for GLP-1 RAs in mitigating post-transplant weight gain, which is a key contributor to metabolic dysfunction in this population.

Although our study was not designed to assess cardiovascular outcomes, the observed improvements in body weight, blood pressure, and lipid profile suggest a potential for cardiometabolic risk modification. This is particularly pertinent for transplant recipients, in whom obesity reflects not only excess adiposity, but also chronic metabolic dysfunction and persistent systemic inflammation—both established contributors to cardiovascular morbidity. The potential cardioprotective value of GLP-1 RAs is supported by large-scale outcome trials demonstrating significant cardiovascular benefits: LEADER reported a 13% reduction in MACEs with liraglutide, primarily due to decreased cardiovascular mortality (11); SUSTAIN-6 showed a 26% MACE reduction with semaglutide, largely attributable to reduced stroke risk (12); and REWIND demonstrated a 12% MACE reduction with dulaglutide, even in a primary prevention cohort (14). Considered alongside the favorable metabolic profile observed in our cohort, these results highlight the potential role of GLP-1 RAs in reducing cardiovascular risk among KTRs with PTDM.

Regarding renal safety, graft function remained stable throughout follow-up, with no significant changes in serum creatinine or eGFR. While statistically neutral, this finding may be clinically relevant, as KTRs typically experience progressive graft function decline—a trend not observed in our cohort. These results are consistent with previous reports in transplant populations (20,25), in which GLP-1 RA therapy was also associated with stable renal function. Additionally, tacrolimus trough levels remained stable in the 20 patients with available data, suggesting no clinically relevant drug interaction or absorption changes with GLP-1 RAs, despite theoretical concerns related to delayed gastric emptying. Nevertheless, given the small sample size and lack of a comparator group, both renal function and tacrolimus findings should be interpreted with caution, as studies of GLP-1 RA safety in KTRs remain limited.

GLP-1 RA therapy was generally well tolerated. Treatment discontinuation occurred in 17% of patients (4/24), all due to gastrointestinal intolerance—mainly nausea—while most patients maintained long-term therapy. Hypoglycemic events occurred exclusively in patients receiving concomitant insulin (3/24; 12.5%), and no cases of pancreatitis, gallbladder disease, or hypersensitivity reactions were observed. These findings reinforce the favorable safety and tolerability profile of GLP-1 RAs in KTRs when accompanied by appropriate monitoring and individualized dosing.

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Study limitations

This study has several limitations inherent to its retrospective, single-center design, including small sample size (n = 24), absence of a control group, and potential unmeasured confounders (e.g., treatment interruptions and lifestyle factors. The inclusion of three different GLP-1 RAs (i.e., dulaglutide, liraglutide, and semaglutide) limits our ability to assess agent-specific effects, as these agents vary in potency, weight-loss efficacy, cardiovascular benefit, and gastrointestinal tolerability. Pooling them may therefore obscure differences in their therapeutic profiles. The exclusively White cohort further restricts generalizability to more diverse populations, and the concomitant use of other antidiabetic agents, including insulin and, in four cases, DPP-4 inhibitors, precludes definitive attribution of observed effects to GLP-1 RA therapy alone. Although no changes in antihypertensive drug classes were noted, changes in dosage of existing medications during follow-up were not systematically recorded; this represents an unquantifiable confounder in assessing the isolated hemodynamic effects of GLP-1 RAs. Additionally, the mean interval of 7.6 years from transplantation to GLP-1 RA initiation introduces a survivor bias, as patients must have survived and maintained graft function long enough to receive this therapy, potentially selecting for a metabolically stable, adherent, and healthier subgroup, and limiting generalizability to the broader PTDM population. Despite these limitations, the study has notable strengths, including a prolonged mean follow-up of 3.2 years (considerably longer than most previous reports), allowing assessment of sustained treatment effects. The homogeneous cohort of exclusively KTRs with PTDM enhances clinical relevance, while the inclusion of multiple GLP-1 RA agents and diverse background regimens reflects real-world clinical complexity and supports the applicability of our findings to routine practice.

In conclusion, this study demonstrated that GLP-1 RA therapy in KTRs with PTDM is associated with significant improvements in total cholesterol and SBP, along with favorable trends in weight reduction and stable renal function over a mean follow-up of 3.2 years. Glycemic control remained unchanged, and the safety profile was acceptable, with gastrointestinal intolerance identified as the most common adverse event. Although these findings support the potential role of GLP-1 RAs in managing this high-risk population, the modest effects on glycemic control and the non-significant weight loss observed underscore the need for larger, prospective controlled studies to confirm long-term cardiorenal benefits and the optimal of these agents in the post-transplant setting.

Author contributions: conceptualization, RN; methodology, RN, LM, and JS; software, RN; validation, LM and JS; formal analysis, RN; investigation, RN, JF, IF, AC; data curation, RN, LM; writing — original draft preparation, RN and LM; review, validation, and editing of the final manuscript, RN, LM, JS; supervision, LM and JS. All authors have read and agreed to the final version of the manuscript.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

Mild and non-persistent fasting plasma glucose elevation in the first trimester of pregnancy is not associated with increased risk of gestational diabetes mellitus and adverse pregnancy outcomes

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ABSTRACT

Objective: To evaluate pregnancy outcomes in women with early gestational diabetes mellitus (eGDM) diagnosed by mild elevation of fasting plasma glucose (FPG) in the first trimester. **Subjects and methods:** This prospective cohort study included 114 pregnant women with first trimester FPG <100 mg/dL (5.6 mmol/L). Women with FPG ≥92 mg/dL (5.1 mmol/L) and <100 mg/dL (5.6 mmol/L) were classified as having eGDM, and FPG was reassessed after approximately 3–4 weeks. Women whose repeated FPG was <92 mg/dL (5.1 mmol/L) were assigned to Group 1 (G1; n = 33) and did not receive eGDM treatment. These participants underwent an oral glucose tolerance test (OGTT) between 24–28 weeks of gestation and, depending on the results, initiated treatment as indicated. Women with eGDM and a second FPG 92-125 mg/dL (5.1–6.9 mmol/L) were assigned to Group 2 (G2; *n* = 31) and immediately started eGDM treatment. The control group (G3; *n* = 50) was composed by pregnant women with FPG <92 mg/dL (5.1 mmol/L) in the first trimester. **Results:** G1 and G3 had lower pre-pregnancy body mass index, less chronic hypertension, lower rates of GDM history, and lower multiparity compared to G2. There was no significant difference in GDM diagnosis by OGTT between G1 and G3 (27.3% vs. 20.0%, p = 0.594). G3 gained more weight (10.2 ± 7.6 kg, 7.5 ± 6.4 kg, and 13.5 ± 6.1 kg for G1, G2, and G3, respectively; p = 0.001). Other outcomes between G1 and G3 were similar. **Conclusion:** Women with eGDM diagnosed by mild, non-persistent FPG elevation in the first trimester had similar outcomes to those with normal early FPG.

Keywords: Gestational diabetes mellitus; early gestational diabetes mellitus; fasting plasma glucose; first trimester

INTRODUCTION

Gestational diabetes mellitus (GDM), as defined by the World Health Organization (WHO), is a carbohydrate intolerance of variable severity first recognized during pregnancy and does not fulfill criteria for overt diabetes (1). The diagnosis is made using a 75-g oral glucose tolerance test (OGTT) between 24–28 weeks of gestation, with only one abnormal value required: fasting plasma glucose (FPG) ≥92 mg/dL (5.1 mmol/L) and <126 mg/dL (7.0 mmol/L); 1-hour ≥180 mg/dL (10.0 mmol/L); 2-hour ≥153 mg/dL (8.5 mmol/L) and <200 mg/dL (11.1 mmol/L). These cutoffs were established by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) and the WHO, based on data from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, which evaluated the relationship between maternal glucose and fetal outcomes (1–3). Although the HAPO study assessed women between 24–32 weeks of gestation and insulin resistance typically increases after late second

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trimester, FPG thresholds of ≥92 mg/dL (5.1 mmol/L) and <126 mg/dL (7.0 mmol/L) were extrapolated to all periods of gestation (1). The use of FPG ≥92 mg/dL (5.1 mmol/L) for early GDM diagnosis is not supported by first-trimester data, so neither FPG nor OGTT diagnoses in early pregnancy are evidence-based (4,5). In Brazil, the Ministry of Health recommends FPG at the first prenatal visit to detect overt diabetes. If FPG is ≥92 mg/dL (5.1 mmol/L) and <126 mg/dL (7.0 mmol/L), GDM is diagnosed without repeat FPG or OGTT (6).

To date, no studies have determined a clear cutoff for first-trimester FPG related to adverse maternal-fetal outcomes. The benefits of diagnosing and treating GDM before 24 weeks of gestation remain uncertain. One study reported that women diagnosed with GDM before 24 weeks had higher risks of pregnancy-induced hypertension, postpartum hemorrhage, postpartum glucose abnormalities, and their offspring had increased rates of prematurity, large-for-gestational-age, and neonatal intensive care unit (NICU) admission (7). A meta-analysis of 13 cohort studies found higher rates of perinatal mortality, neonatal hypoglycemia, and insulin use among women with early-onset GDM compared to late-onset GDM, as well as increased risk of NICU admission in developed countries (8). In a more recent trial, immediate treatment for GDM before 20 weeks in women with risk factors for hyperglycemia modestly reduced the incidence of a composite of adverse neonatal outcomes, particularly for those with higher glycemic values. The study also suggested that early treatment may increase risk of small-for-gestational-age (SGA) infants among women with OGTT results in the lower glycemic range (9).

Therefore, in addition to the uncertain benefits of early treatment, there is concern regarding overtreatment, undernutrition, and increased risk of SGA and NICU admission (9–12). In this study, we aimed to assess maternal-fetal outcomes in women with early GDM (eGDM) diagnosed by mild FPG elevation in the first trimester who did not undergo early intervention.

SUBJECTS AND METHODS

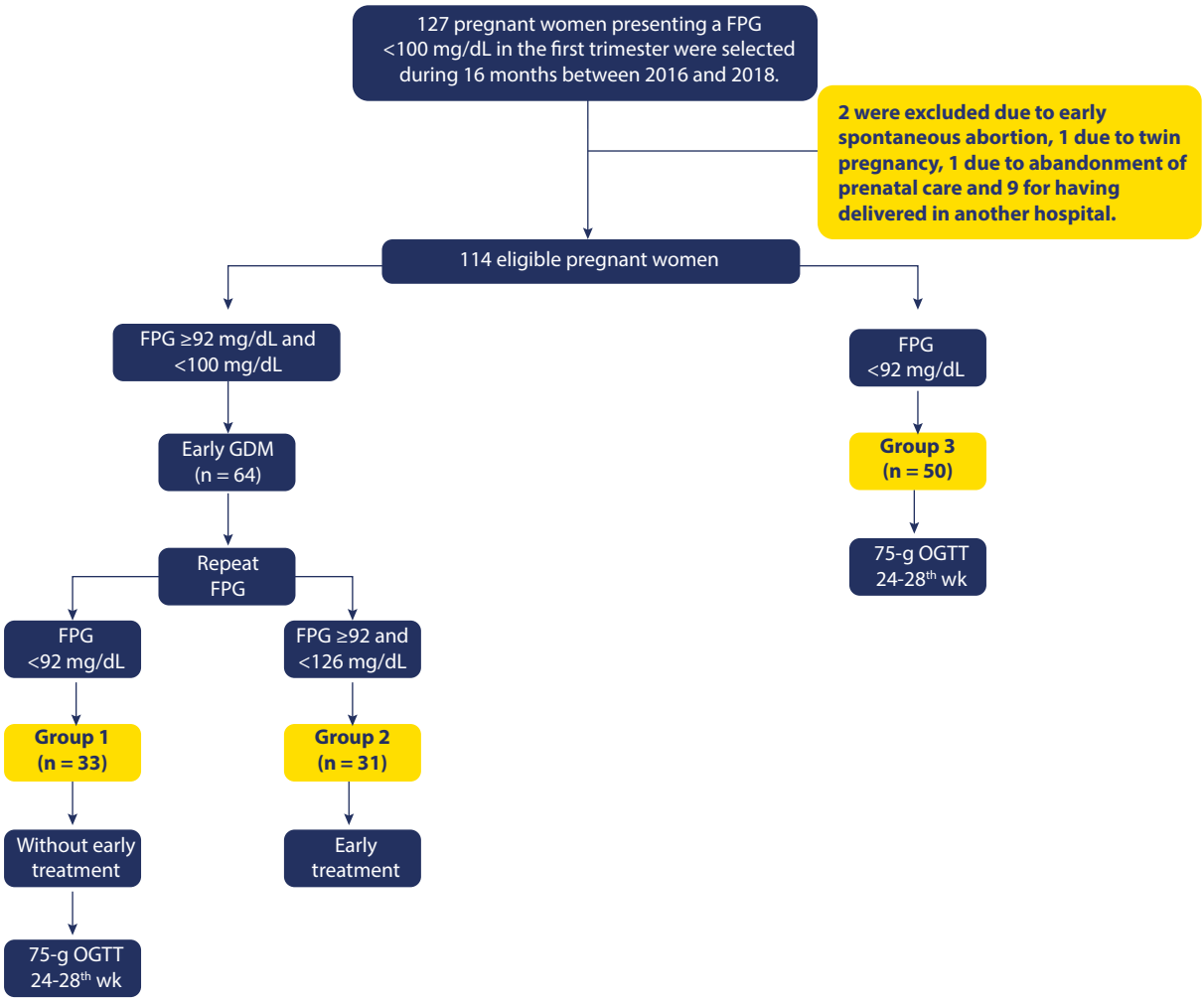
Study design

This was a prospective cohort study. Pregnant women attending prenatal outpatient clinics at a maternity school hospital in Brazil over a 16-month period (2016–2018) with FPG <100 mg/dL (5.6 mmol/L) in the first trimester were included (**Figure 1**). Pregnant women with FPG ≥92 mg/dL (5.1 mmol/L) and <100 mg/dL (5.6 mmol/L) were classified as having eGDM. According to institutional protocol, FPG was repeated approximately 3-4 weeks (13).

Women with eGDM whose second FPG was <92 mg/dL (5.1 mmol/L) were assigned to group 1 (G1) and did not receive eGDM treatment. They underwent routine prenatal care and a 75-g OGTT between 24–28 weeks of gestation. Those with any abnormal OGTT values (FPG ≥92 mg/dL [5.1 mmol/L]; 1-hour ≥180 mg/dL [10.0 mmol/L]; 2-hour ≥153 mg/dL [8.5 mmol/L]) began medical nutrition therapy (14). Blood glucose monitoring devices were provided only for patients on insulin therapy. Diet-treated women were assessed every 2 weeks with FPG and 1-hour post-breakfast plasma glucose. Insulin was initiated for those with FPG ≥95 mg/dL (5.3 mmol/L) and/or 1-hour post-breakfast plasma glucose ≥140 mg/dL (7.8 mmol/L) persisting after at least two weeks on diet. Insulin doses were adjusted every 1–2 weeks by an endocrinologist based on self-monitoring of blood glucose (six times daily) aiming for fasting and preprandial glucose <95 mg/dL (5.3 mmol/L) and 1-hour postprandial glucose <140 mg/dL (7.8 mmol/L) (15).

Women in G1 with normal 75-g OGTT between 24–28 weeks of gestation were followed up to the end of pregnancy, in regular prenatal care until delivery with no specific GDM intervention. Women with eGDM and a second FPG ≥92 mg/dL (5.1 mmol/L) and <126 mg/dL (7.0 mmol/L) were assigned to group 2 (G2) and, for ethical reasons, immediately began GDM treatment with medical nutrition therapy (14). Insulin was added if glycemic control was not achieved on diet alone. The control group (G3) comprised pregnant women with first trimester FPG <92 mg/dL (5.1 mmol/L), selected by convenience from the same outpatient clinics during the inclusion period for G1 and G2. All underwent a 75-g OGTT between 24–28 weeks of gestation. Those with abnormal OGTT values were treated for GDM (14). Women who presented normal OGTT continued standard prenatal care until delivery.

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FPG: fasting plasma glucose; GDM: gestational diabetes mellitus; OGTT: oral glucose tolerance test.

Figure 1. Study flow chart.

The FPG cutoff value of ≥92 mg/dL (5.1 mmol/L) was chosen per IADPSG recommendations for GDM diagnosis (2). The upper limit of <100 mg/dL (5.6 mmol/L) was chosen because some experts suggest that FPG above this threshold is associated with an increased likelihood of developing GDM at 24–28 weeks and presenting unfavorable maternal and fetal outcomes, warranting dietary intervention (plus insulin if necessary) (16,17). Some studies have corroborated this information, demonstrating that over 50% of patients with first-trimester FPG >99 mg/dL (5.5 mmol/L) later develop GDM after 24 weeks gestation (18,19). Regarding the sample size, as the prevalence of mild first trimester FPG elevation in this population is unknown, all eligible women attending the clinic during the 16-month period (2016–2018) were considered for inclusion. Exclusion criteria were prior diabetes mellitus, current GDM treatment, multiple pregnancy, uncontrolled hyperthyroidism, or prior bariatric surgery. All eligible patients agreed to participate.

The research was approved by the local ethics committee (CAAE no. 58475616.3.0000.5275), and all participants provided informed consent.

Data collection

At baseline, participants underwent interviews for clinical and anthropometric data. The International Physical Activity Questionnaire (IPAQ short form) (20,21) and a semi-quantitative food frequency questionnaire (22), both validated in Brazilian Portuguese, were administered. These were repeated between 24–28 weeks to detect changes in diet or activity potentially affecting outcomes. After delivery, maternal

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and fetal outcomes were obtained from medical records. Gestational age and expected delivery dates were established based on the last menstrual period. Ultrasonography (USG) dating was considered in cases where the date of the last menstruation was uncertain or differed by >5 days from the date obtained by USG performed between 6–13 weeks or >10 days from the date based on USG performed between 14–24 weeks.

Outcomes

Maternal outcomes included GDM diagnosis by 75-g OGTT between 24–28 weeks, need for insulin therapy, gestational weight gain, gestational hypertensive disorders (preeclampsia: systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on two or more occasions after 20 weeks with proteinuria [≥1+ dipstick or ≥300 mg24/h]; gestational hypertension: if the criteria for elevated blood pressure were met but without proteinuria; eclampsia: presence of seizures in preeclamptic patients), and cesarean section.

Perinatal outcomes included prematurity (delivery <37 weeks), APGAR <7 at 1 or 5 minutes, large-for-gestational-age (birth weight >90th percentile for sex and gestational age), small-for-gestational-age (birth weight <10th percentile), macrosomia (birth weight ≥4000 g), neonatal hypoglycemia (glucose <36 mg/dL [2.0 mmol/L] in the first 24 hours), shoulder dystocia or birth injury, NICU admission, hyperbilirubinemia (requiring phototherapy), polyhydramnios, and intrauterine fetal or neonatal death.

Statistical analysis

Data were analyzed using SPSS (v. 24.0, IBM Corporation, USA). Means and standard deviations or medians with ranges are reported for continuous variables; counts and percentages for categorical variables. Categorical variables were compared with chi-square or Fisher's exact test (if expected frequency <5). Normality of continuous variables was assessed using the Shapiro-Wilk test. Parametric variables were evaluated with a Student's t-test or analysis of variance (ANOVA), and nonparametric variables were evaluated with the Mann-Whitney U or Kruskal-Wallis test. Binary logistic regression (restricted to G1 and G2) was performed to assess whether higher NICU admission was associated with eGDM with early treatment (G2) or with other factors. Model variables were those with significant univariate differences ($p < 0.05$) between G1 and G2: pre-pregnancy body mass index (BMI), nulliparity, chronic hypertension, and lower carbohydrate intake at 24–28 weeks. Multiple linear regression (restricted to G1 and G2) was used to assess whether lower gestational age at birth was related to eGDM with early treatment (G2) or to other factors. Two models were constructed: the first included variables with significant univariate differences ($p < 0.05$) between G1 and G2; the second added insulin use (since insulin can influence delivery timing). Forward selection was used. Statistical significance was defined as $p < 0.05$.

RESULTS

A total of 127 pregnant women were recruited. Of these, two were excluded due to spontaneous abortion immediately after the first FPG collection, one due to twin pregnancy, one owing to discontinuation of prenatal care at the Institution, and nine because they delivered in another hospital. At the end, 114 pregnant women were eligible for analysis: 33 in G1, 31 in G2, and 50 in G3 (**Figure 1**).

Baseline characteristics are presented in **Table 1**. G2 exhibited a higher pre-pregnancy BMI (30.7 ± 6.0, 26.8 ± 5.5, and 27.5 ± 5.6 kg/m^2 [$p = 0.017$], for G2, G1, and G3, respectively), a greater prevalence of chronic hypertension (48.4, 21.2, and 18.0% [$p = 0.008$], for G2, G1, and G3, respectively) and a higher frequency of personal history of GDM (23.1, 4.8, and 2.8% [$p = 0.023$], for G2, G1, and G3, respectively) than the other groups, along with a lower prevalence of nulliparous women compared to G1 (29.0% vs. 54.5% [$p = 0.047$]). G1 and G3 had similar baseline characteristics.

Total daily caloric intake and macronutrient distribution at baseline and at 24–28 weeks' of gestation are summarized in **Table 2**. Although overall caloric intake was similar across groups, women with early normal FPG (G3) had a higher percentage of lipid intake than the other groups at baseline (23.9 ± 5.0%, 24.0 ± 4.3%, and 26.7 ± 6.1% [$p = 0.028$], for G1, G2, and G3, respectively) and at 24–28 weeks (22.7 ±

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Table 1. Baseline maternal characteristics

Maternal characteristics (n = 114)	G1 (n = 33)	G2 (n = 31)	G3 (n = 50)	p^{*}	$p^{\dagger}$	$p^{\ddagger}$	$p^{\dagger\dagger}$
n	33 (28.9%)	31 (27.2%)	50 (43.9%)				
FPG 1 (mg/dL)	94.0 (92–99)	95.0 (92–99)	83.0 (70–91)	0.000	0.085	0.000	0.000
Gestational age (week)	8.9 (5.1–14.3)	9.4 (5.4–14.7)	9.6 (5–17.6)	0.444	0.619	0.180	0.610
Fasting duration (hour)	10.5 (8–14)	11.0 (8–13)	12.0 (8–13)	0.244	0.781	0.196	0.145
FPG 2 (mg/dL)	86.0 (67–91)	97.0 (92–128)	—	0.000	0.000	--	--
Gestational age (week)	13.1 (8.4–27.1)	12.7 (8.3–21.6)	—	0.519	0.519	--	--
Fasting duration (hour)	10.0 (8–12)	10.0 (8–12)	—	0.926	0.926	--	--
Age (year)	27.7 ± 6.3	30.4 ± 6.4	29.4 ± 6.6	0.229	0.088	0.251	0.474
Pre-pregnancy BMI (kg/m^2)	26.8 ± 5.5	30.7 ±6.0	27.5 ± 5.6	0.017	0.010	0.599	0.018
Non-White (n, %)	14 (42.4)	16 (51.6)	32 (64.0)	0.159	0.617	0.072	0.353
School attendance (n, %)							
≤ 8 years	5 (15.2)	3 (9.7)	6 (12.0)	0.821	0.709	0.747	1.000
9–11 years	19 (57.6)	20 (64.5)	31 (62.0)	0.856	0.616	0.819	1.000
≥ 12 years	9 (27.3)	8 (25.8)	13 (26.0)	1.000	1.000	1.000	1.000
Family income (Brazilian minimum wage)	1.85 (0.26–8.80)	2.05 (0.53–5.56)	1.76 (0.35–0.56)	0.825	0.638	0.783	0.583
Nullipara, n (%)	18 (54.5)	9 (29.0)	22 (44.0)	0.127	0.047	0.377	0.241
Planned pregnancy (n, %)	11 (33.3)	14 (45.2)	18 (36.0)	0.623	0.443	0.819	0.486
History of GDM[§] (n, %)	1 (4.8)	6 (23.1)	1 (2.8)	0.023	0.112	1.000	0.018
History of macrosomia[‖] (n, %)	0	3 (13.6)	2 (7.1)	0.354	0.249	0.526	0.643
Family history of DM[¶] (n, %)	13 (39.4)	11 (35.5)	12 (24.0)	0.302	0.800	0.150	0.315
Chronic hypertension[#] (n, %)	7 (21.2)	15 (48.4)	9 (18.0)	0.008	0.035	0.780	0.006
History of PCOS[**] (n, %)	8 (24.2)	3 (9.7)	8 (16.0)	0.294	0.186	0.401	0.518

BMI: Body mass index; DM: diabetes mellitus; FPG: fasting plasma glucose; GDM: gestational diabetes mellitus; G1: early gestational diabetes mellitus without early treatment; G2: early gestational diabetes mellitus with early treatment; G3: control group; PCOS: polycystic ovaries syndrome.

*Comparison between G1, G2 and G3.

†Comparison between G1 and G2.

‡Comparison between G1 and G3.

††Comparison between G2 and G3.

§Data were collected only in women with previous gestation. G1 (n = 20), G2 (n = 26), G3 (n = 36).

‖G1 (n = 16), G2 (n = 22), G3 (n = 28).

¶Parents or siblings.

#Hypertension diagnosed before pregnancy or before 20 weeks of gestation.

**Polycystic ovaries syndrome (self-declared).

3.8%, 24.2 ± 4.5%, and 26.7 ± 6.6% [p = 0.008], for G1, G2, and G3, respectively). At baseline, G2 consumed more carbohydrates than G3 (57.8% [41.6–76.0] vs. 51.4% [23.8–74.4] [p = 0.038], for G2 and G3, respectively), although this difference was not observed at 24–28 weeks. G1 and G2 had similar dietary patterns at baseline; however, at 24–28 weeks, G2 consumed less carbohydrate than G1 (54.8 ± 6.7% vs. 51.0 ± 4.9% [p = 0.025], respectively). Data on physical activity are described in **Table 2**. At baseline, G1 had a higher percentage of active patients than G3 (57.6% vs. 34% [p = 0.043], for G1 and G3, respectively). By the end of the second trimester, physical activity levels were similar across all groups.

Regarding maternal outcomes are detailed in **Table 3**. FPG, 1h-PG, and 2h-PG during the 75-g OGTT at 24–28 weeks were similar in G1 and G3. The incidence of GDM diagnosed by OGTT at 24–28 weeks was also similar between G1 and G3 (27.3% vs. 20.0% [p = 0.594]). Among women with GDM, a higher percentage of those in G2 (early intervention) required insulin to achieve glycemic control compared to those in G1 and G3 (51.6, 22.2, and 10.0% [p = 0.036], for G2, G1, and G3, respectively).

The G3 gained more weight than the other groups (10.2 ± 7.6, 7.5 ± 6.4, and 13.5 ± 6.1 kg [p = 0.001], for G1, G2, and G3, respectively), with more than half of the women in G3 exceeding the weight gain

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Table 2. Food consumption and level of physical activity at baseline and at 24–28 weeks of gestation

Variables	G1 (n = 33)	G2 (n = 31)	G3 (n = 50)	p*	p†	p‡	p††
Food consumption baseline							
Daily calorie intake (kcal)	1871.5 (224.7–6071.2)	1907.3 (1109.1–4082.0)	1977.4 (605.1–5950.7)	0.864	0.624	0.845	0.669
Protein, %	18.9 (9.9–46.0)	18.2 (8.7–38.2)	20.5 (11.8–37.9)	0.414	0.489	0.533	0.193
Lipids, %	23.9 ± 5.0	24.0 ± 4.3	26.7 ± 6.1	0.028	0.949	0.032	0.034
CHO, %	57.3 (31.8–65.7)	57.8 (41.6–76.0)	51.4 (23.8–74.4)	0.067	0.888	0.073	0.038
Food consumption 24–28 weeks§							
Daily calorie intake (kcal)	2033.70 (992.0–4454.7)	1954.40 (1281.4–3706.3)	1861.61 (1207.0–3278.6)	0.847	0.711	0.453	0.790
Protein, %	22.1 (13.7–34.3)	24.0 (15.3–35.3)	20.2 (10.7–39.1)	0.137	0.141	0.953	0.203
Lipids, %	22.7 ± 3.8	24.2 ± 4.5	26.7 ± 6.6	0.008	0.227	0.003	0.056
CHO, %	54.8 ± 6.7	51.0 ± 4.9	50.9 ± 9.5	0.081	0.025	0.056	0.783
Level of physical activity baseline							
Very active	0	1 (3.2%)	2 (4.0%)	0.619	0.484	0.515	1.000
Active	19 (57.6%)	17 (54.8%)	17 (34.0%)	0.062	1.000	0.043	0.063
Irregularly active A	8 (24.2%)	3 (9.7%)	13 (26.0%)	0.181	0.186	1.000	0.147
Irregularly active B	2 (6.1%)	7 (22.6%)	8 (16.0%)	0.183	0.078	0.302	0.763
Sedentary	4 (12.1%)	3 (9.7%)	10 (20.0%)	0.447	1.000	0.389	0.351
Level of physical activity 24–28 weeks‖							
Very active	1 (3.7%)	1 (3.6%)	0	0.326	1.000	0.397	0.406
Active	15 (55.6%)	15 (53.6%)	17 (41.5%)	0.467	1.000	0.323	0.339
Irregularly active A	3 (11.1%)	6 (21.4%)	9 (21.9%)	0.511	0.469	0.338	1.000
Irregularly active B	4 (14.8%)	5 (17.9%)	12 (29.3%)	0.362	1.000	0.245	0.395
Sedentary	4 (14.8%)	1 (3.6%)	3 (7.3%)	0.285	0.193	0.423	0.641

CHO: Carbohydrate; G1: early gestational diabetes mellitus without early treatment; G2: early gestational diabetes with early treatment; G3: control group.
Level of physical activity by IPAQ (21): Very active–any one of the following criteria: ≥5 days of vigorous activity of at least 30 minutes/day; or ≥3 days of vigorous activity of at least 20 minutes/day plus ≥5 days of moderate-intensity activity or walking of at least 30 minutes/day. Active–any one of the following criteria: ≥3 days of vigorous activity of at least 20 minutes/day; or ≥5 days of moderate-intensity activity or walking of at least 30 minutes/day; or ≥5 days and at least 150 minutes/week of any combination of walking, moderate-intensity or vigorous intensity activities. Irregularly active A–any of the following criteria regarding frequency or duration of activities: ≥5 days/week; or at least 150 minutes/week. Irregularly active B–those individuals who do not meet criteria regarding frequency or duration of activities. Sedentary–those individuals who did not perform physical activity for at least 10 continuous minutes/week.
*Comparison between G1, G2 and G3.
†Comparison between G1 and G2.
‡Comparison between G1 and G3.
††Comparison between G2 and G3.
§G1 (n=28), G2 (n=28), G3 (n=41).
‖G1 (n=27), G2 (n=28), G3 (n=41).

recommended by the Institute of Medicine (IOM) (30.3, 23.6, and 56.0% [p = 0.005], for G1, G2, and G3, respectively) (23,24). No significant difference was observed in weight gain between G1 and G2.

Rates of gestational hypertensive disorders and primary cesarean section were similar across all groups. Fetal and neonatal outcomes are listed in **Table 3**. G2 had a lower median gestational age at delivery (38.7 weeks [22.7–41.3 weeks], 38.0 weeks [24.7–40.7 weeks], and 39.1 weeks [22.1–41.3 weeks] [p = 0.011], for G1, G2, and G3, respectively) and a higher rate of NICU admission (12.9, 41.9, and 18.7% [p = 0.014], for G1, G2, and G3, respectively) than the other groups, but no difference was observed in the rate of prematurity. G1 presented similar fetal and neonatal outcomes to G3. Multivariate analysis (using data from G1 and G2 only) indicated that eGDM treatment (G2), pre-pregnancy BMI, nulliparity, chronic hypertension, and lower carbohydrate intake at 24–28 weeks were not predictors for gestational age at delivery or NICU admission. In model II, insulin use was associated with gestational age at delivery (standardized beta -0.397 [p = 0.018]) (**Supplemental Table S1**).

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Table 3. Maternal and newborns' outcomes

Variables	G1 (n = 33)	G2 (n = 31)	G3 (n = 50)	p^{*}	$p^{\dagger}$	$p^{\ddagger}$	$p^{\dagger\dagger}$
75-g OGTT (mg/dL)							
FPG	84.5 ± 8.1	-	82.5 ± 8.6	0.290	-	0.290	-
1-h PG	127 (77–255)	-	127 (64–197)	0.366	-	0.366	-
2-h PG	109.5 ±21.8	-	110.2 ± 24.5	0.897	-	0.897	-
GA at OGTT (week)	26.9 (23.4–35.7)	-	26.3 (21.7–34.6)	0.156	-	0.156	-
GDM (n, %)	9 (27.3)[§]	31 (100)[‖]	10 (20.0)[§]	-	-	0.594	-
GDM treatment							
Insulin (n, %)	2 (22.2)	16 (51.6)	1 (10.0)	0.036	0.149	0.582	0.028
GA at the start of insulin (week)	32.0 ± 0.8	24.3 ± 7.1	32.3 ± .	0.237	0.161	0.821	0.299
Insulin dose (units/kg/day)	0.4 ± 0.3	0.6 ± 0.3	0.4 ± .	0.592	0.427	0.940	0.487
Basal insulin (n, %)	2 (100)	7 (43.7)	0	0.334	0.471	0.333	1.000
Basal-bolus insulin (n, %)	0	9 (56.3)	1 (100)	0.334	0.471	0.333	1.000
Gestational weight gain (kg)	10.2 ± 7.6	7.5 ± 6.4	13.5 ± 6.1	0.001	0.132	0.031	0.000
Gestational weight gain (n, %)							
Bellow recommended[¶]	7 (21.2)	11 (35.5)	6 (12.0)	0.048	0.269	0.356	0.023
On target[¶]	16 (48.5)	13 (41.9)	16 (32.0)	0.307	0.625	0.168	0.475
Above the target[¶]	10 (30.3)	7 (23.6)	28 (56.0)	0.005	0.577	0.026	0.005
Gestational hypertension (n, %)	0	0	1 (2.0)	1.000	-	1.000	1.000
Preeclampsia (n, %)	5 (15.1)	9 (29.0)	7 (14)	0.208	0.232	1.000	0.150
Eclampsia (n, %)	0	0	0	-	-	-	-
Cesarean delivery (n, %)	16 (48.5)	21 (67.7)	29 (58.0)	0.314	0.137	0.500	0.482
Primary	9 (27.3)	8 (25.8)	13 (26.0)	1.000	1.000	1.000	1.000
Repeated	7 (21.2)	13 (41.9)	16 (32.0)	0.198	0.106	0.325	0.475
GA at delivery (week)	38.7 (22.7–41.3)	38.0 (24.7–40.7)	39.1 (22.1–41.3)	0.011	0.019	0.777	0.008
Prematurity (n, %)	2 (6.1)	5 (16.1)	8 (16.0)	0.383	0.250	0.302	1.000
Male sex (n, %)	16 (48.5)	13 (43.3)	23 (46.0)	0.970	0.801	1.000	1.000
1-min Apgar <7 (n, %)	3 (9.7)	1 (3.3)	2 (4.2)	0.564	0.612	0.376	1.000
5-min Apgar <7 (n, %)	1 (3.2)	1 (3.3)	0	0.311	1.000	0.392	0.385
Birth weight (g)	3315.0 (575–4145)	3185.0 (630–4315)	3240.0 (500–4630)	0.725	0.440	0.795	0.662
Large-for-gestational-age (n, %)	6 (18.7)	5 (16.1)	5 (10.4)	0.517	1.000	0.333	0.502
Small-for-gestational-age (n, %)	3 (9.4)	1 (3.2)	3 (6.2)	0.709	0.613	0.679	1.000
Macrosomia (n, %)	1 (3.1)	3 (9.7)	3 (6.0)	0.551	0.355	1.000	0.670
Neonatal hypoglycemia (n, %)	1 (3.2)	2 (6.4)	0	0.175	1.000	0.392	0.151
Shoulder dystocia or birth injury (n, %)	2 (6.4)	1 (3.2)	1 (2.1)	0.812	1.000	0.558	1.000
NICU (n, %)	4 (12.9)	13 (41.9)	9 (18.7)	0.014	0.021	0.552	0.039
Hyperbilirubinemia (n, %)	3 (9.7)	6(19.3)	3 (6.2)	0.195	0.473	0.674	0.143
Polyhydramnios (n, %)	0	2 (6.5)	0	0.072	0.231	-	0.144
Fetal death (n, %)	2 (6.1)	0	1 (2.0)	0.461	0.493	0.560	1.000
Early neonatal death (n, %)	0	0	0	-	-	-	-
Abortion (n, %)	0	0	1 (2.0)	1.000	-	1.000	1.000

FPG: fasting plasma glucose; GA: gestational age; GDM: gestational diabetes mellitus; G1: early GDM without early treatment; G2: early GDM with early treatment; G3: control group; NICU: neonatal intensive care unit; OGTT: oral glucose tolerance test; PG: plasma glucose.

*Comparison between G1, G2 and G3.

†Comparison between G1 and G2.

‡Comparison between G1 and G3.

††comparison between G2 and G3.

§Diagnosis by OGTT between 24-28 weeks' gestation.

‖Early diagnosis by 2 altered fasting plasma glucose levels.

¶Institute of Medicine recommendation (23,24).

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DISCUSSION

Our findings revealed that women with eGDM (diagnosed by mild, non-persistent FPG elevation in the first trimester) and fewer risk factors (e.g., obesity, multiparity, chronic hypertension) had outcomes comparable to those with early normal FPG, despite not receiving early intervention. To our knowledge, this is the first study to make such a comparison.

The group with eGDM that did not undergo early intervention showed similar fasting, 1-hour, and 2-hour plasma glucose values on the OGTT at 24–28 weeks to those with normal early FPG. The proportion diagnosed with GDM by OGTT at 24–28 weeks was also equivalent. Less than a third of G1 was diagnosed with GDM at 24–28 weeks and underwent intervention from then on, without worse outcomes. More than two-thirds of G1 would have undergone early intervention according to the current WHO GDM protocol, even in the absence of a GDM diagnosis by the present gold standard (75-g OGTT at 24–28 weeks). This is consistent with other studies showing that mild FPG elevations early in pregnancy (≥92 mg/dL [5.1 mmol/L] and <100 mg/dL [5.6 mmol/L]) are frequently not confirmed between 24–28 weeks, and fasting glucose generally declines throughout pregnancy across all pre-pregnancy BMI groups until the nineteenth week of gestation (18,19,25). Likewise, Cosson and cols. (17) showed that women with early fasting hyperglycemia (FPG between 92 mg/dL [5.1 mmol/L] and 125 mg/dL [6.9 mmol/L] before 22 weeks) and no risk factors are unlikely to develop GDM (17). In the TOBOGM study, GDM was newly diagnosed in 67% of women with eGDM who did not have early intervention; nevertheless, the control group in that study included women with risk factors, greater mean gestational age at screening (15.6 weeks), and higher initial FPG values (up to 109 mg/dL [6.0 mmol/L]) (9).

A question could be raised about the possibility that this first FPG of G1 would be slightly increased due to laboratory errors. Pre-analytical variables, such as the time between blood draw and centrifugation, are known to affect glucose measurement. Potter and cols. (26) noted that early centrifugation of 75-g OGTT samples can increase mean glucose readings and raise GDM diagnosis rates of 11.6–20.6%. In our study, all samples across groups were collected after overnight fasting in sodium fluoride tubes and analyzed under the same conditions and laboratory personnel.

It is also worth noting that although pregnant women in G1 did not undergo early intervention, these women gained less weight during pregnancy than those in G3, with most gaining weight below or within the threshold recommended by IOM (23,24). It is not clear if the lower weight gain favorably influenced the outcomes in G1, since it is not defined what is the appropriate weight gain cutoff to achieve better perinatal outcomes in pregnancies complicated or not by GDM (27,28). The rate of chronic hypertension in our study was higher compared with typical values reported in the pregnant population, which may be due to the fact that our maternity is a reference service for pregnant women with chronic hypertension.

Patients from G2 experienced lower gestational age at delivery and higher NICU admission rates than those in G1 or G3. While group allocation alone was not a predictor for NICU admission by multivariate analysis (examining G1 and G2 data only), the observed OR (2.65) suggests a trend potentially limited by sample size and number of events. Notably, the TOBOGM pilot study found an increased NICU admission rate among eGDM patients receiving early intervention (12). The lower gestational age at delivery in G2 likely reflects our institution's protocol, which recommends interrupting pregnancy (by inducing vaginal delivery or cesarean section) in women with GDM in use of insulin at 38 weeks' gestation. This was corroborated by the multivariate analysis (model II), in which only the use of insulin was associated with gestational age at birth. However, this difference did not translate into higher prematurity or adverse neonatal outcomes.

Moreover, previous studies have raised concerns regarding increased SGA incidence in eGDM cases given early treatment. In our study, there was no such increase in SGA in the early treatment group (9,12). Notably, G2 had a greater need for insulin compared to other groups, despite lower gestational weight gain, and similar findings have been reported elsewhere (7–9,29,30). This finding strengthens the position of some

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experts who defend early GDM diagnosis and treatment, positing that such patients present with more severe disease and may benefit from earlier intervention to reduce complications (17,31). However, there are concerns over potential overtreatment and resultant undernutrition (8,10–12,32). Our findings suggest that women with eGDM diagnosed by mild, non-persistent FPG elevation and with fewer risk factors had favorable outcomes even without early intervention, and their insulin use did not differ from control.

When we compared only the group that received early intervention (G2) with those with normal early FPG (G3), we noted that there were also no significant differences in most maternal-fetal outcomes. We speculate that this may be because G2 received early intervention protocol at our Institution. Another hypothesis is that even women with risk factors with mildly elevated FPG in the first trimester may evolve well throughout pregnancy, with or without early treatment. In the TOBOGM study, 33% of eGDM patients with risk factors who did not undergo early intervention no longer met criteria for GDM when assessed by the current gold standard (75-g OGTT at 24-28 weeks) (9). Future studies with larger sample sizes and dedicated designs should seek to address these questions.

Our study has some limitations, including the small number of patients, absence of formal sample size estimation, and the single-center, tertiary-care setting. For ethical reasons, we adhered to institutional protocols, initiating intervention for women with two early FPG readings ≥92 mg/dL (5.1 mmol/L). Nonetheless, this study's strengths include the fact that it was conducted in a center where all participants received care from the same multidisciplinary team using a single protocol. Consequently, it was possible to perform a comparative analysis of various factors that could interfere in the evaluation of gestational outcomes.

In summary, this study found that women diagnosed with eGDM based on mild and non-persistent FPG elevation in the first trimester, and who did not receive early treatment, had outcomes similar to those with early normal FPG. These findings prompt reconsideration of glucose thresholds and the need for early intervention in pregnant women with fewer GDM risk factors. Larger, multicenter, well-controlled studies are warranted to evaluate the true benefit of early treatment for mild first-trimester FPG elevation.

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SUPPLEMENTARY MATERIAL

Supplemental Table S1. Multivariable analyses of predictors that characterized gestational age at delivery in early GDM groups and the need for admission in neonatal intensive care unit

Variables	NICU admission		Gestational age at delivery			
	OR (95% IC)	*p*	Model 1		Model 2*	
			Beta	*p*	Beta	*p*
G2	2.647 (0.64–10.93)	0.18	0.29	0.04	0.000	0.999
Pre-pregnancy BMI (kg/m^2)	1.05 (0.91–1.22)	0.48	-0.08	0.62	-0.181	0.276
Nullipara	1.99 (0.35–11.33)	0.44	0.06	0.67	0.173	0.286
Chronic hypertension	1.09 (0.25–4.78)	0.91	-0.18	0.23	-0.170	0.296
Food consumption 24-28 week: CHO–%.	0.88 (0.76–1.02)	0.10	0.06	0.68	0.209	0.200
Use of insulin					-0.397	0.018

BMI: body mass index; CHO: carbohydrate; GDM: gestational diabetes mellitus; G2: early GDM with early treatment; NICU: neonatal intensive care unit. *Model 2 included the use of insulin.

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original

Hypercholesterolemia induced by hypothyroidism is ameliorated by taurine supplementation through the hepatic AMPK/ACC pathway

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ABSTRACT

Objective: To evaluate the effect of taurine supplementation on the lipid profile of male hypothyroid Wistar rats. **Materials and methods:** Adult male Wistar rats were induced to hypothyroidism by treatment with 0.03% methimazole in drinking water. After 21 days, half of the hypothyroid animals received daily taurine supplementation by gavage (520 mg/kg b.w.), while the other half received water. Control animals received taurine at the same dose or water by gavage for an additional 21 days, totaling 42 days of treatment. The groups were: Control (C), Taurine (T), Hypothyroid (H), and Hypothyroid + Taurine (H+T) (n = 5–20/group). Data were expressed as mean ± SEM, and statistical analysis was performed using two-way ANOVA followed by Tukey's post-test. **Results:** Hypothyroidism increased serum total cholesterol (TC) and LDL levels, which were reduced by taurine supplementation. Hepatic diacylglycerol concentration increased with taurine supplementation, but this effect was not observed in hypothyroid animals. Taurine increased AMPK and ACC phosphorylation in the liver independently of thyroid hormone levels. **Conclusion:** Hypothyroidism induces changes in the lipid profile, particularly increasing TC and LDL cholesterol levels. Taurine supplementation in hypothyroid rats partially reversed lipid alterations and increased AMPK and ACC phosphorylation in the liver, suggesting metabolic benefits.

Keywords: Hypothyroidism; taurine supplementation; lipids serum levels; dyslipidaemia; AMPK; ACC

INTRODUCTION

Hypothyroidism is one of the most common endocrine disorders worldwide and is usually characterised by decreased production of thyroid hormones (TH) by the thyroid gland (1). This dysfunction is associated with a high prevalence of cardiovascular diseases and features related to metabolic syndrome, such as hypertension and dyslipidaemia (2,3). Alterations in lipid metabolism due to hypothyroidism confer a pro-atherogenic profile, with patients typically exhibiting elevated serum levels of total cholesterol (TC) and low-density lipoprotein (LDL), resulting in hypercholesterolemia. TH are known to stimulate lipolysis and the transport of fatty acids (FA) to the liver (3). Additionally, TH links autophagy to mitochondrial fat oxidation, promoting ketogenesis, and stimulates reverse cholesterol transport in the liver (4). However, TH deficiency reduces hepatic triglycerides (TG) content derived from FA and increases lipid uptake by white adipose tissue (WAT) (5). Cholesterol homeostasis is also regulated by TH, which stimulate its hepatic biosynthesis, uptake from circulation and conversion into bile acids (6). The cholesterol-lowering effect of TH can be explained by multiple mechanisms. Triiodothyronine (T3) is known to induce hepatic LDL receptor (LDL-R) gene and protein expression (7) and

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to stimulate cholesterol 7α-hydroxylase (Cyp7A1), the key enzyme in bile acid synthesis from cholesterol (8). Therefore, hypothyroidism is highly associated with non-alcoholic fatty liver disease (NAFLD) and is considered a risk factor for hepatic lipid accumulation (9).

Taurine (β-aminoethanesulfonic acid) is considered a semi-essential amino acid that is not incorporated into proteins and can be found at different concentrations in mammalian tissues. It is widely distributed in skeletal muscle, heart and brain, and its levels may vary in other tissues, such as the liver, depending on dietary intake (10,11). Many studies have shown potential benefits of taurine supplementation in cardiovascular diseases (12), as well as improvements in the lipid profile in obesity, NAFLD and diabetes mellitus (13-15). Taurine has been shown to stimulate FA oxidation via increased peroxisome proliferator-activated receptor γ (PPARγ) protein expression; taurine deficiency, in contrast, leads to impaired metabolism (16). In the same context, a taurine-rich diet was able to reduce the hepatic TG content and increase free FA levels in the liver of high-fat diet-fed mice (17). Furthermore, in a rodent model of obesity induced by monosodium glutamate, taurine supplementation reduced hepatic TG accumulation and serum lipid levels by modulating genes involved in lipolysis (15). Taurine supplementation is also associated with reduced hepatic and serum cholesterol levels, which some authors attribute to decreased HGM-CoA reductase activity, thereby reducing cholesterol synthesis (18) and promoting bile acid conjugation (16). Recent studies have identified taurine deficiency as a hallmark of aging in mice, monkeys and humans. In this context, a reversal of this decline by taurine supplementation may increase healthy lifespan across species (19).

Previous studies have associated lipid alterations observed in hypothyroid patients with the development of atherosclerotic disease (20). Even with levothyroxine treatment, some individuals with hypothyroidism show a delayed reduction in serum lipid levels, making its efficacy in improving the lipid profile by normalizing TH levels alone controversial (21). Increasing evidence suggests that taurine supplementation may decrease the risk of atherosclerosis by reducing oxidative stress (22). Therefore, this study aims to investigate the effect of taurine supplementation in the lipid profile of hypothyroid rats. We found that taurine can restore hypothyroidism-induced hypercholesterolemia, likely via modulation of the hepatic AMPK/ACC pathway.

MATERIALS AND METHODS

Animals

Male Wistar rats (12 weeks of age), weighing approximately 250 g, were randomly housed in groups of five in acrylic cages (18 x 31 x 38 cm) and maintained under controlled conditions from birth: temperature of 23 ± 2 °C, a 12 h:12 h light–dark cycle (lights on at 19:00), with water and standard chow available *ad libitum*.

Study design

This study was approved by the Ethics Committee for Animal Use in Scientific Experimentation (CEUA-CCS; No. IBCCF 080) of the Health Sciences Centre at the Federal University of Rio de Janeiro. Animals received humane care in accordance with the International Guiding Principles for Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences and the International Council for Laboratory Animal Science, Geneva, Switzerland). Some animals were induced to pharmacological hypothyroidism by oral administration of 2-mercapto-1-methylimidazole (MMI; Sigma-Aldrich, USA) at a concentration of 0.03% in drinking water for 21 days, as previously described (23). After hypothyroidism induction, the animals were divided into four groups. Control: animals received water and chow *ad libitum* throughout the experimental period (42 days) and daily water gavage during the last 21 days; Taurine: animals received water and chow *ad libitum* throughout the experimental period and daily taurine gavage (Sigma-Aldrich, USA) at a dose of 520 mg/kg body weight (b.w.) during the last 21 days. This dose was selected based on Allen and cols. (2016), who reported that taurine treatment alone at this dose had no significant effect on the assessed parameters in healthy rats (24); Hypo: animals received MMI in the drinking water throughout the experimental period and daily water gavage during the last 21 days; Hypo + Taurine: animals received MMI in the drinking water

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throughout the experimental period and daily taurine gavage (520 mg/kg b.w.) during the last 21 days. All animals were subjected to daily gavage with drinking water for one week before the start of taurine supplementation for adaptation. Body weight was measured weekly throughout the treatment period. Three independent experiments were performed, each including five animals per group (20 animals per experiment; 60 animals in total). Sample size was calculated using http://www.gpower.hhu.de/.

Glucose tolerance test

The glucose tolerance test (GTT) was performed on the 40th day of treatment. The experimental groups were fasted for eight hours. Blood samples were then collected by tail snip, and fasting glycemia was measured using commercially available test strips and a glucometer (Accu-Check®Active, Roche Diagnostics). Subsequently, a glucose solution (1.75 g/kg b.w.) was administered intraperitonially (i.p.) to each rat. Glycemia was measured again from tail blood samples at 15, 30, 60 and 120 minutes after glucose administration using the same glucometer.

Biological samples collection

After 42 days of treatment, the animals were euthanized by decapitation without prior sedation at 09:00 (dark cycle). Blood was collected from the trunk and centrifuged at 1,200 × g for 20 minutes; serum was separated and stored at −20 ℃. The liver was collected, weighed, frozen in liquid nitrogen, and stored at −70 ℃ for further analyses. WAT depots and brown adipose tissue (BAT) were collected and weighed. Visceral WAT was defined as the sum of retroperitoneal and mesenteric depots; epididymal WAT was collected from the gonadal region, subcutaneous WAT from the anterior subcutaneous region; and interscapular BAT was analysed.

Serum thyroid hormones, total cholesterol, triglycerides and lipoproteins measurements

Serum levels of total T3 and T4 were determined by radioimmunoassay (RIA) according to the manufacturer's instructions (Diagnostic Systems Laboratories Inc., TX, USA). Total cholesterol, triglycerides and HDL levels were measured using colorimetric enzymatic assays, according to the manufacturer's instructions for microplates assays (Bioclin, Belo Horizonte, MG, Brazil). Serum VLDL levels were calculated as triglycerides ÷ 5, and LDL levels were estimated using the Friedewald Equation: total cholesterol - triglycerides ÷ 5 - HDL.

Thin-layer chromatography and high-performance thin-layer chromatography

Liver aliquots (30 mg) were used for total lipid extraction according to a previously described protocol (25), with modifications. A 5 µL aliquot of the extracted lipids were applied to a silica plate (Merck Millipore) as previously described (26). Lipid standards were applied to the plates for identification: for thin-layer chromatography (TLC), free FA, cholesterol, esterified cholesterol, diacylglycerol and triacylglycerol; and for high-performance thin-layer chromatography (HPTLC), sphingomyelin (SM), phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (all standards from Sigma). TLC was performed using a mobile phase composed of hexane–diethyl ether–acetic acid (60:40:1 v/v/v). HPTLC was performed using a mobile phase containing acetone (7.5 mL), methanol (6.5 mL), acetic acid (6 mL), chloroform (20 mL) and water (4 mL). After development, a charring solution (3% $CuSO_4$ and 8% H_3PO_4) was applied to the plates, which were then heated in a hood at 200 ℃ until complete visualization of the spots.

Western blot

Liver samples were homogenized in Tris-HCl buffer (0.0625 M, pH 6.8) containing 10% glycerol, 3% SDS, 1 mM PMSF, 50 mM NaF, 0.01% bromophenol blue and 5% β-mercaptoethanol. Total protein concentration was determined using a bicinchoninic acid (BCA) assay (Thermo Scientific™) according to the manufacturer's instructions for microplate assays, using a bovine serum albumin (BSA) standard curve. A total of 60 µg of protein was loaded onto 7% or 10% polyacrylamide gels and subjected to electrophoresis in a running buffer (25 mM Tris-HCl, 192 mM glycine, 0.1% SDS, pH 8.3) at 120 V. Proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane

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overnight in a transfer buffer (25 mM Tris, 192 mM glycine, 1% SDS, 20% methanol) at 20 V. Membranes were blocked for one hour at room temperature with TBS containing 0.05% Tween 20 (TBST, pH 7.6) and 5% BSA (Sigma-Aldrich) to prevent non-specific binding. Membranes were then incubated overnight at 4 °C under agitation with primary antibodies (1:1500 anti-AMPKα [2603); 1:1500 anti-phospho-AMPKα [Thr172; 50081]; 1:1000 anti-acetyl-CoA carboxylase [3676]; 1:1500 anti-phospho-acetyl-CoA carboxylase [Ser79; 11818]; all from Cell Signaling Technology) (**Table 1**). In the following day, membranes were washed with TBST and incubated for one hour at room temperature under agitation with a peroxidase-conjugated secondary antibody (1:5000 donkey anti-rabbit IgC; Santa Cruz Biotechnology; sc-2317) (**Table 1**), followed by additional washes with TBST. Immunoblots were visualized using ImageQuant™ LAS 4000 (GE Healthcare) after being exposed to a chemiluminescent substrate (Pierce ECL Western blotting substrate, ThermoScientific).

Densitometry

The densitometric analysis of bands obtained from TLC, HPTLC and Western blot experiments was performed using Image J software (National Institute of Health, USA). Results were expressed as a percentage of the control group or the protein constitutive isoform.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test. Statistical significance was set at $p < 0.05$. All analyses were conducted using Graphpad Prism 7.0® (Graphpad Sofware Inc., CA, USA).

RESULTS

Taurine supplementation does not alter thyroid hormone levels and body weight changes induced by hypothyroidism

Serum levels of total T3 (**Figure 1A**) and total T4 (**Figure 1B**) decreased in hypothyroid animals, confirming the effectiveness of the experimental model, and were not altered by taurine supplementation. Hypothyroid rats stopped gaining weight by the end of the third week of MMI treatment (**Figure 1C**), maintaining body weight at approximately 250 g during the following weeks. Taurine supplementation was not able to prevent this alteration. In contrast, control animals, whether supplemented with taurine or not, continued to gain weight until the end of the treatment (**Figure 1C**). GTT was performed on the 40th day of treatment and showed no differences in glycemia or in the area under the curve (AUC) between groups. No substantial effect of taurine supplementation was observed on the glucose profile in either group.

Taurine supplementation increases visceral WAT in a thyroid hormone-dependent manner

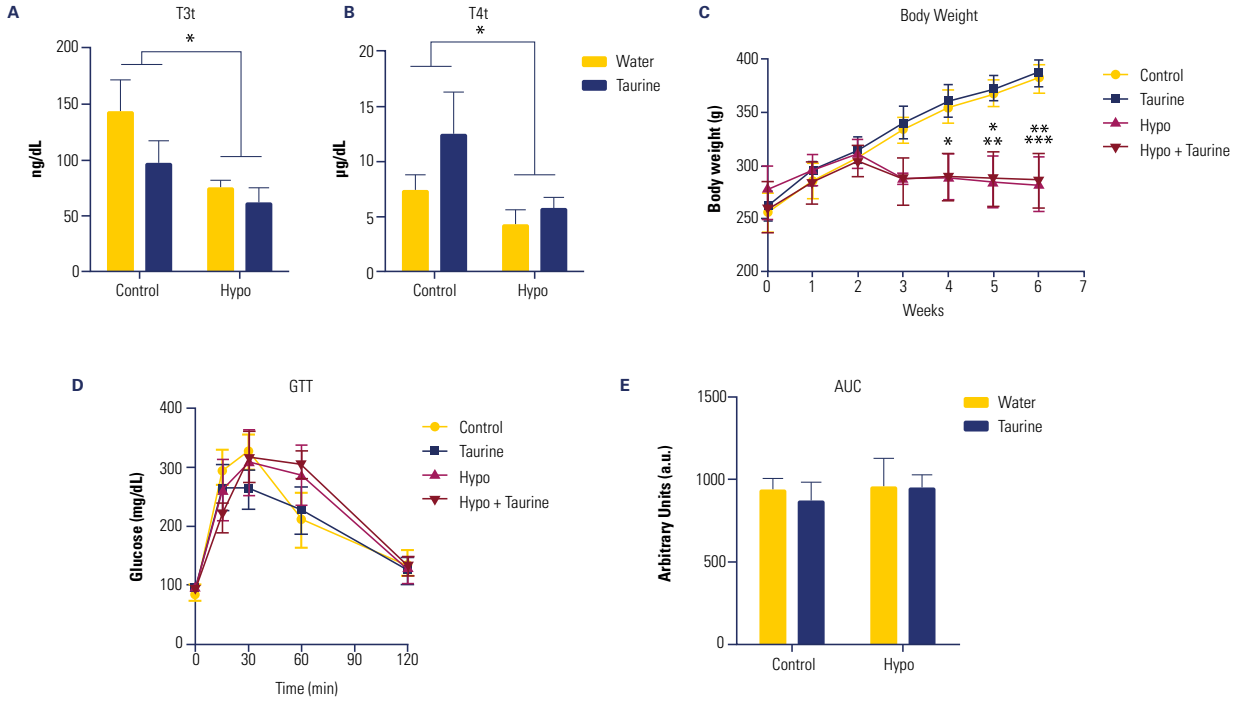
The relative weights of the liver (**Figure 2A**) and BAT (**Figure 2B**) were increased in hypothyroid animals, with no influence of taurine supplementation. In a thyroid hormone-dependent manner, the relative weight of visceral WAT (vWAT) increased only in control animals after taurine supplementation (**Figure 2C**). Despite this observation, the relative weights of subcutaneous (**Figure 2D**) and epididymal WAT did not differ between groups (**Figure 2E**).

Table 1. Antibody specifications for Western blots

Antibody	Dilution	Manufacturer	Catalog number	Specie and type
anti-AMPKα	1:1500	Cell Signaling	Cat#2603	Monoclonal
anti-phospho-AMPKα	1:1500	Cell Signaling	Cat#50081	Monoclonal
anti-ACC	1:1000	Cell Signaling	Cat#3676	Monoclonal
anti-phospho-ACC	1:1500	Cell Signaling	Cat#11818	Monoclonal
donkey anti-rabbit anti-IgG-HRP	1:5000	Santa Cruz Biotechnology	sc-2317	Polyclonal

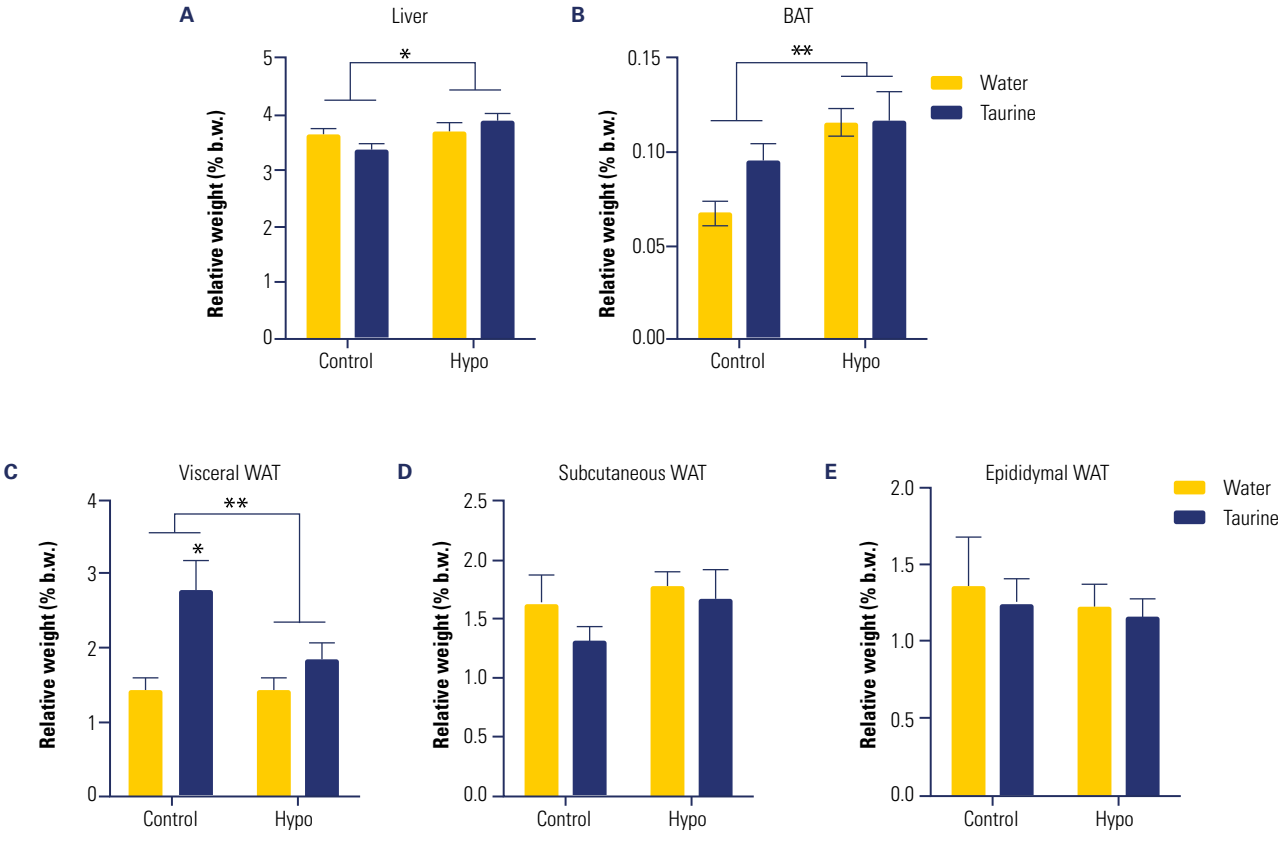
AMPK: AMP-activated protein kinase; ACC: acetyl-CoA carboxylase.

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*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Figure 1. Thyroid hormones serum levels and in vivo analyses.



*$p < 0.05$; **$p < 0.01$

Figure 2. Effect of hypothyroidism and taurine supplementation in the relative weight of liver and adipose tissues depots.

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Hypercholesterolemia induced by hypothyroidism is reversed by taurine supplementation

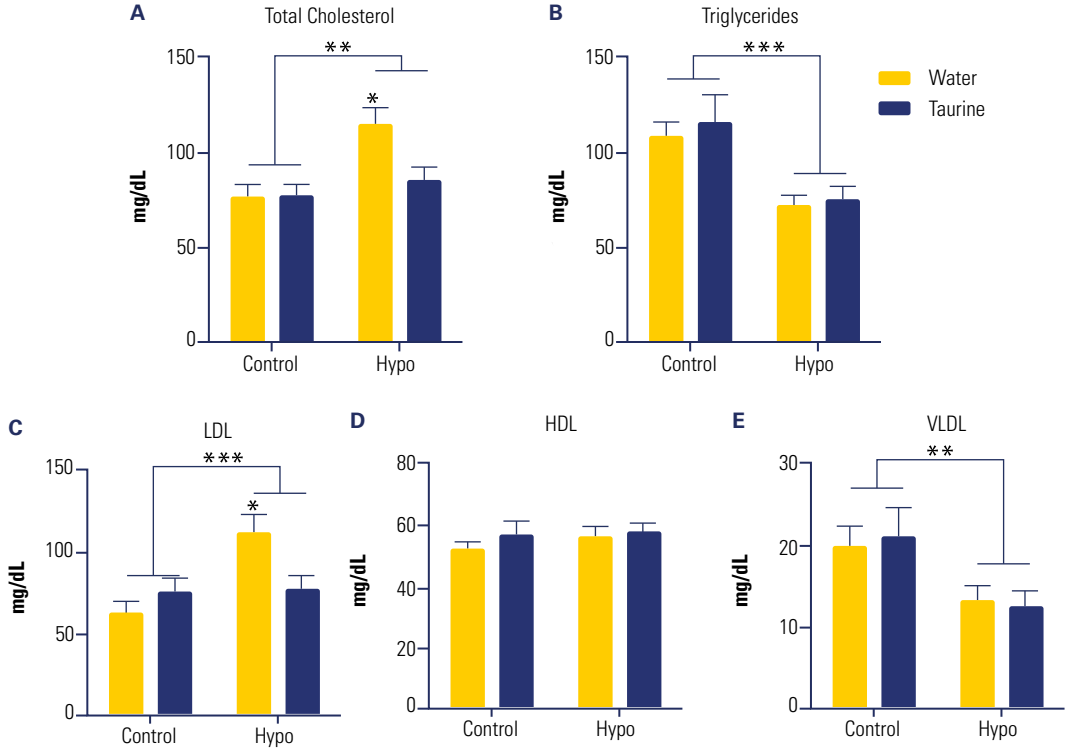
Serum TC levels were increased in hypothyroid animals, and taurine supplementation restored them to control values (**Figure 3A**). Similarly, serum LDL levels were increased in hypothyroid rats and were reduced following taurine supplementation (**Figure 3C**). Serum triglyceride (**Figure 3B**) and VLDL levels (**Figure 3E**) were decreased in hypothyroid animals, without alterations after taurine treatment. HDL levels were not altered by either hypothyroidism or taurine supplementation (**Figure 3D**).

Hepatic diacylglycerol and phosphatidylinositol contents increase with taurine supplementation in a thyroid hormone-dependent manner

In general, hepatic neutral lipids content — including free cholesterol, esterified cholesterol, triglycerides, FA and diacylglycerol — was decreased in hypothyroid rats, with no modulation by taurine supplementation (**Figure 4**). However, control animals supplemented with taurine showed a substantial increase in hepatic diacylglycerol levels, an effect that was not observed in hypothyroid animals, regardless of taurine supplementation (**Figure 4E**). This thyroid hormone-dependent effect of taurine was also observed in hepatic phosphatidylinositol (PI) content (**Figure 5A**). In contrast, hepatic phospholipid SM content (**Figure 5B**) and the phosphatidylethanolamine-to-phosphatidylcholine ratio (PE/PC) (**Figure 5C**) did not differ between groups.

Taurine supplementation increases hepatic AMPK and ACC phosphorylation independently of thyroid hormones levels

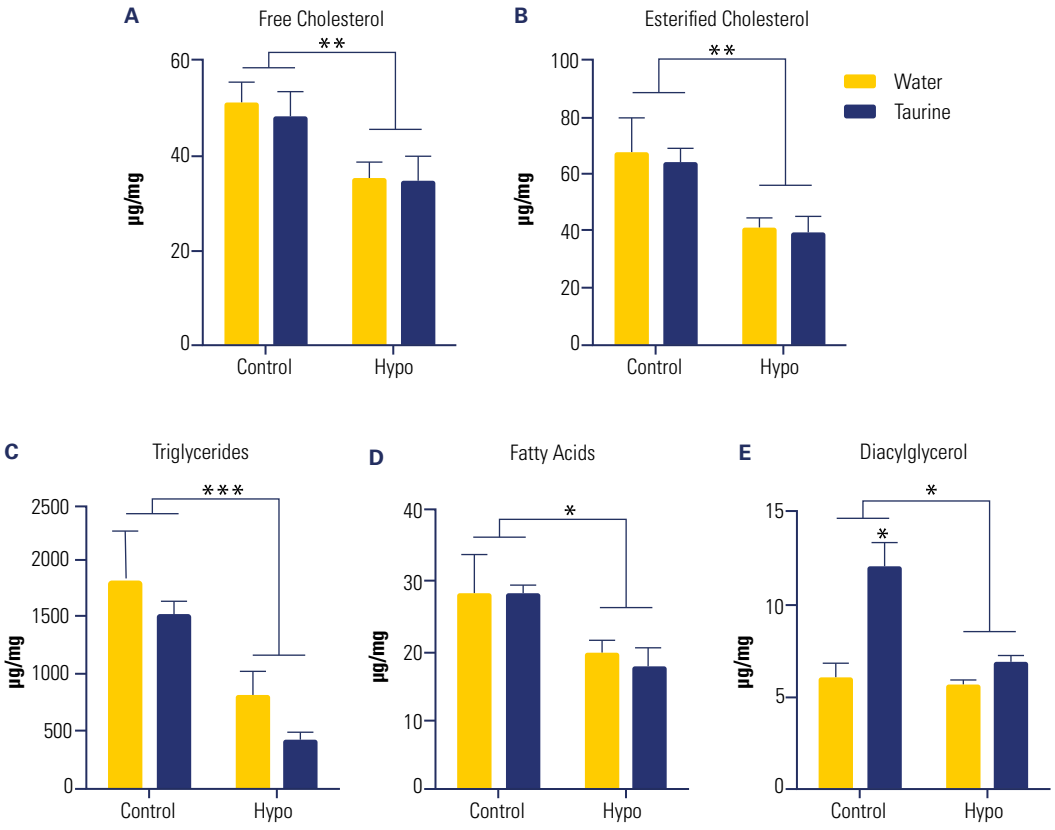
Rats, whether hypothyroid or not, that received daily taurine gavage showed higher levels of phosphorylated AMPK (**Figure 6A**) and ACC (**Figure 6B**) compared with animals that received water only, as demonstrated by the ratio between p-AMPK/AMPK and p-ACC/ACC.



*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

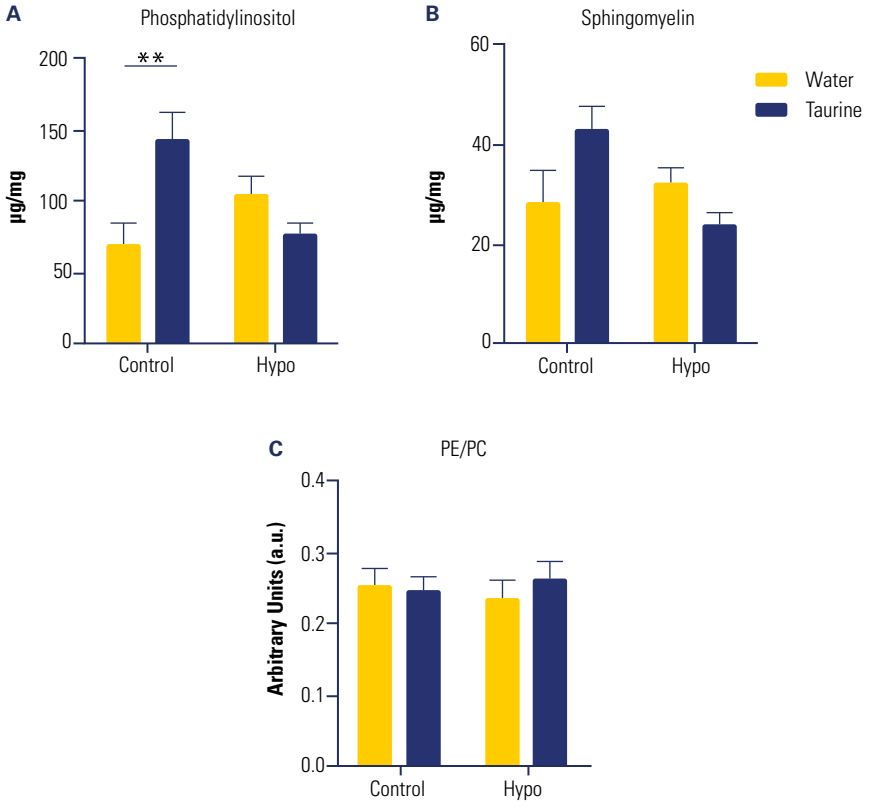
Figure 3. Total cholesterol, triglycerides and lipoproteins serum levels.

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*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

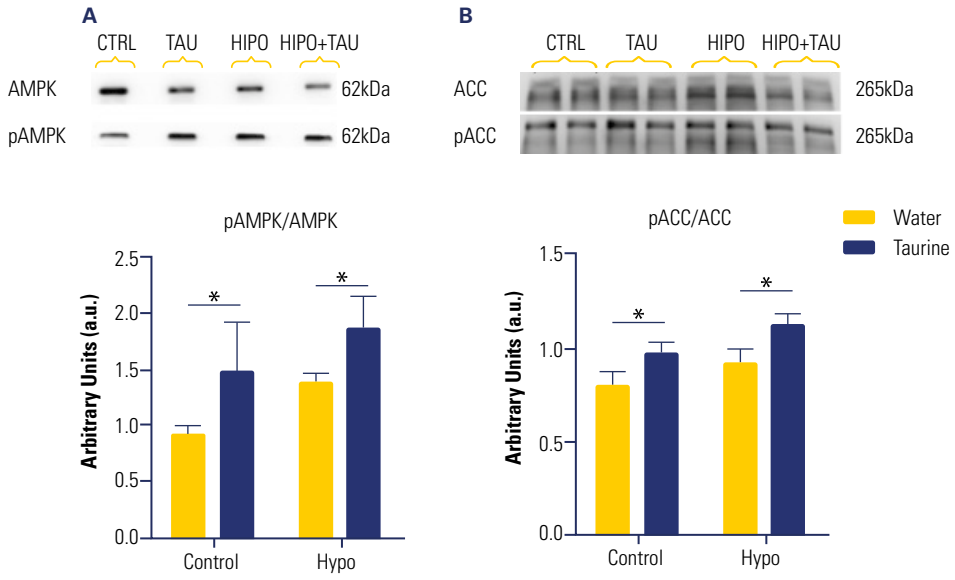
Figure 4. Hepatic lipid content.



**$p < 0.01$

Figure 5. Phospholipids hepatic content.

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*$p < 0.05$

Figure 6. Hepatic protein levels of pAMPK and pACC.

DISCUSSION

The results presented here demonstrate that taurine supplementation during untreated hypothyroidism can reverse hypercholesterolemia, possibly by alterations in hepatic lipid metabolism.

Epidemiological data indicate that approximately 4.6% of the global population has some degree of hypothyroidism, which is commonly associated with hypercholesterolemia and elevated circulating LDL levels (27). Recent studies have identified regulatory factors potentially associated with dyslipidaemia in hypothyroidism, including proprotein convertase subtilisin/kexin type 9, angiogenin-like proteins and fibroblast growth factors (28). Dyslipidaemia associated with hypothyroidism is also frequently observed in conditions such as obesity, diabetes and metabolic syndrome (29). In our study, taurine supplementation did not alter body weight (**Figure 1C**) or glucose metabolism (**Figure 1D**) in either control or hypothyroid groups. However, Kim and cols. reported an anti-obesity effect mediated by inhibition of adipogenesis in animals fed a high-fat diet supplemented with taurine (30). Nevertheless, the role of taurine in weight gain remains controversial, with its effects depending on the animal model and study duration. In our control group fed a normal chow diet supplemented with taurine, visceral WAT mass increased (**Figure 2C**), suggesting that taurine may influence visceral adipogenesis and modulate fat distribution across different depots while maintaining overall body weight. This effect appears to be TH-dependent, as it was not observed in hypothyroid animals (**Figure 2C**). Taurine action in adipose tissue has been associated with the expression of taurine transporters in different depots. In BAT, the taurine receptor is upregulated without changes in mass in obesity models (30), similar to what was observed in our experimental model (**Figure 2B**).

Despite hypothyroidism being associated with insulin resistance in humans (31,32), hypothyroid rats did not show impairments in glucose homeostasis, whether supplemented with taurine or not (**Figure 1D**). Taurine supplementation has also been suggested as a potential dietary strategy for metabolic syndrome management in humans (33). However, our GTT-based data did not show any statistically significant differences in glucose metabolism after taurine treatment.

Regarding lipid metabolism, hypothyroidism is considered one of the major causes of secondary dyslipidaemia (28,34). Accordingly, our data show high TC levels in hypothyroid rats (**Figure 3A**), together with increased circulating LDL levels (**Figure 3C**). For the first time, we showed that taurine supplementation can decrease these lipid levels in hypothyroidism after three weeks (**Figures 3A and 3C**). Previous studies using mice fed a high-cholesterol diet showed that taurine intake for five weeks reduced TC and LDL levels,

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consistent with our findings (34). However, the exact mechanisms by which TH deficiency increases serum cholesterol levels are not fully understood. Some authors suggest that reduced LDL receptor expression during hypothyroidism contributes to elevated serum cholesterol levels. Furthermore, individuals with TH deficiency have an increased risk of atherosclerotic disease due to reduced cholesterol clearance and greater susceptibility to LDL oxidation (35). In fact, de Assis and cols. demonstraded that T3 supplementation modulates liver diurnal transcriptome rhythm, regulating glucose and FA metabolism and controlling hepatic energy turnover (36). Taurine supplementation for two weeks has been shown to increase hepatic LDL receptor gene expression in rodents fed a high-fat diet (37), suggesting a possible mechanism by which this amino acid reduces serum cholesterol levels in our model. Additionally, low circulating TH levels reduce cholesterol conversion into bile acids by decreasing CYP7A1 activity in reverse cholesterol transport (4). Moreover, a previous study showed that chronic taurine intake increased CYP7A1 activity in obese rodents with reduced enzyme activity levels (38). Thus, we propose that the cholesterol-lowering effect of taurine in hypothyroidism may involve some of the mechanisms described; however, further studies are necessary to confirm these hypotheses.

In line with the serum cholesterol data, hepatic levels of free and esterified cholesterol were reduced in hypothyroid rats, and taurine supplementation had no effect on their content (**Figures 4A and 4B**). These findings indicate that, under low TH levels, cholesterol efflux from peripheric tissues to the liver is reduced, resulting in decreased hepatic cholesterol accumulation and clearance. Previous studies have reported modulation of TG content after taurine supplementation in some obesity animal models (15,34), and a clinical trial in individuals with obesity associated seven weeks of taurine intake with reduced plasma TG levels (4). However, a transgenic mice model of obesity showed no alterations in TG content after taurine supplementation (39), suggesting that its effects may vary depending on the experimental model. Furthermore, taurine has been reported to inhibit diacylglycerol acyltransferase enzyme (DGAT) activity (40), the key enzyme responsible for the conversion of DAG into TG. This mechanism may explain the reduction in hepatic TG content observed in dyslipidaemia models supplemented with taurine (41). Nevertheless, further molecular studies are necessary to support this hypothesis. Our data show high hepatic DAG content after taurine supplementation in a TH-dependent manner (**Figure 4E**), which may be explained by reduced hepatic TG synthesis due to DGAT inhibition. While we did not observe changes in serum or hepatic TG levels in our experimental model (**Figures 3B and 4C**), this lack of effect may be related to the duration of taurine treatment.

Although LDL levels are increased in hypothyroidism, circulating HDL levels usually remain unchanged in this disorder (35), including in obesity animal models supplemented with taurine (34). Consistent with previous studies, serum HDL levels were also unaltered in our model (**Figure 3D**). Conversely, VLDL serum levels were significantly lower in hypothyroid animals (**Figure 3E**). This observation may be associated with low FA esterification into TG and their subsequent incorporation into VLDL (38), likely due to the aforementioned low TG content.

In this context, we analysed hepatic phospholipids content among groups. PI levels were higher in control animals treated with taurine compared with other groups (**Figure 5A**), suggesting elevated conversion to DAG. This finding indicates that PI may be preferentially used as a second messenger for cellular functions than being converted into TG. Furthermore, no differences were observed in hepatic SM content or in the PE/PC ratio among groups (**Figures 5B and 5C**), although SM is known to be involved in lipoprotein metabolism (42,43), and the PE/PC ratio has been reported to be modulated by intracellular taurine levels (44).

The effects observed may be regulated by the activation of the hepatic AMPK–ACC pathway. Our study reveals increased phosphorylation of AMPK (Thr172) and ACC (Ser79) in the liver following taurine supplementation, regardless of TH levels (**Figures 6A and 6B**). A previous taurine receptor knockout model showed reduced cardiac phospho-ACC protein expression, as well as decreased CPT1 levels (11). These findings

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support our results and suggest that taurine signalling may be essential for the phosphorylation of these proteins and, consequently, for FA oxidation and lipid metabolism homeostasis. Taurine supplementation has been recently proposed as a potential dietary strategy for the management of metabolic syndrome in humans (33). In fact, Attias and cols. demonstrated in rats with streptozotocin-induced type 2 diabetes that metformin combined with taurine improved metabolic parameters and protected against diabetic complications by means of antioxidative, anti-inflammatory and anti-apoptotic effects (45). These findings support a possible synergistic therapeutic approach involving TH and taurine to mitigate dyslipidaemia associated with hypothyroidism.

In conclusion, our results show that daily taurine supplementation is capable of improving hypercholesterolemia in hypothyroid rats. This alteration may be associated with increased phosphorylation of proteins involved in lipid metabolism, independently of TH levels. Therefore, taurine emerges as a potential candidate for the treatment of dyslipidaemia associated with hypothyroidism. However, further studies are needed to elucidate its underlying mechanism of action.

Authors' contributions: Matos MS performed the experiments and wrote the manuscript; Atella GC supported the thin-layer chromatography experiments; Corrêa da Costa VM contributed to the development of the study, supervised and contributed with funding acquisition.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

Association between triglyceride to high-density lipoprotein cholesterol ratio and new-onset diabetes mellitus

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ABSTRACT

Objective: To examine the association between triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio and the onset of diabetes mellitus (DM) within an ongoing prospective cohort in China. **Subjects and methods:** Participants were categorized into four groups according to their TG/HDL-C ratio quartiles. Kaplan-Meier estimator determined the cumulative incidence during follow-up and generated time-to-event curves. Additionally, a Cox proportional hazards regression analysis assessed the hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) for new-onset DM. A sensitivity analysis was also performed to mitigate the possible effects of reverse causation. **Results:** During a median follow-up duration of 13.67 years, 38,210 individuals developed DM. Kaplan-Meier curves revealed that the cumulative DM incidence across quartiles 1 to 4 was 40.95%, 42.04%, 41.42%, and 44.38%, respectively. The risk of developing DM increased over time according to the baseline TG/HDL-C ratio quartiles. After adjusting for potential confounding variables, the HRs reached 1.045 (95% CI, 1.015–1.076), 1.026 (95% CI, 0.996–1.058), and 1.095 (95% CI, 1.058–1.133) for quartiles 2, 3, and 4, respectively (P for trend < 0.001). These findings were consistent in sensitivity analyses, with HRs of 1.045 (95% CI, 1.016–1.075), 1.026 (95% CI, 0.996–1.058), and 1.095 (95% CI, 1.058–1.133) for quartiles 2, 3, and 4, respectively (P for trend < 0.0001). **Conclusion:** TG/HDL-C ratio is significantly and positively associated with new-onset DM.

Keywords: Triglyceride to high-density lipoprotein cholesterol ratio; insulin resistance; diabetes mellitus

INTRODUCTION

A 2022 report informed that 14% of adults aged 18 and older were living with diabetes mellitus (DM), a significant global increase from 7% in 1990. Additionally, DM was directly responsible for 1.6 million deaths, of which 47% occurred in individuals under 70 (1). Most recent data indicates that DM prevalence in China stands at 11.9% (2), surpassing the 10.5% global average (3). The pivotal pathogenic component of DM is insulin resistance (IR), which is characterized by lack of response to circulating insulin levels leading to fasting hyperglycemia. Research has shown that IR is linked to alterations in lipid and lipoprotein metabolism, resulting in the development of dyslipidemia and the characteristic lipid triad (4), a clinical picture marked by an increase in triglyceride-rich particles, a decrease in high-density lipoprotein cholesterol particles, and an increase in low-density

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lipoprotein cholesterol particles (5). Recent research indicated a strong association between the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio and IR (6,7). However, relevant studies investigating the relationship between the TG/HDL-C ratio and DM risk are scarce. Thus, our objective is to examine the association between the TG/HDL-C ratio and DM onset within an ongoing prospective cohort study based in China.

SUBJECTS AND METHODS

Study population

Data were obtained from the Kailuan study (registration number in the Chinese clinical trial registry: ChiCTR-TNRC-11001489) (Registration Date: 2011-08-24), a prospective cohort investigation conducted within the Kailuan community in Tangshan, China. Briefly, the study involved 101,510 participants, aged between 18 and 98 years, who completed the initial survey from June 2006 to October 2007. Participants underwent assessments through questionnaires, physical examinations, and laboratory tests. Subsequently, all participants were followed up biennially to update the aforementioned information. In the present study, we excluded 9,456 individuals with a history of myocardial infarction, 327 individuals with a history of cancer, and 1,281 individuals with incomplete baseline data regarding triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) levels. Thus, a total of 90,446 participants were included in the current analysis.

Data collection and grouping

Baseline characteristics were collected by trained professionals using a questionnaire that has been published previously (8). Participants' smoking and drinking statuses were categorized as current, former, or never users. Regular physical activity was defined as engaging in exercise at least four times per week, minimum duration of 20 minutes per session. Educational level was classified into five categories: illiterate, primary education, lower secondary education, upper secondary education, and higher education. Various biochemical indices, including total cholesterol (TC), TG, HDL-C, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), serum creatinine (Scr), and high-sensitivity C-reactive protein (hs-CRP), were assessed. TG/HDL-C ratio was calculated by dividing the TG serum concentration by that of HDL-C. Based on the TG/HDL-C ratio quartiles, participants were divided into four groups: quartile 1 (TG/HDL-C ratio ≤ 0.56), quartile 2 (0.56 < TG/HDL-C ratio ≤ 0.83), quartile 3 (0.83 < TG/HDL-C ratio ≤ 1.32), and quartile 4 (TG/HDL-C ratio > 1.32) (Figure 1).

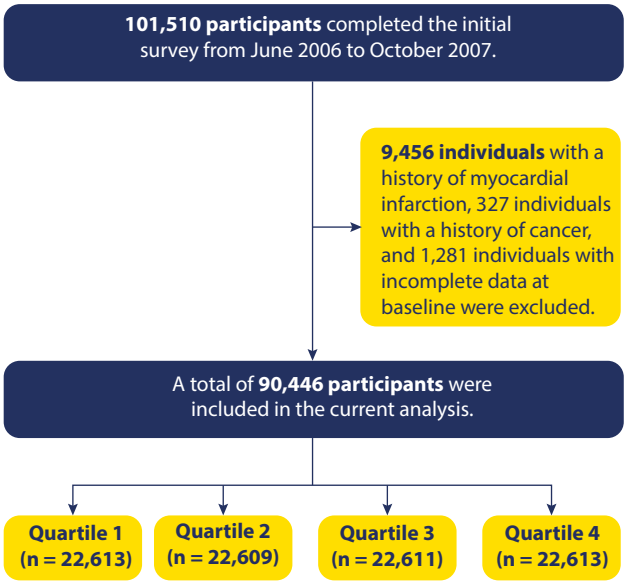


Figure 1. Study flowchart.

DM diagnosis and follow-up

The study endpoint was defined as a new DM diagnosis. The diagnostic criteria employed aligned with the latest diabetes diagnosis criteria (9). Study participants were monitored from the initial assessment until either a new diabetes diagnosis, date of the final physical examination, or death, depending on which event occurred first.

Statistical analysis

All statistical analyses were conducted on SAS version 9.4. Continuous variables are presented as mean ± standard deviation when they conform to a normal distribution; otherwise, they are reported as median and interquartile range. Categorical variables are shown as numbers and percentages. Differences among the Kaplan-Meier estimates for groups were

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assessed using the log-rank test. Cox proportional hazards regression analysis was performed to estimate the hazard ratios (HRs) along with their 95% confidence intervals (CIs) for occurrence of the primary endpoint. A sensitivity analysis was conducted to eliminate the potential influence of reverse causation.

RESULTS

Baseline characteristics

Table 1 presents the baseline characteristics for the 90,446 study participants. Mean age was 51.36 years, with a standard deviation of 12.74 years, and 72,014 (79.62%) participants were male. Patients with a higher TG/HDL-C ratio exhibited elevated levels of body mass index, Scr, hs-CRP, blood pressure, and FPG. Hypertension, hyperlipemia, and chronic kidney disease showed greater prevalence in the sample, as did current smokers and drinkers. Notably, these participants were more likely to use hypotensive drugs and hypolipidemic drugs.

Risk of new-onset DM

During a median follow-up period of 13.67 years, 38,210 cases of new-onset DM were reported. Kaplan-Meier curves indicated that the cumulative DM incidence in quartile 1–4 was 40.95%, 42.04%, 41.42%, and 44.38%, respectively (Figure 2). Additionally, the DM incidence rates in quartile 1–4 were 32.71, 34.10, 33.91, and 37.30 per 1000 person-years (Table 2).

Hazard ratios and 95% confidence intervals

Risk of developing DM increased over time by baseline TG/HDL-C ratio quartiles in univariate model, with

Table 1. Baseline characteristics

Variables	Total (n = 90,446)	Quartile 1 (n = 22,613)	Quartile 2 (n = 22,609)	Quartile 3 (n = 22,611)	Quartile 4 (n = 22,613)	*P* Value
Age, years	51.36 ± 12.74	51.39 ± 13.44	51.33 ± 12.98	51.79 ± 12.58	50.94 ± 11.90	<0.0001
Male, n (%)	72,014 (79.62)	16,756 (74.10)	17,769 (78.59)	18,299 (80.93)	19,190 (84.86)	<0.0001
BMI, kg/m^2	24.93 ± 3.48	23.40 ± 3.23	24.54 ± 3.29	25.46 ± 3.34	26.33 ± 3.34	<0.0001
TC, mmol/L	4.92 ± 1.12	4.83 ± 0.97	4.94 ±1.00	5.04 ± 1.02	4.88 ± 1.43	<0.0001
TG, mmol/L	1.24 (0.88-1.87)	0.70 (0.57-0.85)	1.07 (0.92-1.23)	1.47 (1.25-1.73)	2.63 (2.04-3.74)	<0.0001
HDL-C, mmol/L	1.55 ± 0.40	1.78 ± 0.44	1.58 ± 0.34	1.46 ± 0.33	1.36 ± 0.35	<0.0001
LDL-C, mmol/L	2.34 ± 0.90	2.20 ± 0.92	2.39 ± 0.85	2.43 ± 0.89	2.33 ± 0.93	<0.0001
TG/HDL-C ratio	1.14 ± 1.33	0.41 ± 0.10	0.69 ± 0.08	1.04 ± 0.14	2.40 ± 2.17	<0.0001
Scr, umol/L	91.69 ± 29.41	86.94 ± 23.54	92.72 ± 27.89	92.08 ± 29.83	95.02 ± 34.71	<0.0001
hs-CRP, mg/L	0.80 (0.30-2.23)	0.62 (0.22-2.01)	0.73 (0.29-2.04)	0.88 (0.33-2.30)	1.00 (0.40-2.59)	<0.0001
SBP, mmHg	130.18 ± 20.79	126.28 ± 20.74	129.46 ± 20.66	131.41 ± 20.63	133.58 ± 20.42	<0.0001
DBP, mmHg	83.19 ± 11.71	80.45 ± 11.34	82.65 ± 11.42	83.97 ± 11.65	85.71 ± 11.79	<0.0001
FBG, mmol/L	5.08 ± 0.70	4.96 ± 0.67	5.06 ± 0.67	5.12 ± 0.70	5.17 ± 0.72	<0.0001
Hypertension, n (%)	38,409 (42.47)	7,420 (32.81)	9,213 (40.75)	10,303 (45.57)	11,473 (50.74)	<0.0001
Hyperlipemia, n (%)	52,581 (58.14)	8,016 (35.45)	9,066 (40.10)	13,484 (59.63)	22,015 (97.36)	<0.0001
Chronic kidney disease, n (%)	12,335 (13.64)	2,059 (9.11)	3,545 (15.68)	3,129 (13.84)	3,602 (15.63)	<0.0001
Hypotensive drugs, n (%)	6,682 (7.39)	1,064 (4.71)	1,395 (6.17)	1,861 (8.23)	2,362 (10.45)	<0.0001
Hypolipidemic drugs, n (%)	5,889 (6.51)	925 (4.09)	1,262 (5.58)	1,615 (7.14)	2,087 (9.23)	<0.0001
Income >800 yuan/month, n (%)	12,974 (14.34)	3,503 (15.49)	2,925 (12.94)	3,191 (14.11)	3,355 (14.84)	<0.0001
High education background, n (%)	18,615 (20.58)	5,176 (22.89)	4,241 (18.76)	4,481 (19.82)	4,717 (20.86)	<0.0001
Regular physical exercise, n (%)	82,500 (91.21)	20,777 (91.88)	20,749 (91.77)	20,519 (90.75)	20,455 (90.46)	<0.0001
Current smoker, n (%)	31,367 (34.68)	7,452 (32.95)	7,121 (31.50)	7,918 (35.02)	8,876 (39.25)	<0.0001
Current drinker, n (%)	34,195 (37.81)	8,661 (38.30)	7,621 (33.71)	8,426 (37.27)	9,487 (41.95)	<0.0001

Abbreviations: BMI: body mass index; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein; LDL-C: low density lipoprotein; TG/HDL-C ratio: triglyceride to high-density lipoprotein cholesterol ratio; FBG: fasting blood glucose; Scr: serum creatinine; hs-CRP: high sensitivity C-reactive protein, SBP: systolic blood pressure; DBP: diastolic blood pressure.

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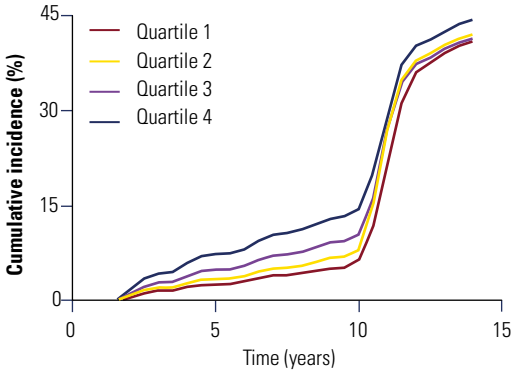


Figure 2. Cumulative incidence of new-onset DM in different quartiles.

hazard ratios (HRs) of 1.072 (95% CI: 1.042–1.103), 1.061 (95% CI: 1.031–1.092), and 1.177 (95% CI: 1.145–1.211) for quartiles 2, 3 and 4, respectively (*P* for trend < 0.001). After adjustment for potential confounding factors, the HRs reached 1.058 (95% CI: 1.028-–.089), 1.043 (95% CI: 1.012–1.075), and 1.118 (95% CI: 1.080–1.156) for quartiles 2, 3 and 4, respectively (*P* for trend < 0.001) (**Table 2**). The results remained consistent across three sensitivity analyses (**Table 3**).

Table 2. Risk and hazard ratios for new-onset DM

	Case/Total	Incidence rate, per 1000 person-years (%)	Model 1	Model 2	Model 3
Quartile 1	9,271/22,613	32.71	1	1	1
Quartile 2	9,521/22,609	34.10	1.072 (1,042-1,103)	1.104 (1,073-1,136)	1.058 (1,028-1,089)
Quartile 3	9,380/22,611	33.91	1.061 (1,031-1,092)	1.114 (1,082-1,146)	1.043 (1,012-1,075)
Quartile 4	10,038/22,613	37.30	1.177 (1,145-1,211)	1.219 (1,185-1,255)	1.118 (1,080-1,156)
P for trend			<0.001	<0.001	<0.001

Model 1 was unadjusted. **Model 2** was adjusted for age and sex. **Model 3** was adjusted for age, sex, body mass index, total cholesterol, low-density lipoprotein cholesterol, serum creatinine, high sensitivity C-reactive protein, systolic blood pressure, diastolic blood pressure, history of hypertension, history of hyperlipemia, history of chronic kidney disease, hypotensive drugs usage, hypolipidemic drugs usage, income level, education level, physical activity, status of smoking, and status of drinking.

Table 3. Sensitivity analysis

	Model 1	Model 2	Model 3
Analysis 1			
Quartile 1	1	1	1
Quartile 2	1.066 (1.036-1.098)	1.101 (1.069-1.133)	1.058 (1.028-1.090)
Quartile 3	1.047 (1.017-1.078)	1.102 (1.070-1.135)	1.038 (1.007-1.070)
Quartile 4	1.148 (1.115-1.181)	1.193 (1.159-1.228)	1.102 (1.065-1.141)
P for trend	<0.001	<0.001	<0.001
Analysis 2			
Quartile 1	1	1	1
Quartile 2	1.073 (1.042-1.105)	1.105 (1.072-1.138)	1.044 (1.013-1.076)
Quartile 3	1.065 (1.034-1.097)	1.115 (1.082-1.149)	1.027 (0.995-1.059)
Quartile 4	1.173 (1.138-1.208)	1.213 (1.177-1.249)	1.085 (1.047-1.124)
P for trend	<0.001	<0.001	<0.001
Analysis 3			
Quartile 1	1	1	1
Quartile 2	1.071 (1.042-1.102)	1.104 (1.073-1.135)	1.058 (1.028-1.089)
Quartile 3	1.061 (1.032-1.092)	1.114 (1.082-1.146)	1.043 (1.012-1.075)
Quartile 4	1.177 (1.145-1.211)	1.219 (1.185-1.255)	1.118 (1.080-1.156)
P for trend	<0.001	<0.001	<0.001

Model 1 was unadjusted. **Model 2** was adjusted for age and sex. **Model 3** was adjusted for age, sex, body mass index, total cholesterol, low-density lipoprotein cholesterol, serum creatinine, high sensitivity C-reactive protein, systolic blood pressure, diastolic blood pressure, history of hypertension, history of hyperlipemia, history of chronic kidney disease, hypotensive drugs usage, hypolipidemic drugs usage, income level, education level, physical activity, status of smoking, and status of drinking.
Analysis 1 excluded participants experienced DM during the first 2 years of follow-up. **Analysis 2** excluded participants experienced atherosclerotic cardiovascular diseases during follow-up. **Analysis 3** excluded all-cause death during follow-up.

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DISCUSSION

Our study confirmed a significant association between the TG/HDL-C ratio and the risk of new-onset DM. Risk of developing DM increased over time according to the baseline TG/HDL-C ratio quartiles. Moreover, the association between TG/HDL-C ratio and DM onset persisted after adjusting for potential confounding variables, with the results remaining consistent across sensitivity analyses.

Similarly, Chen and cols. performed a retrospective cohort study involving 114,787 adults from the Rich Healthcare Group in China. Their results indicated a positive correlation between the TG/HDL-C ratio and DM risk, with a HR of 1.159 (95% CI: 1.104, 1.215). Additionally, they identified a non-linear relation between the TG/HDL-C ratio and DM onset, with an inflection point at a TG/HDL-C ratio of 1.186 (10). Several studies explored the association between TG/HDL-C ratio and DM risk across different genders. In this context, Kim and cols. examined data from 80,693 individuals within the Korean NHIS-HEALS cohort database, revealing that an elevated TG/HDL-C ratio was significantly correlated to an increased risk of new-onset DM in both men and women. Specifically, compared with the first tertile, the HRs (95% CIs) for new-onset DM in the second and third tertiles reached 1.17 (1.06–1.30) and 1.47 (1.34–1.62) for men, and 1.20 (1.02–1.42) and 1.52 (1.30–1.78) for women, respectively (11). Conversely, Qin and cols. analyzed data from 116,855 individuals and found that a higher TG/HDL-C ratio was significantly associated with DM incidence only in men, with a HR of 1.30 (95% CI: 1.03–1.64) (12).

Since type 2 diabetes mellitus (T2DM) is the most common form of DM (13,14), several studies have investigated the association between TG/HDL-C ratio and the risk of T2DM. Liu and cols. performed a longitudinal retrospective cohort study involving 2,571 participants, finding that higher TG/HDL-C ratio quintiles were associated to increased HRs for T2DM onset compared with the lowest quintile, with HRs of 1.35 (95% CI, 0.85–2.17), 1.31 (0.83–2.06), 1.85 (1.20–2.85), and 2.10 (1.38–3.20), respectively. Additionally, they identified a non-linear relation between the TG/HDL-C ratio and T2DM risk, with the curve slope diminishing after reaching a cutoff point of 2.54 (15). Zheng and cols. examined data from 1,460 participants in the Beijing Longitudinal Study, revealing HRs (95% CI) of 1.90 (1.12–3.23), 2.75 (1.58– 4.80), and 2.84 (1.69– 4.77) for TG/HDL-C ratios of 0.87–1.30, 1.31–1.74, and ≥1.75, respectively, in comparison to those with TG/HDL-C ratios below 0.87 (16). Wang and cols. analyzed 15,453 Japanese individuals in a cohort study, finding a positive correlation between the baseline TG/HDL-C ratio and T2DM the risk, with a HR of 1.19 (95% CI, 1.09–1.30). Smoothed curve fitting and two-stage linear regression analysis indicated a J-shaped relationship between the baseline TG/HDL-C ratio and T2DM risk. Additionally, a baseline TG/HDL-C ratio exceeding 0.35 was positively correlated with T2DM development, yielding a HR of 1.2 (95% CI, 1.10–1.31) (17). Liu and cols. analyzed data from 7,791 participants in the REACTION cohort study, finding a positive association between TG/HDL-C ratio and T2DM risk, with an odds ratio (OR) of 1.49 (95% CI, 1.26–1.78) (18).

Prediabetes is identified by blood glucose levels that exceed normal ranges yet fall short of the criteria for diabetes diagnosis, and signifies an elevated risk for DM progression (19). Sun and cols. conducted a study involving 15,017 individuals diagnosed with prediabetes. During a median follow-up period of 3.05 years, 1,731 participants (11.46%) were eventually diagnosed with DM. Their results revealed a significant association between TG/HDL-C ratio and DM development in prediabetic individuals (HR = 1.111, 95% CI: 1.061, 1.164). Those with the highest TG/HDL-C ratios exhibited an increased risk of DM progression compared with those with the lowest ratios (HR = 1.397, 95% CI: 1.200, 1.627) (*P* for trend < 0.001) (20).

Gestational diabetes mellitus (GDM) constitutes one of the most prevalent complications during pregnancy (21). Several studies have specifically focused on the correlation between TG/HDL-C ratio and GDM. Wang conducted an analysis involving 636 women with singleton pregnancies, revealing a GDM prevalence of 17.30% (n = 110). He found that the TG/HDL-C ratios were significantly elevated in the GDM group (1.24 [0.96–1.81]) compared with non-GDM (1.04 [0.80–1.39]) (*P* < 0.01). Additionally, the

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TG/HDL-C ratio was independently associated with GDM risk (OR = 1.64, *P* = 0.02) (22). You and cols. conducted a secondary analysis involving 590 women with singleton pregnancies, indicating a positive association between TG/HDL-C ratio and GDM incidence (OR = 1.77, 95% CI: 1.32–2.38, *P* = 0.0001). Their study also found that the TG/HDL-C ratio serves as a reliable GDM predictor, with an area of 0.7863 (95% CI: 0.7090–0.8637) under the receiver operating characteristic curve. Optimal cut-off value for TG/HDL-C ratio in detecting GDM was 2.2684, yielding a sensitivity of 72.97% and a specificity of 75.05% (23).

In conclusion, a higher TG/HDL-C ratio is strongly associated with new-onset DM development in non-diabetic individuals. Our research indicates that managing and maintaining the TG/HDL-C ratio may be helpful in lowering the risk of developing T2DM.

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Ethics statement: the project protocol was approved by the ethics committee of the Ethics Committee of the Kailuan Medical Group and was approved by the guidelines of the Helsinki Declaration, and all study individuals in this project signed an informed consent form at enrollment.

Disclosure: no potential conflict of interest relevant to this article was reported.

Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

Prognostic factors and survival in parathyroid carcinoma: a 50-year single-center cohort study

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ABSTRACT

Objective: This study aims to analyze the clinical characteristics and prognostic factors of patients with parathyroid carcinoma (PC) treated at a tertiary institution. **Subjects and methods:** This retrospective cohort included 30 patients with PC who were treated between 1970 and 2023 at a single reference center. Clinical, pathological, and survival data were reviewed and prognostic factors were evaluated. **Results:** Of the 30 cases, 16 (53.3%) were women. Mean age at diagnosis was 45.9 years, median follow-up was 60 months and mean tumor size was 33 mm. Median follow-up was 60 months. Disease recurrence occurred in 16 (53.3%) cases, with disease persistence in three (10.0%). Median disease-free survival was 80 months, with a 57% overall survival rate. Neither gender nor age showed significant impact on survival. Patients with lower-stage tumors (T1 by the AJCC/UICC and T1+T2 by the Shaha classification) had significantly better overall survival ($p = 0.004$ and $p = 0.0018$, respectively) and disease-free survival ($p = 0.03$ and $p = 0.04$, respectively) than those with more advanced stages ($p = 0.03$ and $p = 0.04$, respectively). Additionally, patients who experienced tumor recurrence after 36 months had significantly better survival outcomes than those with earlier recurrences ($p = 0.02$). **Conclusion:** Advanced tumor stage (T3 by the Shaha or T2 and above by the AJCC/UICC) is associated with higher mortality in patients with PC. Furthermore, a disease-free interval exceeding 36 months emerged as a relevant factor associated with improved overall survival.

Keywords: Parathyroid carcinoma; survival rate; prognostic factors; disease-free survival

INTRODUCTION

Parathyroid carcinoma (PC) is a rare malignancy, accounting for less than 1% of primary hyperparathyroidism cases, with five- and 10-year mortality rates that may reach from about 80% to 50%, respectively (1,2). It continues to significantly challenge its diagnosis, treatment, classification, and staging.

Differentiating between benign and malignant parathyroid disease can often be difficult from a clinical, radiological, and even histological perspective due to the considerable overlap between these neoplasms. As a result, a definitive diagnosis is frequently established only postoperatively or in case of pathological evidence of metastatic disease. Nevertheless, suspicion of PC can be raised preoperatively based on specific clinical and laboratory findings (3). This distinction is critical as the management of these conditions differs significantly, and preoperative biopsy is generally contraindicated due to the risk of tumor seeding, except in cases with documented metastatic disease. Therefore, in cases with suspected PC, *en bloc* resection (removal of the parathyroid gland along with the ipsilateral thyroid lobe and central neck compartment

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dissection) or, at minimum, resection with negative margins is recommended (4).

Several diagnostic classifications for PC have been proposed over the years. In 1973, Schantz and Castleman described a set of histological criteria for PC diagnosis, including capsular or vascular invasion, the presence of fibrous bands, mitotic figures, and a trabecular growth pattern (5). Currently, the diagnostic gold standard relies on histopathological features such as vascular, lymphatic, and/or perineural invasion, local invasion into adjacent structures, or metastasis (6).

The limited data available regarding the biological behavior and prognosis of PC has hindered the production of an universally accepted staging system that reliably predicts disease-specific survival. However, with the prospective recording and evaluation of new prognostic variables, a formal staging system may be developed in the near future (7).

Thus, the rarity of this disease continues to limit the definition of its diagnostic criteria and prognostic factors, which remain under construction via retrospective analyses. Reporting new case series and their clinical outcomes is crucial for advancing this knowledge.

This study aims to find potential prognostic factors — including clinical, laboratory, and histopathological characteristics — associated with PC in a single academic referral center dedicated to managing this disease.

SUBJECTS AND METHODS

This retrospective cohort study was carried out at *Hospital das Clínicas* at *Faculdade de Medicina da Universidade de São Paulo* (HCFMUSP) — an university public hospital and a reference center for parathyroid disease — with patients who were treated for PC between January 1970 and April 2023.

Demographic data, histopathological features from postoperative pathological analysis, timing, type and outcome of treatment, and use of complementary or adjuvant therapies were evaluated. The following laboratory parameters were analyzed: total serum calcium (CaT) (reference range: 8.4–10.2 mg/dL), ionized calcium (4.49–5.29 mg/dL), and parathyroid hormone (PTH) (15–68.3 pg/mL).

Inclusion criteria comprised patients who had clinical follow-up and/or underwent surgical procedures at the institution during the study period whether as initial treatment or for recurrence. Only patients whose main treatment modality was surgery were included, even if they underwent adjuvant or complementary treatments (such as chemotherapy, radiotherapy, immunotherapy, and/or image-guided interventional ablation), regardless of clinical and/or pathological stage.

In cases in which malignancy was suspected prior to the initial procedure, *en bloc* resection was the recommended surgical approach. This was defined as parathyroidectomy combined with ipsilateral thyroid lobectomy and ipsilateral central neck dissection, preferably performed as a single, uninterrupted specimen.

The diagnosis of PC was based on one or more of the following histopathological criteria: invasion of adjacent structures, unequivocal vascular invasion, presence of metastases, or a previous histopathological diagnosis of PC with recurrence. Atypical tumors or cases with inconclusive findings were excluded from this cohort. All pathology reports were re-evaluated during this study to standardize pathological staging, using the eighth edition of the TNM classification system from the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) (7), in addition to the system proposed by Shaha. (8) The TNM classification is defined as follows: the primary tumor (T) may be unassessable (Tx) or absent (T0); Tis denotes an atypical parathyroid neoplasm of uncertain malignant potential; T1 tumors are confined to the parathyroid gland with limited soft tissue extension; T2 indicates invasion into the thyroid; T3 corresponds to invasion into the recurrent laryngeal nerve, trachea, esophagus, skeletal muscle, thymus, or adjacent lymph nodes; and T4 represents invasion into major blood vessels or the spine. Regarding regional lymph nodes (N), Nx indicates unassessable nodes; N0, the absence of regional lymph node metastasis; and N1, the presence of such metastases, subdivided into N1a (central or superior mediastinal nodes) and N1b (lateral cervical or retropharyngeal nodes). For distant metastasis (M), M0 denotes absence and M1, the presence of distant

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spread. Currently, there are insufficient data to establish an anatomical or prognostic staging system for parathyroid carcinoma. On the other hand, According to Shaha's classification, the staging system for parathyroid carcinoma is defined as follows: T–Tx: not defined by the author; T1: primary tumor <3 cm; T2: primary tumor >3 cm; T3: tumor of any size with invasion of adjacent soft tissues (such as the thyroid or striated muscle); and T4: extensive central compartment disease invading the trachea or esophagus, or recurrent parathyroid carcinoma. N–Nx: not defined by the author; N0: no regional lymph node metastasis; and N1: presence of regional lymph node metastasis. M–Mx: not defined by the author; M0: no evidence of distant metastasis; and M1: evidence of distant metastasis. Based on these parameters, Stage I corresponds to T1N0M0, Stage II to T2N0M0, Stage IIIa to T3N0M0, Stage IIIb to T4N0M0, Stage IIIc to any T, N1M0, and Stage IV to any T, any N, M1.

For patients who underwent initial surgery at external institutions, a paraffin block review was performed by the same pathology team at HCFMUSP whenever possible to maximize the standardization of histopathological assessment. In cases in which this was unfeasible due to unavailable material, specimens from subsequent surgeries were evaluated by the same team to confirm the diagnosis of PC.

Additional variables included age at diagnosis, symptom duration at diagnosis, tumor size and volume, serum calcium and PTH levels at diagnosis and during follow-up, total follow-up time, and time to any recurrence or persistence of disease.

The data were collected and treated on REDCap (Research Electronic Data Capture), hosted at HCFMUSP (9,10).

Statistical analyses were performed on GraphPad Prism, version 10.5 (GraphPad Software, San Diego, CA, USA). The distribution of continuous numerical variables was tested by the Kolmogorov-Smirnov test. Variables with normal distribution are shown as means with minimum and maximum values or standard deviations and compared using Student's *t*-test or ANOVA. Non-normally distributed variables are shown as medians with first (Q1) and third (Q3) interquartile ranges and analyzed by the Mann-Whitney or Kruskal-Wallis tests, as appropriate. Categorical variables are shown as frequencies and compared by the chi-squared or Fisher's exact tests, when applicable. Survival analysis was performed using Kaplan-Meier curves and compared by the log-rank (Mantel-Cox) test. A significance level of 5% was adopted for all p-values.

This study was approved by the Institutional Review Board (Research Ethics Committee of HCFMUSP) and registered at the National Ethics Committee on Human Research under protocol number CAAE 29266720.9.0000.0068.

RESULTS

The study sample consisted of 30 cases. **Table 1** summarizes their characteristics.

This study observed no statistically significant differences when comparing sex with overall survival (Log-rank, p-value = 0.55) or disease-free survival (Log-rank, p-value = 0.74).

Table 1. Patients' demographic and clinical data

Sex		
Male	14	46.7%
Female	16	53.3%
Age at diagnosis	Mean = 45.9 years	Min–Max = 17–64 years
Time from symptoms to diagnosis	Mean = 24.7 months	Min–Max = 1–96 months
Size (largest diameter) of the tumor	Mean = 36 mm	Min–Max = 15–77 mm
Tumor volume	Median = 6.5 cm^3	Q1–Q3 = 3.3–8.0 cm^3
Time to recurrence (locoregional and distant)	Median = 12 months	Q1–Q3 = 8.5–54.0 months
Time to locoregional recurrence	Median = 12 months	Q1–Q3 = 7–36 months
Follow-up time	Median = 60 months	Q1–Q3 = 15.0–95.5 months

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This research analyzed patients in two groups: those younger and those older than 45 years of age (a cut-off chosen based on the mean age of the cohort). Serum calcium, PTH levels, and time to recurrence showed no statistically significant differences between these groups. Although younger patients had smaller tumors, this failed to impact survival outcomes. This research observed no statistically significant difference between these age groups regarding disease-specific mortality versus being alive or deceased from other causes (Fisher's exact test, p-value = 0.69). Similarly, it found no significant difference for these outcomes between genders (Fisher's exact test, p-value = 0.69). The comparison of disease-free survival between age groups found no relevant associations (Log-rank, p-value = 0.66).

The median disease-free survival totaled 80 months, after which the overall survival rate equaled 57% (**Figure 1**).

The mean total CaT and PTH levels (with ranges) totaled 14.5 mg/dL (11–19 mg/dL) and 1,470 pg/mL (158–3,654 pg/mL), respectively. Higher serum calcium levels showed no association with statistically significant impact on survival. In the gender-based analysis, no assessed variable reached statistical significance, although CaT levels tended to be slightly higher among men.

This study found no correlation between tumor size (greater or smaller than 33 mm) and overall (Log-rank, p-value = 0.96) or disease-free survival (Log-rank, p-value = 0.57).

Regarding mortality (death from disease vs. not deceased from disease by the end of follow-up), this study found no correlation with vascular invasion (Fisher's exact test, p-value = 0.55). Similarly, we observed no statistically significant correlation between vascular invasion and overall (Log-rank, p-value = 0.21) or disease-free survival (Log-rank, p-value = 0.34), despite an apparent trend.

For AJCC/UICC staging, **Table 2** summarizes the distribution by T and N stages. Regarding distant metastasis, 28 patients (93.3%) had no evidence of distant metastases (M0), whereas only two (6.7%) showed systemic disease (M1). Statistical analysis compared patients with T1 and T2 tumors, excluding T3 cases and those with distant metastasis at diagnosis due to the small sample size for proper statistical inference. This comparison found a significant difference for mortality (Fisher's exact test, p-value = 0.004) and disease-free survival (Log-rank, p-value = 0.03) (**Figure 2**).

This study also evaluated patients according to the Shaha staging system (**Table 2**). It excluded four patients due to insufficient diagnostic information for accurate staging classification (such as maximum tumor size). This staging model, grouping T1 and T2 together (due to sample size) versus T3, observed a statistically significant difference between the groups,

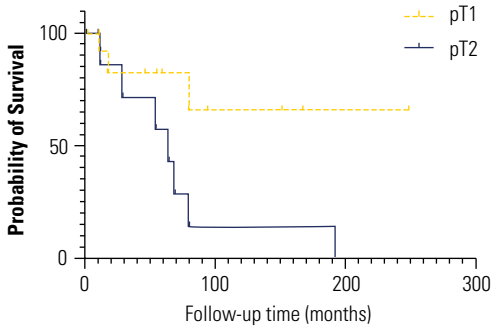


Figure 2. Kaplan-Meier curve for disease-free survival among patients with T1 and T2 stages in the AJCC/UICC classification.

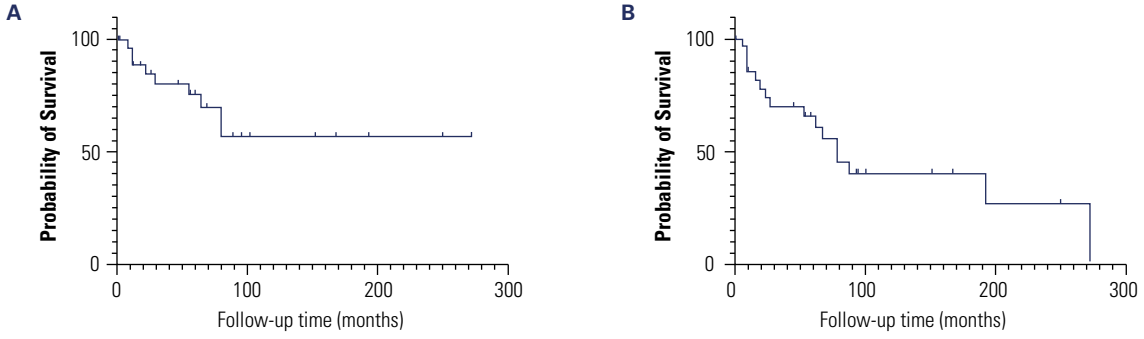


Figure 1. Kaplan-Meier curve for (**A**) overall survival and (**B**) disease-free survival in patients diagnosed with parathyroid carcinoma.

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with higher mortality (Fisher's exact test, p-value = 0.0018) and shorter disease-free survival (Log-rank, p-value = 0.04) in the latter group (**Figure 3**).

Finally, this study analyzed the impact of time to recurrence on survival regardless of whether the recurrence was locoregional or distant. Considering only patients who experienced recurrence at any point during follow-up and applying temporal cut-offs at 12 and 24 months, we found no statistically significant difference in mortality between these groups (Log-rank, p-value = 0.06 and 0.10, respectively). However, analysis of the 36-month time point found a statistically significant difference in mortality (Log-rank, p-value = 0.02), indicating lower survival among patients who recurred within this period (**Figure 4**).

Table 2. Distribution of cases by T and N staging according to the AJCC/UICC and the classification proposed by Shaha

	AJCC/UICC	Shaha
Initial T		
T1	15 (50.0%)	9 (34.6%)
T2	8 (26.6%)	5 (19.2%)
T3	3 (10.0%)	9 (34.6%)
T4	0 (0.0%)	2 (7.7%)
Tx	4 (13.3%)	Not defined by the authors
Initial N		
N0	29 (93.3%)	25 (96.1%)
N1	1 (3.3%)	1 (3.8%)
Nx	0 (0.0%)	Not defined by the authors
Staging		
I	Not defined by the authors	9 (34.6%)
II	Not defined by the authors	5 (19.2%)
IIIa/b	Not defined by the authors	10 (38.5%)
IV	Not defined by the authors	2 (7.7%)

DISCUSSION

Several studies using population-based databases have investigated prognostic factors in patients with PC. Sadler and cols. analyzed a cohort of patients diagnosed with PC over the years and found that the

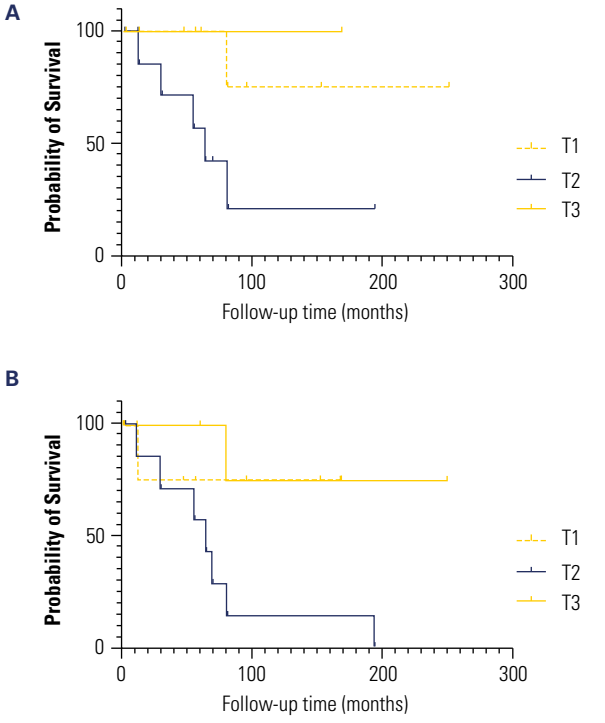


Figure 3. Kaplan-Meier curves for (**A**) overall survival and (**B**) disease-free survival among patients with T1, T2, and T3 stages in the Shaha classification.

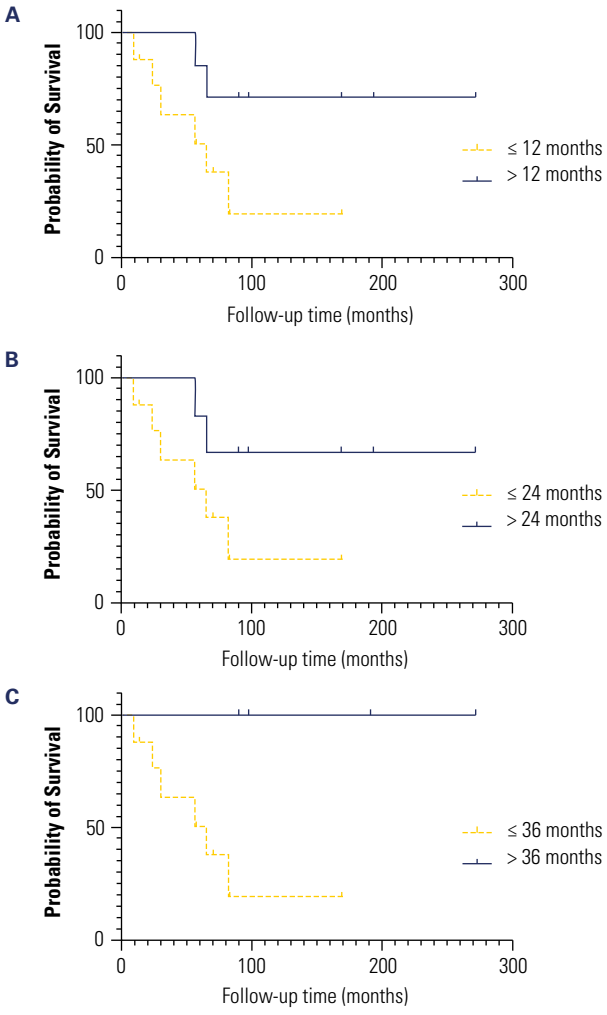


Figure 4. Kaplan-Meier curves for overall survival of patients who showed recurrence during follow-up according to the time separation point at which they occurred: (**A**) 12, (**B**) 24, and (**C**) 36 months.

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presence of metastatic lymph nodes at diagnosis and age were associated with disease survival (11). Similarly, Asare and cols., analyzing the same population-based dataset, observed an association between age, sex, and tumor size with survival in patients with PC (12). However, this cohort showed no such associations, probably due to its small sample size.

Ohkuwa and cols., in a recent single-center study with a patient number comparable to ours, found three predictive factors for recurrence and prognosis in patients with PC: Ki-67 index, weight of the affected gland, and presence of necrosis on histopathological examination (13). According to the authors, a Ki-67 index ≥4.1%, gland weight ≥4,890 mg, and the presence of necrosis may be associated with distant metastasis and increased recurrence risk.

The literature has no consensus on tumor size as a prognostic factor in PC. Hsu and cols. concluded that a tumor size ≥3 cm is associated with lymph node metastasis (14). Additionally, Asare and cols. correlated tumors ≥4 cm with increased mortality risk (12). Qian and cols., in a multicenter study using surveillance and epidemiological data from 604 PC patients, confirmed that tumors ≥3.5 cm were associated with worse overall survival (15). However, Ohkuwa and cols., in a smaller study, failed to confirm this association (13). Similarly, Lee and cols. also found no relationship between tumor size and survival (16). The HCFMUSP cohort in this study was also unable to establish such associations.

To address the need for standardized classification and subgrouping of patients based on prognosis (staging), several systems have been proposed over time. Notable among them are those proposed by Shaha and Shah (8), Talat and Schulte (17,18), and more recently, the AJCC/UICC TNM staging system (7).

Despite many proposed staging systems, all still require validation before they can be widely adopted. The TNM system (AJCC/UICC), for example — widely used for many cancer types — currently includes no detailed PC subclassifications within stages, mainly due to insufficient data. The Shaha system, on the other hand, differs by incorporating tumor size. The Schulte system stratifies prognosis based on histopathological features, such as vascular invasion. Thus, while all these systems aim to stratify patients prognostically, they emphasize different disease characteristics. Validation and analysis of such systems in various cohorts and for distinct clinical or pathological variables are essential to strengthen existing models and contribute to the overall understanding of the disease.

Within our cohort, comparing T1 and T2 tumors (excluding patients with M1 disease at diagnosis) according to the AJCC/UICC TNM system obtained a significant association with survival, confirming that tumors extending beyond the parathyroid gland are linked to worse survival outcomes.

The analysis using the Shaha system grouped T1 and T2 cases as the limited number of patients and the relatively short follow-up period made statistically significant analysis challenging. Nevertheless, this study observed that patients with early-stage tumors (T1 and T2) had better overall and disease-free survival than those with more advanced tumors (T3) by this classification.

A widely used histopathological parameter to predict prognosis in other head and neck cancers refers to the presence of vascular invasion. Although this is often a decisive prognostic criterion in other tumor sites, this analysis observed no such association. This may be because vascular invasion is a diagnostic criterion for PC, and the qualitative assessment — classified simply as present or absent — without quantification in this study may have limited its prognostic evaluation. This, for example, hindered a more robust assessment of the Schulte system. Perhaps, applying a grading system for vascular invasion (e.g., invasion in ≤5 *foci versus* >5 *foci*) would have found a significant association with disease-free survival.

Another prognostic factor, first highlighted by Schantz and Castleman in 1973 (5), is the time to recurrence. However, due to small sample sizes in most series, some authors have been unable to corroborate this finding. Therefore, while clinically relevant, this aspect remains controversial. Despite having been suggested decades ago and representing an important biological marker of PC aggressiveness, the relationship between disease-free interval and mortality has been insufficiently investigated in recent years.

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A study reviewing 47 years of institutional data, including 37 cases from 1966 to 2009, found no association between mortality and time to first recurrence. Instead, it associated mortality with the presence of lymph node or distant metastases, higher calcium levels at recurrence, and higher levels of hypocalcemia-inducing medications (19). Conversely, a 2022 literature review with studies from 1966 to 2019, including a total of 73 patients, suggested that disease-free interval was indeed a survival predictor (20).

Similarly, a previous smaller cohort from the same institution has shown that the time between initial treatment and development of recurrent disease (locoregional or distant) negatively affected overall survival rates (21). The difference was marginal and statistically insignificant at 24 months but became clearly significant for recurrences within 36 months, resulting in poor survival (five-year median). This expanded cohort corroborated this finding. The evaluation of survival regarding time to disease recurrence (locoregional or distant) observed no significant (although borderline) relationship with mortality when recurrence occurred before or after 12 or 24 months. However, the analysis of recurrence within 36 months of the initial treatment confirmed a statistically significant association with mortality. This result reinforces the idea that time to recurrence is related to patient survival, especially when recurrence occurs within the first three years of follow-up. The possible explanation is that PC may have different molecular mechanisms and biological behaviors.

Although the occurrence of parathyroid carcinoma is relatively uncommon, HCFMUSP has gained substantial experience in managing these patients over a 50-year period. Despite the challenges inherent to evaluating such a rare condition, the surgical approach and its outcomes, along with the underlying principles, have remained largely consistent from the beginning of the cohort to the present day (21,22). From the outset, the recommended management for these patients has been *en bloc* resection to maximize the likelihood of cure and effective treatment. Over time, however, greater experience has been achieved in identifying patients with an increased suspicion of malignancy, enabling more oncologically appropriate therapeutic strategies.

The clinical implications of these findings lie in the potential use of staging and recurrence timing as practical tools for risk stratification and patient counseling. By integrating these parameters into clinical decision-making, physicians in referral centers may enhance follow-up strategies and provide more accurate prognostic information to patients with PC.

Despite representing one of the larger single-center PC cohorts in the literature — especially given the rarity of the disease — this study has certain limitations. First, its small sample size limited its ability to perform robust analyses. Although its small sample size represents an inherent limitation due to the rarity of parathyroid carcinoma, these data contribute valuable clinical insights that may support future meta-analyses or multicenter studies aimed at increasing statistical power and refining treatment strategies. The fact that the cohort comes from a single institution and that some initial surgeries were performed elsewhere adds to this limitation. Moreover, due to the general lack of knowledge about the disease, no standardized treatment protocol was consistently applied, with management strategies varying according to the attending team's judgment over a long observation period. Although inherent to the long study period and the rarity of parathyroid carcinoma, this study mitigated such variability by applying adequate surgical and pathological criteria throughout the cohort. Future efforts should aim to develop standardized treatment protocols or even to set up a national registry to facilitate comparisons across institutions and improve patient outcomes. Finally, the lack of centralized histopathological review — particularly for patients initially treated at external centers — hindered a more uniform pathological assessment (81% of the cases had pathology slides available from either primary or subsequent operations for histopathological re-evaluation).

In conclusion, PC presents with heterogeneous biological behavior, with some patients surviving for decades and an indolent disease course in a significant proportion of cases. It is associated with potentially severe clinical findings and carries a considerable risk of recurrence, including the possibility of late metastases. Additionally, patients diagnosed with

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T3 (according to the Shaha classification) or T2 and above PC (according to the 2017 AJCC/UICC TNM staging system) have higher mortality than those with earlier stages of the disease. Furthermore, a disease-free interval longer than 36 months proved to be a relevant factor associated with improved overall survival. These findings, derived from one of the largest Brazilian referral centers, add valuable information to the limited global literature on this rare malignancy and underscore the importance of further multicenter and collaborative studies to refine prognostic assessment and management.

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original

Timing of 17-hydroxyprogesterone measurement during the standard-dose Synacthen test in pediatric patients evaluated for non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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ABSTRACT

Objective: We aimed to evaluate the diagnostic utility of the 30-minute 17-hydroxyprogesterone (17-OHP) measurement during the standard-dose Synacthen test. **Subjects and methods:** This retrospective study analyzed the medical records of patients aged 0–18 years who underwent Synacthen testing for suspected non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency between 2000 and 2024. **Results:** Among 150 patients included, the median age was 13 years (range 5.5–18), and 85.3% were female. Twenty-nine patients exhibited a peak stimulated 17-OHP level ≥ 10 ng/mL. In three of these patients, the 30-minute 17-OHP level was within normal limits, whereas all had diagnostic 17-OHP levels at 60 minutes. No patients exhibited a diagnostic 17-OHP level at 30 minutes with a non-diagnostic value at 60 minutes. Using a basal 17-OHP cut-off value of 3.78 ng/mL, sensitivity and specificity were 89.7% and 94.2%, respectively. The V281L variant was the most frequently identified pathogenic variant. **Conclusion:** The 60-minute 17-OHP measurement demonstrated greater diagnostic sensitivity than the 30-minute measurement in this cohort and may provide adequate diagnostic information in pediatric patients evaluated for non-classical congenital adrenal hyperplasia, as no additional cases were identified exclusively at the 30-minute time point.

Keywords: 17-hydroxyprogesterone; Synacthen; congenital adrenal hyperplasia; 21-hydroxylase deficiency

INTRODUCTION

The short (standard-dose) Synacthen test, also known as the corticotropin stimulation test, is the gold standard for diagnosing primary adrenal insufficiency. Serum cortisol and 17-hydroxyprogesterone (17-OHP) levels are analyzed at baseline and at 30 and/or 60 minutes following corticotropin administration (125 µg for patients < 2 years of age and 250 µg for those >2 years of age), preferably as an intravenous bolus, with a peak cortisol level <18 µg/dL indicating adrenal insufficiency (1,2). A baseline or stimulated 17-OHP concentration >100 ng/mL is diagnostic for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21-OHD-CAH). In the non-classical form (NC-CAH), basal 17-OHP is generally between 2 and 100 ng/mL (2). However, the assessment of basal, 30-minute, and/or 60-minute cortisol and 17-OHP concentrations using the standard-dose Synacthen test when 17-OHP is < 100 ng/mL is an established practice used to confirm the diagnosis of 21-OHD-CAH (2). Several publications recommend measuring cortisol and 17-OHP at 0 and 60 minutes in the Synacthen test, while in the United Kingdom, sampling at 30 minutes is also recommended (3).

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Comparison of basal and stimulated 17-OHP levels usually allows differentiation between normal individuals, heterozygotes, patients with NC-CAH, and patients with classical CAH, though overlapping values exist across these groups (4). A basal 17-OHP < 2 ng/mL and a stimulated 17-OHP < 10 ng/mL exclude CAH with high probability, while a stimulated 17-OHP of 10–100 ng/mL supports the diagnosis of 21-OHD NC-CAH (2,4).

Several studies have evaluated the adequacy of the 60-minute cortisol value alone in demonstrating cortisol insufficiency during short or standard-dose Synacthen tests, finding the 60-minute value sufficient (5–7). To our knowledge, no studies in children compare 17-OHP levels at 30 and 60 minutes in the standard-dose Synacthen test for diagnosing 21-OHD CAH. Therefore, this study sought to assess the diagnostic contribution of the 30-minute 17-OHP measurement compared with the 60-minute value in pediatric patients undergoing the standard-dose Synacthen test for suspected 21-OHD non-classical congenital adrenal hyperplasia (NC-CAH). Omitting the 30-minute measurement, while not reducing total test duration, could decrease the number of blood samples, thereby improving patient comfort and reducing costs.

SUBJECTS AND METHODS

In this retrospective study, we analyzed the medical records of patients aged 0–18 years who were admitted to the Pediatric Endocrine Departments at İnönü University, Cumhuriyet University, and Dicle University Medical Faculties between 2000 and 2024. Included patients presented with hirsutism, early axillary and/or pubic hair growth, menstrual irregularities, acne, a basal laboratory 17-OHP level ≥ 2 ng/mL, and/or advanced bone age, and underwent Synacthen testing with a pre-diagnosis of 21-OHD NC-CAH. Classical forms (salt-wasting or simple virilizing) of 21-OHD were excluded. Salt wasting CAH due to 21-hydroxylase deficiency typically presents early in life and is fatal if untreated. The simple virilizing form may present with pronounced bone age advancement, clitoromegaly in girls, and macrogenitalia in boys. Patients with clinical and genetic features of classical 21-OHD-CAH were excluded.

Patients with other enzymatic defects, such as 11β-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase deficiency, or POR deficiency, may have elevated 17-OHP levels. Individuals with symptoms indicative of these defects (e.g., hypertension, ambiguous genitalia, skeletal deformities, elevated 11-deoxycortisol) and/or genetic results inconsistent with 21-OHD NC-CAH were excluded. Blood sampling for adrenal hormone analysis was performed at 08:00. In menstruating girls, samples were taken during the early follicular phase (between days 3 and 5 after the onset of spontaneous bleeding). None of the patients had taken medication potentially interfering with hormone testing, such as oral contraceptives, gonadotropin-releasing hormone analogues, metformin, or cortisone, for at least three months prior to sampling.

Basal cortisol and 17-OHP levels were measured at baseline. A 250 µg intravenous bolus of Synacthen was then administered, and stimulated cortisol and 17-OHP concentrations were measured at 30 and 60 minutes post-administration. 17-OHP and cortisol levels were analyzed using the MAGLUMI 2000 (Shenzhen New Industries Biomedical Engineering Co., China) fully automated chemiluminescent immunoassay analyzer. A peak stimulated cortisol <18 µg/dL was considered inadequate (1); a peak 17-OHP ≥ 10 ng/mL was suggestive of 21-OHD NC-CAH (4). For patients with a peak 17-OHP ≥ 10 ng/mL, the timing of this value was recorded, as were the genetic analysis results.

Patients were stratified into two groups based on peak 17-OHP during Synacthen testing: Group 1 (biochemically suggestive of NC-CAH; peak 17-OHP ≥ 10 ng/mL) and Group 2 (controls; peak 17-OHP < 10 ng/mL). Baseline hormone test results were compared between groups. To address potential biochemical overlap between NC-CAH and heterozygous carriers, *CYP21A2* genetic analyses within Group 1 were retrospectively reviewed. The genetic testing methods and corresponding molecular results were systematically recorded. The study protocol was approved by the local ethics committee.

Statistical analysis

Statistical analyses were performed using SPSS for Windows (v. 25.0, SPSS, USA). The normality of

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quantitative data was assessed with the Kolmogorov-Smirnov test and histogram plots. Two-group comparisons were performed using the Mann-Whitney U test. Receiver operator characteristics (ROC) curves were generated to determine sensitivity and specificity of baseline 17-OHP. Numerical data are presented as median and range, qualitative data as number (percentage), with $p < 0.05$ considered statistically significant.

RESULTS

Of the 150 patients analyzed, the median age was 13 years (5.5–18), and 128 (85.3%) were female. The most common presentation was early axillary and/or pubic hair growth (n = 70, 46.7%), followed by hirsutism (n = 52, 34.7%), menstrual irregularity (n = 20, 13.3%), acne (n = 5, 3.3%), and precocious puberty (n = 3, 2%). Median baseline DHEA-S was 207.5 µg/dL (14.9–864); median baseline 17-OHP was 1.68 ng/mL (0.15–57.4); median 30-minute 17-OHP was 2.815 ng/mL (0.15–70.1); median 60-minute 17-OHP was 3.16 ng/mL (0.15–80).

Twenty-nine patients (Group 1) exhibited peak 17-OHP ≥ 10 ng/mL during Synacthen testing. In 26 of these, both 30- and 60-minute 17-OHP levels were ≥ 10 ng/mL. Three patients had normal 30-minute 17-OHP, while 60-minute 17-OHP was ≥ 10 ng/mL in all three. No patient had a 60-minute 17-OHP <10 ng/mL with a 30-minute value ≥ 10 ng/mL. In Group 1, median 30-minute 17-OHP was 17.20 (1.9–70.10) ng/mL; median 60-minute 17-OHP was 25 (10.1–80.0) ng/mL. There was no statistically significant difference between median 30- and 60-minute levels.

The median age of Group 1 was 9.8 (5.5–17.6) years, and Group 2 was 13 (5.6–18) years; this difference was not statistically significant (p = 0.389). Fifteen (51.7%) of 29 patients in Group 1 and 57 (47.1%) of 121 in Group 2 were prepubertal. Median basal DHEA-S in Group 1 was 184 (14.9–572) µg/dL, and in Group 2 209 (15–864) µg/dL (p = 0.148). Median basal 17-OHP in Group 1 was 9.32 (0.74–57.4) ng/mL; in Group 2 it was 1.42 (0.15–7.56) ng/mL (p < 0.001).

ROC analysis demonstrated that basal 17-OHP significantly predicted a positive test result (p < 0.001, Figure 1). Using 3.78 ng/mL as the cut-off for basal 17-OHP, sensitivity was 89.7% and specificity was 94.2%.

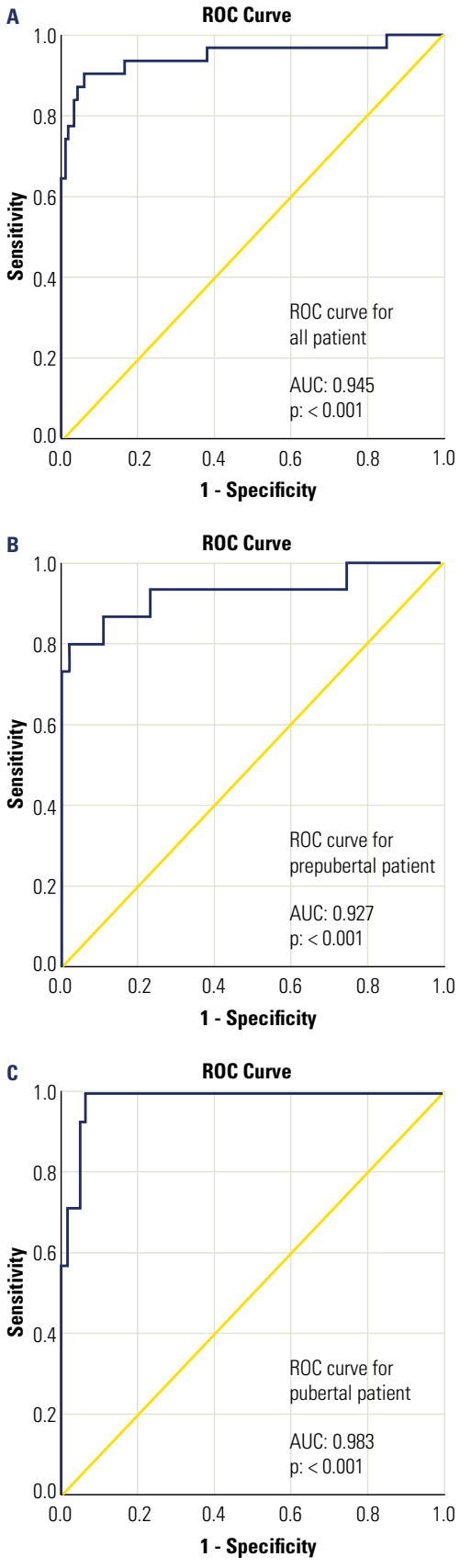


Figure 1. ROC analysis of basal 17-OHP level in predicting standard dose Synacthen test positivity and summary of analysis. (A) All patients group; (B) Prepubertal patients group; (C) Pubertal patients group.

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The patients were stratified by pubertal status (prepubertal versus pubertal). The optimal cut-off values identified by ROC analysis for the groups were 2.525 and 3.85 ng/mL for the prepubertal and pubertal groups, respectively. These resulted in sensitivity and specificity values of 86.7% and 89.5% (prepubertal) and 92.86% and 93.75% (pubertal), respectively. Using the commonly recommended cut-off of 2 ng/mL (4), sensitivity was 93.1% and specificity 68.6% for the whole group; sensitivity was 86.7% and specificity 80.7% for prepubertal, and sensitivity 100% with specificity 57.8% for pubertal children (**Figure 1**).

A peak cortisol ≥18 µg/dL was considered an adequate response. In Group 1, 11 of 29 patients had an inadequate cortisol response. Of the 18 with adequate cortisol, 15 had both 30- and 60-minute responses ≥18 µg/dL; three had 30-minute cortisol <18 µg/dL but 60-minute ≥18 µg/dL. No patients had 60-minute cortisol <18 µg/dL but a 30-minute value ≥18 µg/dL. In Group 2, no patients exhibited inadequate peak cortisol (**Table 1**). *CYP21A2* molecular analysis was performed in 21 of 29 Group 1 patients: 9 (42.8%) had biallelic mutations (homozygous or compound heterozygous), 6 (28.5%) were isolated heterozygous carriers, and 6 (28.5%) had no mutations detected using available methodologies (**Table 1**).

DISCUSSION

Non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency is approximately ten times more prevalent than classical CAH, with clinical features well recognized in women but often missed in men, contributing to the gender imbalance observed in many studies (8–11). In the present study, 81.8% of genetically confirmed 21-OHD NC-CAH (homozygous and compound heterozygous) and 86.2% of those with Synacthen test peak 17-OHP ≥10 ng/mL were female.

Comparing the 29 patients with peak 17-OHP ≥ 10 ng/mL and the 121 with < 10 ng/mL, no differences in age or basal DHEA-S were observed, whereas basal 17-OHP was significantly higher in those testing positive. Similar findings were reported by Gönç and cols. (12), where basal 17-OHP was higher among patients with peak Synacthen 17-OHP ≥ 10 ng/mL, while DHEA-S was not significantly different. Using 3.78 ng/mL as the cut-off for basal 17-OHP, sensitivity and specificity were 89.7% and 94.2%, respectively. In similar studies conducted in Turkey, Sahmay and cols. (13) determined the cut-off value of basal 17-OHP to be 2.25 ng/mL (50.4% sensitivity and 84% specificity) and Cengiz and cols. (14) reported it to be 3.19 ng/mL (75% sensitivity and 51.6% specificity). The specificity of the current study's cut-off was superior for predicting a positive test. Using the commonly accepted 2 ng/mL cut-off, sensitivity was found to be 93.1% with a specificity of 68.6% in predicting a positive test result. In our study, the optimal cut-off value was 2.525 ng/mL in prepubertal patients and 3.85 ng/mL in pubertal patients, and specificity increased with the use of these values without a significant decrease in sensitivity. Nonetheless, screening tests are generally biased towards sensitivity, as it is deemed more important not to miss a diagnosis, at the cost of accepting lower specificity.

Raising the cut-off value increases the specificity of this test at the expense of lower sensitivity, although some patients with the condition can be missed. Therefore, based on our findings, we cannot confirm whether the cut-off value should be increased. Additionally, given the lack of a uniform genetic gold standard across all patients and the potential overlap between NC-CAH and heterozygous carriers, these ROC-derived cut-off values should be interpreted as exploratory and hypothesis-generating rather than definitive for clinical practice.

Previous studies assessing cortisol response at 30 and 60 minutes during the standard-dose Synacthen test have shown that 60-minute cortisol levels were adequate (≥ 18 µg/dL) in patients with inadequate at 30 minutes, with no case being found in which the 30-minute cortisol response was adequate while the 60-minute cortisol response was not (5–7). In these studies, 4.8–8.6% of patients could be diagnosed with cortisol insufficiency and incorrectly treated if a decision was made based on the 30-minute value alone, and it therefore possible to conclude that the 60-minute cortisol value should definitely be measured and may be sufficient for diagnosis (5–7).

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Table 1. Patients with peak 17-OHP ≥ 10 ng/mL

Patient	Sex	Age at presentation (years)	Complaint	Basal ACTH pg/mL	Basal DHEA-S mcg/dL	17-OHP Basal ng/mL	17-OHP 30-min ng/mL	17-OHP 60-min ng/mL	Cortisol Basal mcg/dL	Cortisol 30-min mcg/dL	Cortisol 60-min mcg/dL	Genetic analysis	Genotype variation	Phenotype
P1	F	7.5	Pubic hair	37.1	86.7	22.3	24.4	24.8	4.4	9.64	12.3	Whole gene sequence analysis	IVS2-13C>G heterozygousExon 7 V281L heterozygous	NC-CAH
P2*	F	6	Pubic hair	17.6	88.7	11	25	25	10.9	20	22	NGS-PCR and MLPA	In2G(C>G) homozygous	NC-CAH
P3*	M	9.8	Pubic hair	22.8	310	9	14.9	19.2	8.57	13.7	16.3	NGS-PCR and MLPA	In2G(C>G) homozygous	NC-CAH
P4	F	9.25	Pubic hair	131	23.3	23.9	30.2	32	10	15.2	17.5	Whole gene Exon sequence analysis and intron 2	Exon 7 V281L homozygous	NC-CAH
P5	F	15.75	Hirsutism	30.7	25.8	50	70.1	80	9.4	10.6	11.8	Whole gene Exon sequence analysis and intron 2	Exon 7 V281L heterozygousExon 4 I172N heterozygous	NC-CAH
P6	F	5.5	Pubic hair	30	15	2.67	10.2	10.1	11.38	20.16	22.4	Whole gene Exon sequence analysis and intron 2	Exon 7 V281L heterozygous	NC-CAH
P7*	F	7.88	Pubic hair	74.7	91	57.4	58.8	67.7	9.2	9.5	10.4	Whole gene Exon sequence analysis and intron 2	Exon 7 V281L heterozygous	NC-CAH
P8*	F	7.88	Pubic hair	105	68.1	12.5	15.9	16.2	9.9	10.9	11.2	Whole gene Exon sequence analysis and intron 2	Exon 7 V281L heterozygous	NC-CAH
P9	F	15.3	Hirsutism	57.6		15.2	28.4	41.2	15.4	16.8	20.1	Whole gene sequence analysis	Exon 7 V281L homozygous	NC-CAH
P10	M	13.2	Acne	57	444	6.87	22.4	26.5	11.1	14.4	15.6	Whole gene sequence analysis	Exon 7 V281L homozygous	NC-CAH
P11	F	6.5	Pubic hair	67	57	20	63.8	70.8	16.5	18.3	19.9	Whole gene sequence analysis	Exon 4 I172N heterozygousExon 7 V281L heterozygous	NC-CAH
P12	F	7.5	Pubic hair	32	90	13.6	23.1	71.6	12.1	16.3	17.4	Whole gene Exon sequence analysis and intron 2	Exon 10 P453S homozygous	NC-CAH
P13	F	14	Hirsutism	29.3	282	9.1	11.7	13.4	18	22.8	27.4	Whole gene Exon sequence analysis and intron 2	Exon 10 A434V homozygous	NC-CAH
P14	F	13.5	Hirsutism	41.5	572	5	25	32	8.3	10.8	11.4	NGS-PCR	Exon 8 Q318X heterozygous	NC-CAH
P15	F	8.83	Pubic hair	72.8	77.2	5.59	35	36.9	10.6	26	31	NGS-PCR	Exon 7 V281L homozygous	NC-CAH
P16	F	16.3	Hirsutism	56.3	450	27	24	50	11.4	11	9.85	Whole gene sequence analysis	None detected	NC-CAH
P17	F	13.7	Hirsutism	19.5	279	3.93	13.1	17.3	14	17.8	21	Whole gene sequence analysis	None detected	NC-CAH
P18	F	8.11	Precocious puberty	13.2	15	6.96	29.14	35.48		17.2	19.8	-	None detected	NC-CAH
P19	F	9.8	Pubic hair	85	166	0.74	1.9	31.4	18.74	26.5	29.4	NGS-PCR	None detected	NC-CAH
P20	F	14.11	Hirsutism	20.9	523	9.18	13.38	18.72	10.3	24.7	32.2	-	None detected	NC-CAH
P21	M	7	Pubic hair	25	212	1.77	3.39	13	13	25.9	34	Whole gene sequence analysis	None detected	NC-CAH
P22	F	17.6	Hirsutism	20	408	5	17.2	17.7	11.7	20.2	22.5	Not tested	-	NC-CAH
P23	F	16.5	Hirsutism	60.1	184	11.82	12.87	12.34	19.8	22.9	24.6	Not tested	-	NC-CAH
P24	F	7	Pubic hair	182	34.5	9.32	10.38	11.7	23.8	30.8	31.8	Not tested	-	NC-CAH
P25	F	16.1	Hirsutism	16	191	9.47	13.32	13.72	14.9	23.8	27.4	Not tested	-	NC-CAH
P26	F	17.2	Hirsutism	18.5	558	3.8	4.41	65.37	28	21.2	24.5	Not tested	-	NC-CAH
P27	F	17.11	Hirsutism	25	391	12.43	16.38	16.9	13.5	19.6	23.6	Not tested	-	NC-CAH
P28	F	14.4	Menstrual irregularity	13.3	161	6.01	10.71	12.07	12.6	18	24.6	Not tested	-	NC-CAH
P29	M	9.1	Pubic hair	99.2	-	50.9	60.1	63.9	12	13.1	13.2	Not tested	-	NC-CAH

ACTH: adrenocorticotropin hormone; F: female; M: male; adrenocorticotropin hormone; DHEA-S: dehydroepiandrosterone sulfate; 17-OHP: 17-hydroxyprogesterone; NGS-PCR: next-generation sequencing-polymerase chain reaction; MLPA: multiplex-ligation-dependent-probe-amplification. NC-CAH: non-classical congenital adrenal hyperplasia. *P2 and P3 are siblings; P7 and P8 are twins.

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In the present study, among patients with peak 17-OHP ≥ 10 ng/mL, 18 had an adequate cortisol response; in three, only the 60-minute value was sufficient, while in 15 both measurements were sufficient. Notably, all patients with elevated peak 17-OHP had 60-minute 17-OHP ≥ 10 ng/mL, with three having normal 30-minute values.

Thus, our results support the conclusion that the 60-minute cortisol and 17-OHP measurements more reliably capture the peak hormonal response than the 30-minute measurement. While omitting the 30-minute measurement does not reduce the overall test duration, it significantly improves clinical efficiency by reducing the number of invasive blood samples required. In the pediatric population, minimizing invasive procedures is essential for improving patient comfort and reducing procedure-related anxiety. Eliminating unnecessary intermediate measurements also reduces healthcare costs and laboratory workloads.

Nevertheless, our observation that the 60-minute measurement detected all positive cases is based on a relatively small cohort of 29 patients with elevated 17-OHP. This sample size may have limited the statistical power to definitively conclude that an early peak at 30 minutes does not occur in NC-CAH. Hence, despite the 60-minute measurement being more reliable, the results may not be generalizable to all clinical settings without further validation in larger populations.

In terms of genetic findings, the V281L pathogenic variant was the most common genotype among our 21-OHD NC-CAH patients. This findings is persistent with the literature, as V281L variations have been reported as the most common genetic variation (34–55.9%) in the *CYP21A2* gene in patients diagnosed with 21-OHD NC-CAH in different studies conducted in Turkish, Spanish, Greek, and French populations (15–18). The V281L variant was homozygous in four patients, compound heterozygous in three, and heterozygous in another three. As further genetic analysis (e.g., MLPA) was not performed in the patients with heterozygous variation, compound heterozygosity could not be excluded, suggesting patients may be NC-CAH or symptomatic carriers. Evidence has also indicated peak 17-OHP levels in 21-OHD CAH carriers in the ranges of 4–10 or 5–15 ng/mL (19,20).

We acknowledge that using a peak 17-OHP threshold of 10 ng/mL may include some heterozygous carriers, as seen in six of our tested patients. The overlap between symptomatic heterozygotes and NC-CAH patients is a recognized diagnostic challenge, particularly with immunoassays (e.g., chemiluminescent immunoassay), which may overestimate 17-OHP compared to LC-MS/MS. However, the primary focus of this study was to assess the 30- versus 60-minute temporal dynamics of the peak response rather than to establish new diagnostic criteria.

This study has several limitations. Its retrospective design and the relatively small number of positive Synacthen test results may have limited generalizability. Patient selection based on clinical judgment may have also introduced bias. Genetic confirmation was not available for all patients, and genetic analyses utilized different methods (e.g., PCR, NGS, and MLPA) over an extended period. These methods vary in sensitivity, particularly for large deletions or complex rearrangements in the *CYP21A2* gene. Lastly, while MLPA was used in some cases to increase the detection rate of deletions, the lack of a standardized genetic protocol for all patients may have led to an underestimation of some variants.

In conclusion, our findings suggest that the 60-minute 17-OHP measurement may capture the peak response in most pediatric patients evaluated for suspected 21-OHD NCCAH. However, prospective studies with larger cohorts and comprehensive genetic confirmation are required before definitive modifications of current testing protocols can be recommended. If further validated, this approach may reduce testing costs, decrease laboratory workload, and improve patient comfort.

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original

Comprehensive insights into bone health, hormonal patterns, and fracture risks among men with drug resistant epilepsy: a crucial evaluation

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ABSTRACT

Objective: To evaluate bone density and microstructure, fragility fractures, and hormones in men 50 years and older receiving antiseizure medications (ASMs). **Subjects and methods:** Study population included 39 men with difficult-to-control epilepsy and 42 healthy men as control group. Spine and hip areal bone mineral density (aBMD), body composition and vertebral fracture assessment (VFA) were evaluated using Dual-Energy X-Ray Absorptiometry (DXA). Volumetric bone mineral density (vBMD) and microarchitecture of trabecular and cortical compartments were evaluated using High-Resolution Peripheral Quantitative Computed Tomography (HR-pQCT). Physical activity was tested by the International Physical Activity Questionnaire (IPAQ). The fracture risk was estimated by fracture risk assessment tool (FRAX). **Results:** ASMs patients showed lower cortical bone thickness (Ct.Th) in distal tibia (1.196 ± 0.295 vs. 1.341 ± 0.241 mm, p = 0.03), more fractures (12 vs. 2, p = 0.003) mostly morphometric vertebral fractures, and a higher risk for major and hip fractures based on FRAX. Hormonal changes included higher median parathyroid hormone (PTH) (50.4 vs. 24 pg/mL, p < 0.001) possibly associated with vitamin D insufficiency, (25(OH)D levels below 30 ng/mL in 43.6% of ASMs patients), lower median Estradiol that was an independent factor for fractures (p = 0.04) and bioavailable Testosterone in the lowest quartile in 52 % of them. **Conclusion:** Men on ASMs exhibit lower Ct.Th and hormone changes that may contribute to bone fragility. While clinical fractures are infrequent, it is crucial to actively search for morphometric fractures, either during DXA or through the more available method of spine X-rays.

Keywords: Epilepsy; antiseizure medications; bone mineral density; body composition

INTRODUCTION

Pharmacoresistant epilepsy, which affects 30% of all individuals with epilepsy, presents a considerable challenge in epilepsy management. This condition is defined by the inability to secure sustained seizure control after adequate trials of two well-chosen, well-tolerated, and properly administered antiseizure medications (ASMs) schedules, whether as monotherapies or in combination (1). For such patients, the complexity of treatment increases, often necessitating the use of a combination of ASMs, aiming to maintain effectiveness while minimizing side effects. Studies have shown that ASMs that induce liver enzymes through cytochrome P450, such as carbamazepine, oxcarbazepine, phenobarbital, phenytoin, and topiramate, are more negatively associated with bone mineral density (BMD) than non-enzyme-inducing drugs like gabapentin, lacosamide, lamotrigine, levetiracetam, pregabalin, and valproate (2–4). Additionally, ASMs usage has been correlated with lowered levels of vitamin D and sex steroids, with restrictions on physical activity and the presence of sarcopenia potentially contributing to

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increased bone loss and fracture risks in this demographic (5,6).

The unpredictable nature of epileptic seizures elevates the risk of falls, significantly increasing the chance of fractures and adding to both the physical morbidity and the psychological and social impact on the patient (5,7). This underpins the importance of optimizing bone health within the epileptic community.

This study aimed to investigate bone density and microstructure, physical activity, the occurrence of fragility fractures, and the hormonal profiles in men aged 50 and above with epilepsy who are on ASMs therapy.

SUBJECTS AND METHODS

Study design and patient selection

This observational, cross-sectional study was performed in a single referral center for the treatment of epilepsy in southern Brazil, the Instituto Estadual do Cérebro Paulo Niemeyer (IECPN). The inclusion criteria were male adults 50 years and older with epilepsy who had been treated at IECPN outpatient clinic for at least two years. Women were excluded from this study because menopause would interfere with interpretation of bone events. The following risk factors for the development of osteoporosis were excluding criteria: vegetarian or vegan diet; regular use of medications that might interfere with bone homeostasis, such as glucocorticoids, sex hormones, antidepressants, proton pump inhibitors, antipsychotics, as well as antiosteoporosis drugs; malignant neoplasia in the previous five years; chronic diseases affecting the kidneys or the respiratory or cardiac systems; cerebral palsy; and any condition favoring immobility. Patients with a body mass index (BMI) below 18 kg/m^2 or above 40.0 kg/m^2 were also excluded. The control group was composed of healthy men 50 years and older, recruited from family members or friends of patients who participated in research studies including DXA and HRPQCT evaluation that were developed in the osteometabolism unit of the Endocrinology Division of HUCFF-UFRJ. The present study was approved by the local research ethics committee (CAAE: 39122720.1.0000.8110), and all participants signed an informed consent form before being included in the study. Laboratory and imaging tests were collected at a single time point.

Data collection

All participants completed a questionnaire that addressed their medical history, information about previous fractures and also parental hip fractures, falls and injuries; smoking; alcoholism; data on physical activity considered in IPAQ; and intake of dairy products and vitamin D or calcium supplements. Demographic data on weight (W) and height (HT), which were used to calculate BMI (BMI=W/HT^2), as well as age and other variables, were also collected.

Laboratory

Blood samples were drawn between 8 AM and 10 AM after an overnight fast. Glucose (reference values: 60–99 mg/dL), calcium (8.5–10.1 mg/dL), phosphorus (3.0–4.5 mg/dL), albumin (3.5–5.5 g/dL), gamma glutamyl transferase (GGT; 12–45 U/L), and creatinine (0.8–1.3 mg/dL) were measured using colorimetric methods. The glomerular filtration rate (GFR) was estimated using the chronic kidney disease epidemiology collaboration equation (8). Chemiluminescent assays using commercial kits Ebran® were used to measure total alkaline phosphatase (ALP; 27–100 U/L) and the following hormones: free thyroxine (FT4; 0.54–1.24 ng/dL), thyrotropin (TSH; 0.38–5.33 µUI/mL), 25-hydroxyvitamin D (25(OH)D; 20–60 ng/mL), intact parathyroid hormone (PTH; 12–88 pg/mL), estradiol (E2; < 33 pg/mL), sex hormone-binding globulin (SHBG; 13.2–89.5 nmol/L), and testosterone (T; 175–761 ng/dL). Bioavailable testosterone (Bio T; 50–59 years: 99.3–288.3 ng/dL, 60-69 years: 84.1–269.9 ng/dL, 70–79 years: 74.6–251.4 ng/dL) was calculated based on total T, SHBG, and albumin using a validated equation (9).

Physical Activity Questionnaire (IPAQ)

Physical activity practice was estimated using the International Physical Activity Questionnaire (IPAQ), a method that has been validated for the Brazilian population, including elderly males (10). The participants were asked about the frequency and duration of the activities in which they engaged in a typical week.

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The responses were used to categorize the participants into the following three groups based on their level of physical activity: inactive, active, and very active.

Dual-Energy X-Ray Absorptiometry (DXA)

Areal bone mineral density (aBMD), body composition and vertebral fracture assessment (VFA) were carried out on all participants using the same equipment Prodigy Advanced Plus, GE-Lunar, EUA. BMD (g/cm^2) was measured at lumbar spine (LS), femoral neck (FN), and total hip (TH). The T-score is defined as the number of standard deviations from the patient's BMD and the mean reference value of the normal young population. Based on their lowest T-score, the participants were classified as having osteoporosis (T ≤ -2.5 SD), low BMD or osteopenia (T-score between < -1 and > -2.5 SD), or normal BMD (T ≥ -1 SD). The precision error (coefficient of variation, %CV) and the least significant change (LSC = 2.77 x precision error) of the DXA Prodigy Advance were calculated based on the ISCD (International Society of Clinical Densitometry) orientation. Thirty patients (age range 20-65 years) were scanned twice for spine, hip and total body. After the first scan, each patient was removed from the table and repositioned for the second scan. All measurements were entered in the equation available on the ISCD site https://iscd.org/resources/calculators/. Results obtained for the Root Mean Square Standard Deviation (RMS-SD) and the LSC CI 95% were 0.010 g/cm^2 and 0.028g/cm^2 for lumbar spine, and 0.012 g/cm^2 and 0.034 g/cm^2 for total hip. For longitudinal studies, we assume changes at or higher than 1.5% for the lumbar spine and 2.3% for the total hip as significant.

Body composition provided data on fat and lean mass. To estimate muscle mass, appendicular lean mass (ALM; lean mass in the arms and legs) was calculated and adjusted for BMI and height (ALM/BMI and ALM/HT^2, respectively).

Non-clinical vertebral fractures were actively sought via VFA using a visual scale to detect deformities compatible with morphometric vertebral fractures. All exams were conducted and evaluated by a professional with extensive experience in densitometry, according to the International Society for Clinical Densitometry guidelines (11).

FRAX

The fracture risk assessment tool (FRAX), which has been validated for the Brazilian population (12), was employed to estimate the probability of hip and/or major osteoporotic fractures (which include hip, vertebrae, distal forearm, and proximal humerus fractures) in the next 10 years of life.

High-Resolution Peripheral Quantitative Computed Tomography (HR-pQCT)

Volumetric bone density and microarchitecture of the trabecular and cortical compartments were evaluated at the distal radius (non-dominant arm) and distal tibia using a first-generation high-resolution peripheral quantitative computed tomography (HR-pQCT) system called Xtreme CT I (Scanco Medical AG, Brüttisellen, Switzerland). From both sites, 110 slices with a nominal resolution (voxel size) of 82 µm were obtained. The first slice was 9.5 mm and 22.5 mm proximal to the reference line for the distal radius and distal tibia, respectively. The parameters obtained were nominated following the recommendations of a recent consensus (13). Volumetric bone density was measured at the entire bone [Tt.BMD], cortical bone [Ct.BMD] and trabecular bone [Tb.BMD]. Microstructural parameters included: cortical thickness [Ct.Th: ratio of cortical bone volume to the outer bone surface], trabecular number [Tb.N], trabecular bone volume fraction [BV/TV, derived from trabecular density/1200 mg hydroxyapatite], trabecular thickness [Tb.Th:(BV/TV)/Tb.N], trabecular separation [Tb.Sp: (1-BV/TV)/Tb.N], and trabecular inhomogeneity [Tb.1/N.SD, the standard deviation of 1/Tb.N, which reflects the inhomogeneity of the network]. Unlike DXA, there are no standardized values for healthy young people regarding volumetric density or microstructure parameters. Therefore, the exam does not define T-scores or Z-scores.

Quality control included daily phantom measurements to ensure the stability of volumetric density measurements (QC1), and weekly phantom assessments were conducted to verify long-term scanner stability and consistency of structural measurements (QC2). Patients positioning, movement artifacts and image examination were performed and analyzed by

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the same experienced professional according to the manufacturer's recommendations and described in detail by Whittier and cols. (13).

There are few reports on CV% RMS and LSC values, but acceptable values for CV% widely cited for the X-treme CT1 reproducibility were described by Mikolajewicz and cols. (14), and vary from 0.8-2.0% for vBMD, 2.3-4.0% for bone area and 1.6-4.9% for trabecular and cortical microstructure. The HR-pQCT evaluation allowed to differentiate patients with and without fractures, despite only some of the measures were significantly larger than the LSC (Tt.vBMD; Tb.vBMD; Ct.Th and Ct.vBMD at the tibia only.

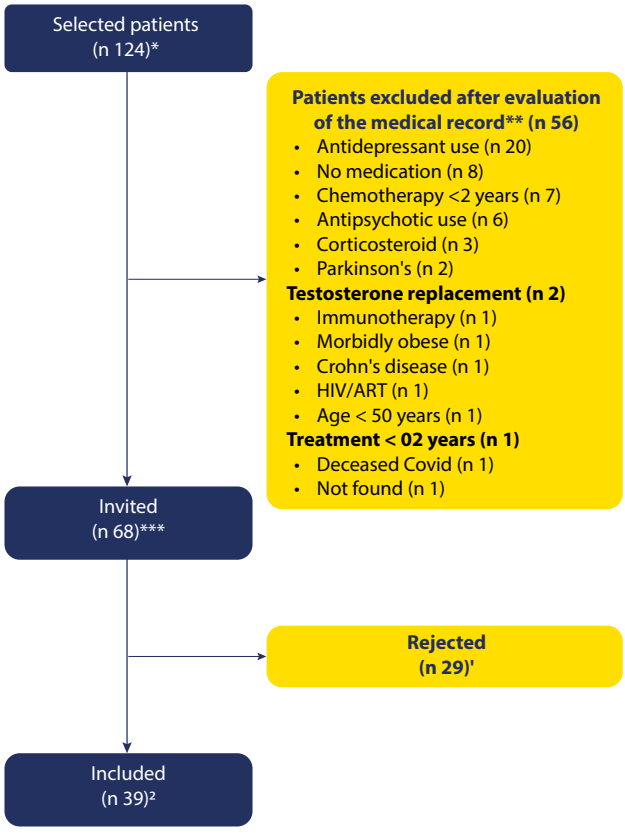
Considering the difficulty to estimate CVs for every HR-pQCT parameter, we assume, for longitudinal studies, CV values from 1-2% for density measurements and 3-5% for microarchitectural parameters.

Statistics

The statistical analyses were performed using SPSS version 23.0 for MacOS (SPSS Inc., Chicago, IL, USA). In the descriptive analysis, the categorical variables were expressed as frequency and percentage, and the numerical variables were expressed as mean ± SD. The Kolmogorov-Smirnov test defined the variables with a normal distribution. The student's t-test or the Mann-Whitney U test was performed to compare the numerical variables between the groups. The chi-square test or the Fisher's exact test was used to compare the categorical variables. Multivariate analysis was performed to test the contribution of PTH, Estradiol, Cortical bone thickness and IPAQ in the occurrence of fragility fractures. A *p*-value < 0.05 was considered significant.

RESULTS

The medical records of 124 male epileptic patients treated at the IECPN outpatient clinic between January and December 2021 were screened for inclusion in this study. After initial analysis, 56 patients were excluded based on predefined exclusion criteria. Subsequently, the remaining 68 patients were contacted by phone and invited to participate in the study. Of these, 39 completed all assessments as outlined in the study protocol (see **Figure 1**). The control group



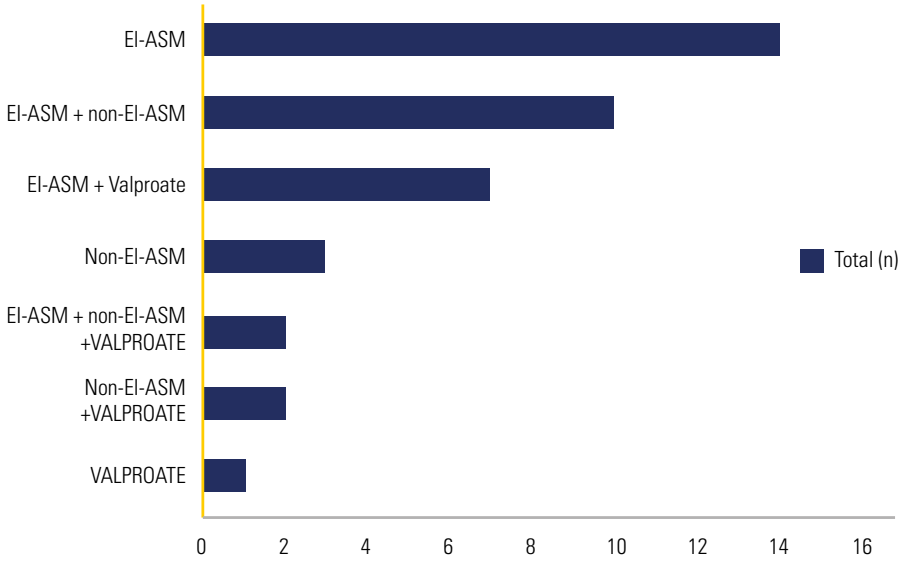
*Selected medical records of male patients >50 years of age treated at the IECPN epilepsy outpatient clinic from January to December 2021. **Exclusion criteria found, ***Number of individuals invited to participate via telephone. ¹ Individuals who refused to participate. ² Individuals included in the survey after signing the TCLE.

Figure 1. Flow chart of patient's selection

consisted of 45 healthy men. No significant differences were observed between the ASMs and control groups in terms of age, BMI, or the prevalence of type 2 diabetes mellitus (12.7% of the ASMs group and 16.3% of the control group) treated only with metformin.

All the Study patients included had drug-resistant epilepsy and commonly required a combination of both enzyme-inducing antiseizure medications (EI-ASMs) (e.g., carbamazepine, oxcarbazepine, phenobarbital, phenytoin, topiramate) and non-enzyme-inducing antiseizure medications (non-EI-NASMs) (e.g., lamotrigine, levetiracetam, valproate). Specifically, 84.6% of patients were treated with EI-ASMs, 30.7% received valproate, and 43.5% were prescribed non-EI-ASMs. Additionally, 64% of patients used multiple drugs to manage their seizures (**Figure 2**). The ASMs treatment varied from 2 to 60 years, median duration 9 years.

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n: number of participants in each drug association; EI-ASMs: enzyme-inducing antiseizure medications (Carbamazepine, Oxcarbazepine, Phenobarbital, Phenytoin and Topiramate); non-EI-ASMs: non-enzyme-inducing antiseizure medications.

Figure 2. Combination of antiseizure medications

The etiology of epilepsy was most commonly observed were benign CNS tumors, followed by vascular causes and mesial temporal sclerosis (MTS). Imaging exams revealed nonspecific changes unrelated to epilepsy in six patients and no changes in three patients.

Regarding metabolic parameters all participants had serum calcium, phosphorus, creatinine, TSH, and FT4 levels within normal limits, with GFR at or above 60mL/min/1.73 m^2. In comparison with controls, ASMs patients had higher GFR and liver enzymes (total alkaline phosphatase and GGT).

The 25(OH)D levels were below 20 ng/mL in 18% of the ASMs group and 9% of the control group. No participant had 25(OH)D values below 12 ng/mL, consistent with severe vitamin D deficiency. Serum PTH levels were higher in the ASMs patients.

Total testosterone levels were within normal limits for all participants, and the median values were even higher in the ASMs group. SHBG levels, only measured in ASMs patients, was in the highest quartile (above 70 nmol/L) in 34.3% of them. As a consequence, bioavailable testosterone was in the lowest quartile in 86% and below the age-adjusted reference values in 22% of them. Estradiol was significantly lower in the ASMs group than in the control group. These results are presented in **Table 1**.

According to the IPAQ results, the ASMs group was less active than the control group. A comparison of the body composition of the groups revealed no significant difference in lean mass, which mostly reflected the muscle mass of the total body, arms, and legs. These results are shown in **Table 2**.

There was no difference in the frequence of normal BMD, low BMD (osteopenia), and osteoporosis between ASMs patients (58%, 39%, and 3%) and control (52%, 48%, and 0%), respectively. No statistically significant differences were found in areal BMD at the lumbar spine or hip between these groups (**Table 3**).

Fractures were more frequent in the ASMs group; five referred prevalent fractures (three at the radius and two at the clavicle), and seven were diagnosed with morphometric vertebral fractures using VFA. Meanwhile, only two participants in the control group had morphometric vertebral fractures. These findings were consistent with the FRAX results, which showed that the ASM group had a higher risk of major osteoporotic and hip fractures than the control group (**Table 3**).

The HRpQCT results showed that the ASM group had lower Ct.Th at the distal tibia than the control group. No significant difference was detected in the trabecular compartment (**Table 4**).

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Table 1. Comparison of biochemical data between epileptic patients and controls

	ASM (N = 39)	Control (N = 42)	P value
Age (years)	56 (54; 67)	58 (55.3; 64)	0.98
Height (cm)	170 (159; 183)	170 (150; 183)	0.99
Weight (kg)	78.3 (62.2; 118.7)	77.98 (56; 118)	0.91
BMI (kg/m^2)	27.1 ± 3.4	27.0 ± 4	0. 98
GFR (mL/min/1.73 m^2)	95.9 ± 16.4	86.1 ± 12.1	**0.004**
Calcium (mg/dL)	9.5 (9.1; 10)	9.2 (8.8; 9.5)	**0.01**
Phosphorus (mg/dL)	3.5 ± 0.8	3.4 ± 0.5	0.50
Albumin (g/dL)	4.2 ± 0.4	4.1 ± 0.3	0.10
GGT (U/L)	58 (32; 87)	33 (25; 56)	**0.026**
ALP (U/L)	175.5 ± 86.2	94.6 ± 44.6	**0.026**
25(OH)D (ng/dL)	30.4 (23.6; 42)	26 (22.7; 29.2)	**0.009**
25(OH)D below 20 ng/mL (%)	18	9.3	0.44
PTH (pg/mL)	50.4 (37.2; 68.6)	24 (17.9; 28.8)	**<0.001**
Total Testosterone (ng/dL)	402.5 ± 147.2	323.1 ± 108.6	**0.026**
Estradiol (pg/mL)	25 (18; 31)	49.7 (37; 63)	**<0.001**
FT4 (ng/dL)	0.89 ± 0.2	1.08 ± 0.15	**0.02**
TSH (µUI/mL)	2.0 (1.4; 2.7)	1.8 (1; 2.1)	0.28

Values are expressed as mean ± SD or median (1st-3rd quartiles). BMI (body mass index), GFR (glomerular filtration estimated by CKD-EPI equation), GGT (gamma glutamyl transferase), 25(OH) (25-hydroxyvitamin D), PTH (parathyroid hormone), ALP (alkaline phosphatase FT4 (free thyroxine), TSH (thyroid stimulating hormone).

Table 2. Comparison of body composition and physical activity between epileptic patients and controls

	ASM (N = 39)	Control (N = 42)	P value
Total body fat mass (%)	32.47 ± 7.39	31.29 ± 4.43	0.42
Total body lean mass (kg)	50.64 ± 5.43	51.26 ± 7.67	0.71
Arms lean mass (kg)	6.26 ± 0.94	6.64 ± 1.16	0.16
Legs lean mass (kg)	17.06 ± 2.47	17.03 ± 3.61	0.96
Appendicular lean mass*	23.31 ± 3.23	23.67 ± 4.61	0.72
ALM/H^2	8.08 ± 0.87	8.28 ± 1.37	0.49
ALM/BMI	0.03 ± 0.01	0.03 ± 0.01	0.28
IPAQ (%)**			**0.003**
Group 1 (high activity)	14.3	46.2	
Group 2 (moderate activity)	37.1	42.3	
Group 3 (low activity)	48.6	11.5	

Values expressed as mean ± SD or number of subjects – n (%), *Lean mass in the arms and legs (ALM), ALM/BMI (adjusted for BMI), ALM/H^2 (adjusted for height) **IPAQ (International Physical Activity Questionnaire).

Table 3. Comparison of bone densitometry and fractures between epileptic patients and controls

	ASM (N = 39)	Control (N = 42)	P value
Lumbar spine BMD (g/cm^2)	1.183 (1.060; 1.243)	1.172 (1.100; 1.301)	0.59
Femoral neck BMD (g/cm^2)	0.953 (0.924; 1.135)	0.975 (0.916; 1.050)	0.21
Total hip BMD (g/cm2)	1.047 (0.766; 1.252)	1.060 (0.996; 1.110)	0.45
Patients with fractures: N (%)	12 (30.8)	2 (4.7)	**0.003**
FRAX major fractures (absolute risk)[1]	2.00 (1.60; 3.10)	1.85 (1.60; 2.13)	**0.019**
FRAX hip fractures (absolute risk)[2]	0.30 (0.10; 1.00)	0.20 (0.10; 0.50)	**0.023**

Values are expressed as Median (1st-3rd quartiles) or number of subjects – n (%); BMD (areal bone mineral density); [1]FRAX assessment thresholds for ten-year probability of a major osteoporotic fracture, [2]FRAX assessment thresholds for ten-year probability of hip fracture.

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Table 4. Comparison of bone density and microstructure evaluated by HR-pQCT between epileptic patients and controls

	ASM group (N = 39)	Control group (N = 42)	P value
Distal radius			
Tt.BMD (mg HA/cm^3)	339.07 ± 78.20	328.98 ± 47.52	0.51
Ct.BMD (mg HA/cm^3)	895.87 ± 75.15	869.14 ± 44.49	0.07
Ct.Th (mm)	0.860 ± 0.243	0.834 ± 0.183	0.61
Tb.BMD (mg HA/cm^3)	176.63 ± 37.27	180.00 ± 26.29	0.66
BV/TV (1)	0.147 ± 0.031	0.150 ± 0.022	0.66
Tb.N (mm^{-1})	1.961 ± 0.282	2.055 ± 0.246	0.14
Tb.Th (mm)	0.075 ± 0.016	0.074 ± 0.012	0.92
Tb.Sp (mm)	0.444 ± 0.075	0.418 ±0.055	0.09
Tb.1/N.SD (mm)	0.200 ± 0.065	0.188 ± 0.062	0.43
Distal tíbia			
Tt.BMD (mg HA/cm^3)	294.71 ± 59.01	302.20 ± 46.01	0.55
Ct.BMD (mg HA/cm^3)	897.07 ± 57.17	895.38 ± 40.87	0.88
Ct.Th (mm)	1.196 ± 0.295	1.341 ± 0.241	**0.03**
Tb.BMD (mg HA/cm^3)	165.74 ± 31.11	161.70 ± 33.75	0.61
BV/TV (1)	0.138 ± 0.026	0.135 ± 0.028	0.62
Tb.N (mm^{-1})	1.909 ± 0.306	1.920 ± 0.327	0.88
Tb.Th (mm)	0.073 ± 0.014	0.070 ± 0.009	0.27
Tb.Sp (mm)	0.464 ± 0.082	0.466 ± 0.093	0.94
Tb.1/N.SD (mm)	0.214 ± 0.049	0.214 ± 0.055	0.97

Values expressed as mean ± SD, BMD: bone mineral density; Tt.BMD (vBMD of entire bone), Ct.BMD (cortical vBMD), Ct.Th (cortical thickness), Tb.BMD (trabecular vBMD), BV/TV (bone volume-to-total volume ratio), Tb.N (trabecular number), Tb.Th (trabecular thickness), Tb.Sp (trabecular separation), Tb.1/N.SD (inhomogeneity of trabecular network).

Multivariate analysis identified Estradiol as an independent and significant factor for fractures (p=0.04), and PTH influence tended towards significance (p = 0.07), while Ct.Th and IPAQ were not associated with fractures.

DISCUSSION

This study identified a higher prevalence of clinical fractures and morphometric vertebral fractures in men with difficult-to-control epilepsy as compared to controls, unrelated to decreased bone mineral density. Lower cortical bone thickness detected by HR-pQCT and hormonal changes may contribute to the bone fragility.

The predominant use of EI-ASMs arises from the fact that this class of medication is available free of charge in our public health system, enabling access to treatment for patients with low socioeconomic status. Chronic ASMs use frequently causes asymptomatic or transient elevations of liver enzymes, which have been described in 75-95% of patients (15,16). According to the authors, patients chronically treated with EI-ASMs showed high ALP activity, which could correspond to increase in bone isoenzyme and reflect clinical or subclinical osteomalacia, or it might have hepatic origin and reflect hepatic induction, not necessarily hepatocyte injury (17). The ASM group we studied had higher levels of liver enzymes, although the bone ALP isoenzyme was not measured. ASM group also showed lower FT4 despite normal TSH, which is in accordance with literature (18) and do not indicate functional hypothyroidism or have a negative influence on bone metabolism.

Bone health is often compromised (19), although mechanism is not fully understood. However, an established hypothesis is that ASMs such as carbamazepine, phenytoin, and oxcarbazepine cause hepatic enzyme induction via cytochrome P450, resulting in vitamin D catabolism and disturbances in sex steroids associated with increased SHBG concentrations (20).

Regarding bone health, ideal levels of vitamin D should guarantee maximum intestinal

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calcium absorption and adequate bone mineralization and prevent secondary hyperparathyroidism. An Institute of Medicine report (21) concluded that 25(OH)D levels ≥ 20 ng/mL are adequate for most people. In contrast, the Endocrine Society (22) asserted that the ideal level (especially for people at risk of fractures) is ≥ 30 ng/mL (vitamin D sufficiency) and that a level between 20 and 30 ng/mL corresponds to vitamin D insufficiency and a level <20 ng/mL represents vitamin D deficiency.

In the present study, none of the participants had severe vitamin D deficiency, as defined by 25(OH)D levels below 10–12 ng/mL (23), or clinical manifestations of osteomalacia (e.g., diffuse bone pain, skeletal deformities, pseudofractures) (24). However, considering that ASM patients are at risk of osteoporosis, the ideal vitamin D level should not be lower than 30 ng/mL, which occurred with 17 (43.6%) of them. This probably contributed to decreased intestinal calcium absorption and higher PTH levels in ASM patients. We cannot exclude they might have lower calcium intake, but it is worth note that all these disturbances favor secondary hyperparathyroidism (25,26). The negative impact of chronic hyperparathyroidism on bone density, quality, and fractures is well recognized. Bone resorption exceeds bone formation, leading to a negative imbalance in bone turnover and bone loss, mostly in the cortical compartment (27). A study of 287 elderly Brazilian women identified a significant association between vitamin D deficiency, cortical bone loss, and fragility fractures (28). It is worth note that alterations in cortical bone have been associated with the severity of vertebral fractures (29) and that ASM group had decreased cortical thickness at the distal tibia and vertebral fractures.

Regarding sex steroids, it is worth mentioning that estradiol is the most important regulator of bone remodeling, including in men (30). The conversion of androgens to estrogens is the major source of circulating estrogens in men and depends on cytochrome P450 aromatase. This enzymatic reaction occurs in adipose tissue and other tissues including brain and bone cells, and the local concentration of estradiol seems to contribute significantly to skeletal homeostasis (31). We found decreased estradiol in ASM patients, probably related to lower aromatase activity. On the other hand, the decreased conversion might explain higher total testosterone levels in ASM patients. Meanwhile, the modulation of androgen action depends on SHBG, as an increase in sex steroids binding decreases the free and bioavailable fractions of testosterone. Age-related increase in SHBG and decrease in bioavailable testosterone and estradiol were associated to bone loss in Brazilian men (32) and also with fragility fractures in elderly men (33). Furthermore, only higher SHBG (but not decreased sex steroids) was associated with fractures in a large cohort described by Cawthon and cols. (34). We found bioavailable testosterone below the normal reference values in 22% of the ASM patients and in the lower 1st–2nd quartiles in 86%. The discrepancy between total and bioavailable testosterone in epileptic patients using ASMs has been attributed to an increase in SHBG (35,36). SHBG was not evaluated in the controls, but SHBG levels were in the highest quartile in about one third of ASM patients. Thus, these hormonal changes probably contributed to fractures in ASM patients.

Drug-resistant epilepsy significantly compromises the quality of life and restricts usual activities. Regarding the assessment of body composition, previous studies have used different methods and obtained varied results. Szałwińska and cols. (37) used bioelectrical impedance to study 60 epileptic patients (18–73 year old) who had at least one year of treatment but did not find significant differences in body mass, lean body mass, free fat mass, total fat, or visceral fat when compared to a control group. Sarangi and cols. (38) also evaluated epileptic patients using bioelectrical impedance and found no significant differences between any of the study groups in terms of body composition parameters, even when the parameters were compared based on new and old ASMs. In the present study, all participants were evaluated for body composition using DXA, the most recommended method for this purpose. Despite being less active according to IPAQ evaluation, ASM patients did not show alteration in body composition. Although they were not evaluated with test for muscle function, the measurement of total and appendicular lean mass did not suggest sarcopenia in ASM patients (39). Furthermore, there were

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no differences in body fat mass which could influence the conversion of testosterone into estradiol.

People with epilepsy are at risk of developing osteoporosis (4), and prolonged ASM use is a risk factor that contributes to this condition (40). Shiek Ahmad and cols. (41) described changes in BMD in pairs of 48 same-sex twin/age-matched sibling that were discordant regarding the use of ASM. Compared with non-IE-ASM users, ASM users had lower FN, TH, and trochanter BMD. In addition, ASM users had higher rates of TH and whole-body bone loss per year than non-users. In the present study, about 50% of the ASM and control groups had normal BMD, and only 3% of the ASM group was classified as having osteoporosis based on DXA. Thus, bone density added little information on bone fragility associated with fractures

Compared to the general population, the risk of fractures was described as 2 to 6 times higher according to Petty and cols. (42). Falls during seizures and imbalances caused by ASM side effects result in increases in forearm and hip fractures in patients with epilepsy. The hip may be at greater risk due to a fall pattern in which patients may not be able to use their arms protect themselves. Crisis-related fractures represent only 25% to 43% of all fractures, indicating that other factors also contribute to increased risk (43–45). In a meta-analysis, Vestergaard (46) evaluated the effects of epilepsy on changes in BMD and the risk of fractures, concluding that low BMD in patients with epilepsy did not sufficiently explain the increased risk of fractures and suggesting that the involvement of microarchitectural changes could lead to a reduction in bone strength. Rolvien and cols. (47) used DXA and HR-pQCT to perform a skeletal assessment of a six-year-old girl with epileptic encephalopathy accompanied by severe juvenile osteoporosis and multiple skeletal fractures and found combined trabecular and cortical bone loss. We did not find studies of HR-pQCT in epileptic males. Thus, our study is the first to demonstrate cortical bone changes in a group of ASM men, who also presented increased serum PTH and decreased bioavailable testosterone and estradiol, and more fractures than control group, even considering only non-clinical vertebral fractures, that were unrelated to falls (18% in ASM and 4.7% in controls).

We recognize as the main limitation the small number of ASM patients included, which limits the generalizability of the results. Meanwhile, the strengths of the study included the careful selection of the sample, which enabled the creation of a homogeneous group of patients; stability in terms of the use of medications for at least two years; and the exclusion of important confounding factors, such as menopause. Although it was not possible to test associations between bone, ASMs and hormones due to the small sample size, we considered the contribution of calciotropic hormones and cortical bone changes to the higher prevalence of fractures, especially morphometric vertebral fractures, which were not associated with falls.

In conclusion, people with epilepsy should be examined for fractures and hormonal changes. In most cases, there is no clinical fracture, and a morphometric fracture is present. Although studies can be conducted using VFA, spine X-rays may be more adequate due to cost and accessibility, since DXA contributed little to the assessment of bone fragility. Overall, the active investigation of morphometric fractures is recommended for ASM males 50 years and older to help identify patients with bone fragility and start anti-osteoporosis medications.

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Consent to participate: informed consent was obtained from all individual participants included in the study.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

LncRNA *GSEC* impedes the reprogramming of glucose metabolism in papillary thyroid carcinoma by inhibiting the IGF2BP2/GLUT1 axis

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ABSTRACT

Objective: To investigate the expression pattern of lncRNA *GSEC* in papillary thyroid carcinoma (PTC) and elucidate its functional role and molecular mechanism in regulating glycolytic metabolism and malignant progression. **Materials and methods:** The expression levels of the *GSEC* gene in thyroid cancer were determined through bioinformatics analysis of the TCGA database, and potential downstream regulatory genes were predicted. *GSEC* mRNA levels in normal thyroid cells and thyroid cancer cells were detected by qRT-PCR. Cellular functions, including proliferation, invasion, and migration, were evaluated using CCK-8 assays, Annexin-V/PI staining, western blot, EdU staining, Transwell assays, and scratch tests. Glycolytic activity was assessed by western blot, glucose uptake assays, and measurements of extracellular lactate levels. Subcutaneous and metastatic papillary thyroid carcinoma (PTC) models were established in nude mice to investigate the effects of lncRNA *GSEC* expression on tumor growth and metastasis. **Results:** LncRNA *GSEC* expression was significantly downregulated in thyroid cancer. Overexpression of *GSEC* in PTC-1 cells effectively inhibited their proliferation, invasion, and migration. *GSEC* downregulated GLUT1 expression by inhibiting IGF2BP2, thereby disrupting glycolysis and suppressing tumor growth and invasion. *In vivo* assay demonstrated that *GSEC* overexpression significantly reduced tumor glycolysis, retarded tumor growth, and inhibited metastasis. **Conclusion:** LncRNA *GSEC* is a key factor in the progression of PTC. By modulating the IGF2BP2/GLUT1 axis, *GSEC* affects cancer cell glycolysis, presenting a new potential target for metabolic intervention strategies.

Keywords: GLUT1; IGF2BP2; glucose metabolism; lncRNA *GSEC*; PTC

INTRODUCTION

The incidence of thyroid cancer, the most common endocrine malignancy, is sharply increasing worldwide (1,2). Papillary thyroid carcinoma (PTC) is the most frequent subtype, accounting for nearly 80% of all thyroid cancer cases (3). Typically, PTC is viewed as a relatively indolent malignancy. Standard treatments include thyroidectomy, radioactive iodine ablation, and thyroid-stimulating hormone (TSH) suppression therapy. These approaches generally yield a 10-year survival rate exceeding 95% (4). However, clinical data reveal that 5–20% of patients may still experience recurrence or develop local or distant metastases post-treatment. This recurrence rate significantly complicates the challenge of achieving a complete cure for PTC (5). Thus, determining the underlying mechanisms that drive PTC progression is crucial for enhancing clinical outcomes and improving patient prognosis.

Cancer cells often rewire their metabolism to support rapid growth and spread. Even when oxygen is abundant, these cells tend to break down glucose through a process called aerobic glycolysis, which is

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known as the "Warburg effect" (6). This unusual metabolic strategy not only fuels the rapid division of cells but also helps them invade surrounding tissues and migrate to distant sites. By altering cellular signaling pathways and the tumor microenvironment, this metabolic shift ultimately drives cancer progression (7). In PTC cells, the upregulation of key glycolytic enzymes, including PKM2, and glucose transporters, such as GLUT1 and GLUT3, suggests robust glycolytic metabolism (8). This metabolic shift is likely driven by the overexpression of hypoxia-inducible factor 1α (HIF-1α), which promotes glycolytic activity and supports the manifestation of the Warburg effect in PTC (9). However, despite indications of unusually active glycolytic metabolism in PTC, the regulatory mechanisms at work remain unclear.

Long noncoding RNAs (lncRNAs) are RNA molecules with transcripts of approximately 200 base pairs that do not encode proteins; they can regulate gene expression at multiple levels and are closely related to the development, metastasis, and prognosis of many cancers (10,11). Hence, lncRNAs have become a popular research topic in the field of cancer in recent years. Among the vast array of lncRNAs, *GSEC* has emerged as a molecule of significant interest. *GSEC* features a distinctive G-quadruplex structure that enables it to modulate gene expression and cellular functions through interactions with proteins or other RNA molecules (12-14). *GSEC*, an lncRNA, interacts with the RNA helicase *DHX36* to influence cancer cell migration and invasion (13); it also upregulates PFKFB3, increasing glycolytic metabolism in sepsis and driving the activation of inflammation in neutrophils (15). These results highlight the potential regulatory function of *GSEC* in glycolysis. Research on the role of lncRNA *GSEC* in PTC is rather scarce, and how *GSEC* affects the development of PTC and whether it is involved in the regulation of glycolytic metabolism in PTC have not yet been determined.

Motivated by the gaps in knowledge regarding lncRNA *GSEC*, our research focused on its actions within the context of PTC and its effects on glycolytic processes. Our experiments demonstrated that *GSEC* levels are notably lower in PTC tissues. Upon overexpression of *GSEC* in PTC cells, we observed a significant decrease in the activity of the IGF2BP2/GLUT1 axis, which in turn halted glycolytic reprogramming and the malignant trajectory of cancer. In summary, our study not only highlights *GSEC* as a key player in PTC progression but also reveals its molecular links to cancer cell glycolysis, paving the way for the development of *GSEC*-targeted metabolic interventions for PTC.

MATERIALS AND METHODS

Sample collection

A total of 5 pairs of PTC tissue samples and adjacent noncancerous tissue samples were collected for this study. All the samples were obtained from The Affiliated Yixing Hospital of Jiangsu University between 2024.12 and 2025.5. The sample collection process strictly adhered to the guidelines of the hospital's Ethics Committee, and informed consent was obtained from all patients. The inclusion criteria were as follows: patients pathologically diagnosed with PTC; patients aged between 18 and 80 years; patients with no history of other malignant tumors; and patients who had not undergone radiotherapy or chemotherapy prior to surgery. Adjacent noncancerous tissues were collected from sites >0.8 cm away from the tumor edge. All surgically resected PTC tissues and adjacent tissue samples were processed within 30 minutes after collection. Samples intended for western blot analysis were immediately frozen in liquid nitrogen and subsequently transferred to a -80 °C freezer for long-term storage. Samples for IHC (immunohistochemistry) were fixed in 4% paraformaldehyde (PFA) and stored at 4 °C.

Bioinformatics

We retrieved transcriptomic data for thyroid cancer (THCA) and normal thyroid tissues from the TCGA (https://portal.gdc.cancer.gov/) and GTEx (https://www.gtexportal.org) databases, which included 425 normal and 513 cancer samples. Differential expression analysis was executed using the "edgeR" package, with |logFC| > 1.5 and padj < 0.05 as the thresholds to detect differentially expressed mRNAs. The target gene *GSEC* was identified through an exhaustive literature review. The potential target genes of lncRNA

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GSEC were predicted using the starBase website. Pearson correlation analysis was subsequently used to evaluate the correlation between lncRNA *GSEC* and IGF2BP2 expression.

Cell culture

The normal human thyroid cell line Nthy-ori 3-1 (CL-0817) and the thyroid cancer cell lines HTh-7 (CL-0647), KTC-1 (CL-0649), and TPC-1 (CL-0643) were obtained from Procell Bio Co., Ltd. (China). The human thyroid squamous cell carcinoma cell line SW579 (SNL-451) was purchased from SUNNBIO (China). Nthy-ori 3-1 and KTC-1 cells were cultured in complete RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin (P/S). HTh-7 and TPC-1 cells were cultured in complete DMEM supplemented with 10% FBS and 1% P/S. SW579 cells were cultured in Leibovitz's L-15 medium supplemented with 10% FBS and 1% P/S. Nthy-ori 3-1, HTh-7, KTC-1, and TPC-1 cells were cultured at 37 °C with 5% CO_2, while SW579 cells were cultured at 37 °C in 100% air.

Cell transfection

The pcDNA3.1 empty vector (Vector), pcDNA3.1-*GSEC* expression plasmid (LncRNA *GSEC*-OE), pcDNA3.1-*IGF2BP2* expression plasmid (*IGF2BP2-OE*), pLKO.1-Puro empty vector (sh-NC), and pLKO.1-*GLUT1* knockdown plasmid (sh-*GLUT1*) were designed and synthesized by GenePharma (China). In accordance with the manufacturer's instructions, these plasmids were transfected into TPC-1 cells using Lipofectamine 2000 (Invitrogen, USA). After 48 hours of transfection, the culture medium was replaced with complete medium supplemented with 800 μg/mL G418 (overexpression group) or 1.5 μg/mL puromycin (knockdown group), and the medium was changed every 3 days. The screening was continued for 14 days until individual clones were visible. We selected individual clones for expansion culture and maintained the concentration at half (400 μg/mL G418 or 0.5 μg/mL puromycin). After wtable expression was verified by qRT–PCR and western blot analysis, the cells were used for subsequent experiments (passages ≤ 15 generations).

RNA extraction and qRT–PCR

To isolate total RNA from tissue samples and cultured cells, we used Ribozol RNA extraction reagent (Thomas Scientific, USA). For the reverse transcription step, a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) was used. qRT–PCR was subsequently performed using SYBR Green PCR Master Mix (Applied Biosystems) on an Applied Biosystems real-time PCR system. β-Actin was selected as the reference gene, and the relative expression levels of the target genes were determined using the $2^{-\Delta\Delta Ct}$ method. The primers used are detailed in **Table 1**.

Table 1. Primer sequence

Gene	Sequence (5′-3′)
β-*actin*	Forward: GGCTGTATTCCCCTCCATCG
	Reverse: CCAGTTGGTAACAATGCCATGT
GSEC	Forward: CCCACCCTGCTCACCTAAAC
	Reverse: TTCAATTTGAGGCCCCAGGA
GLUT1	Forward: GGCCAAGAGTGTGCTAAAGAA
	Reverse: ACAGCGTTGATGCCAGACAG
IGF2BP2	Forward: AGTGGAATTGCATGGGAAAATCA
	Reverse: CAACGGCGGTTTCTGTGTC

CCK-8 assay

Cell viability was assessed using a CCK-8 kit (Dojindo, Japan). We seeded approximately 1×10^4 TPC-1 cells into each well of a 96-well plate. To track cell proliferation, we added 100 μL of a 10% CCK-8 solution to the cells at 0, 24, and 48 hours and incubated them for 3 hours. We then measured the absorbance at 450 nm using a Power Wave XS microplate reader (Bio-Tek, USA).

Apoptosis analysis

Transfected TPC-1 cells were plated in 6-well plates at a density of 1×10^6 cells per well and cultured for 48 hours at 37 °C. Apoptosis was assessed using an Annexin V-FITC/PI kit (Yeasen, China). The cells were resuspended in 100 μL of 1× binding buffer after the supernatant was removed. Afterward, 5 μL of Annexin V-FITC and 10 μL of PI were added, mixed gently, and incubated in the dark at room temperature for 10–15 minutes. Finally, 400 μL of 1× binding buffer was added, and the cells were analyzed using a BD Calibur flow cytometer.

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Western blot analysis

We extracted total protein from tissue samples and cultured cells using a RIPA Lysis Buffer Kit (Santa Cruz, USA). The proteins were separated by SDS–PAGE and transferred to PVDF membranes. The membranes were incubated with primary antibodies overnight at 4 °C. The primary antibodies used were anti-Bcl-2 (Abcam; ab182858), anti-Bax (Abcam; ab32502), anti-cleaved caspase3 (Abcam; ab32042), anti-MMP2 (Abcam; ab92536), anti-MMP9 (Abcam; ab76003), anti-GLUT1 (Abcam; ab115730), anti-hexokinase II (Abcam; ab209847), anti-PKM2 (Thermo Fisher; PA5-28700), anti-IGF2BP2 (Abcam; ab124930), and anti-β-actin (Abcam; ab8226). The membranes were then incubated with an HRP-conjugated secondary antibody (Abcam; ab205718) for 2 hours at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) kit (Takara, Japan), and protein expression was quantified using Quantity One software.

EdU staining

Cell proliferation was assessed using an EdU Proliferation Kit (Abcam; ab19801). Transfected TPC-1 cells were seeded into 96-well plates at 1×10^4 cells per well and cultured for 48 hours at 37 °C. The EdU reaction mixture provided in the kit was added to the cells, which were then incubated in the dark for 30 minutes. Excess dye was removed by washing with PBS, and cell proliferation was detected using a flow cytometer (BD Calibur, USA).

Cell migration assay

TPC-1 cells were plated in 48-well plates and grown to confluence. Sterile 200 µL pipette tips were used to introduce scratches into the monolayer. The wells were then washed with PBS to clear away cell debris. The cells were then maintained in serum-free or low-serum medium at 37 °C. The scratch areas were imaged at 0 and 48 hours using a microscope. The scratch width and healing area were measured using ImageJ software.

Transwell assay

Following starvation, the transfected TPC-1 cells were adjusted to an appropriate density and seeded into the upper chamber of Transwell inserts coated with Matrigel. The lower chamber contained a chemoattractant. After 48 hours of culture, the cells were fixed with 4% paraformaldehyde (PFA) for 30 minutes and stained with crystal violet for 10 minutes. The cells were subsequently washed 2–3 times with PBS. The number of cells that migrated to the lower side of the membrane was determined by counting the cells in 3–5 random fields under a microscope.

Glucose uptake determination

TPC-1 cells subjected to various transfection strategies were incubated with 100 µM 2NBDG (APExBIO, USA) at 37 °C for 30 minutes to assess glucose uptake. After incubation, the cells were centrifuged at 1000 rpm for 5 minutes, and excess 2NBDG was removed by washing with PBS. Glucose uptake was then analyzed using a flow cytometer (BD Calibur, USA).

Lactate concentration assessment

To determine the lactate levels in the supernatant of the transfected TPC-1 cells, we used a Lactate Assay Kit (Beyotime, China). We collected the supernatant and added 50 µL to each well of a 96-well plate. We then added 50 µL of WST-8 colorimetric working solution to each well, mixed well, and incubated the plate at 37 °C in the dark for 30 minutes. After incubation, we measured the absorbance at 450 nm and calculated the lactate concentration based on a standard curve.

In vivo assay

We obtained 12 male BALB/c nude mice (5–6 weeks old) from GemPharmatech (China) and acclimated them for two weeks under standard conditions (12-hour light/dark cycle; 55% relative humidity). Human PTC cells, transfected with either a control vector or lncRNA *GSEC*-OE, were injected subcutaneously into the flanks of the mice at a dose of 2×10^7 cells per mouse to create a PTC xenograft model. The tumor volumes were measured every five days, and the mice were sacrificed after 20 days. The tumors were then excised, weighed, photographed, and fixed in 4% paraformaldehyde for histological analysis. For the metastasis model, luciferase-labeled PTC cells were injected

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into the tail veins of mice, and their *in vivo* growth and metastasis were tracked using a small-animal *in vivo* imaging system at designated time points.

Histological analysis

Patient samples or tumor tissues collected from tumor-bearing mice were fixed in 4% PFA for 24 hours, embedded in paraffin and cut into 5 μm sections. Hematoxylin and eosin (H&E) staining and TUNEL staining were conducted to explore pathological changes and proliferation in the tumors. IHC staining was performed to detect the expression of anti-cleaved caspase3 (Abcam; ab32042) and anti-IGF2BP2 (Abcam; ab124930) in the tumor tissue.

Statistical analysis

All experiments were conducted in triplicate to ensure reliability. The results are presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS v.19.0 software (IBM, USA). Student's *t* test was used to compare two groups, while one-way ANOVA was used for comparisons among multiple groups. Pearson correlation analysis was performed to evaluate the correlation between *GSEC* and IGF2BP2 expression levels. Significance was defined as $P < 0.05$ (*) or $P < 0.01$ (**).

RESULTS

Reduced expression of lncRNA *GSEC* in thyroid cancer

We initiated our research by leveraging bioinformatics tools to examine lncRNA *GSEC* expression in thyroid cancer. Compared with that in normal thyroid tissues, *GSEC* expression in thyroid cancer tissues was reduced ($P<2.22e-16$) (**Figure 1A**). Subsequent analysis of *GSEC* expression in normal human thyroid cells (Nthy-ori 3-1) and diverse thyroid cancer cell lines (HTh-7, KTC-1, TPC-1, and SW579) revealed a marked disparity. The percentage of *GSEC* was notably greater in Nthy-ori 3-1 cells but markedly lower in the cancer cell lines (**Figure 1B**). These results indicate that *GSEC* expression is generally downregulated in thyroid cancer cells. In this study, we focused on further investigating the potential role of lncRNA *GSEC* in TPC-1 cells.

Overexpression of lncRNA *GSEC* inhibits PTC progression

To elucidate the impact of *GSEC* on PTC progression, we overexpressed the *GSEC* gene in TPC-1 cells and successfully established a cell line with high *GSEC* expression, designated lncRNA *GSEC*-OE (**Figure 2A**). We first examined the effect of *GSEC* expression on the viability of TPC-1 cells. The results of the CCK-8 assay (**Figure 2B**) revealed that after the culture period exceeded 24 hours, the viability of TPC-1 cells overexpressing *GSEC* was significantly lower than that of the control and empty vector (Vector) groups. Additionally, apoptosis assays (**Figures 2C–D**) revealed that the apoptosis rate of TPC-1 cells increased significantly upon overexpression of *GSEC*, exceeding 10%. After *GSEC* was overexpressed, western blot analysis revealed a significant decrease in the expression of the antiapoptotic protein Bcl-2, along with a marked increase in the expression of the proapoptotic proteins Bax and cleaved caspase3 (**Figure 2E**). This shift in protein expression suggests that *GSEC* overexpression can trigger apoptosis. To further assess cell proliferation, we used EdU staining and flow cytometry. The results revealed that the percentage of EdU-positive cells was significantly lower in the lncRNA *GSEC*-OE group than in the control and vector groups (**Figures 2F–G**), indicating that *GSEC* overexpression inhibited cell proliferation. We also investigated the invasive and migratory capabilities of the cells. Using Transwell assays and western blot analysis, we observed that the invasive potential of cells with

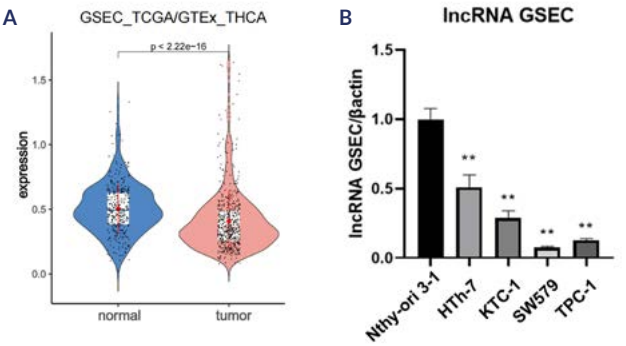


Figure 1. Reduced expression of LncRNA *GSEC* in thyroid cancer. (**A**) Expression levels of LncRNA *GSEC* in thyroid cancer obtained from the TCGA database; (**B**) qRT-PCR was performed to detect LncRNA *GSEC* expression in human normal thyroid cells (Nthy-ori 3-1) and human thyroid cancer cell lines (HTh-7, KTC-1, SW579, and TPC-1). *$P < 0.05$; **$P < 0.01$.

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elevated *GSEC* expression substantially decreased (**Figures 2H–I**). This reduction was accompanied by a notable decrease in the expression of matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) (**Figure 2J**). Furthermore, wound-healing assays revealed that the migration rate of the lncRNA *GSEC*-OE group was only approximately 20% at 48 hours, whereas that of the control and vector groups was nearly 70% (**Figures 2K-L**). These results highlight the inhibitory effect of *GSEC* overexpression on cell invasion and migration.

Given that metabolic reprogramming is a hallmark of cancer progression, we explored how *GSEC* expression affects glycolysis. Our qRT–PCR analysis revealed that overexpressing *GSEC* significantly reduced *GLUT1* mRNA levels (**Figure 2M**). Western blot analysis further revealed that the protein levels of GLUT1, HK2, and PKM2 were notably lower in *GSEC*-overexpressing cells than in control and vector-transfected cells (**Figure 2N**). These proteins are pivotal for glycolysis, and their reduced expression suggests that *GSEC* overexpression may disrupt the glycolytic pathway in cancer cells. The overexpression of *GSEC* notably affected cancer cell metabolism. Specifically, flow cytometry using 2-NBDG revealed a significant reduction in glucose uptake by cancer cells (**Figures 2O-P)**. This metabolic shift was further evidenced by a marked decrease in lactate production in the cell supernatant (**Figure 2Q**). Collectively, these results indicate that *GSEC* likely influences cancer progression by affecting glycolysis.

Overexpression of lncRNA *GSEC* downregulates IGF2BP2 expression

To elucidate the molecular underpinnings of how lncRNA *GSEC* affects cancer progression, we used the starBase platform to pinpoint IGF2BP2 as a likely target regulated by *GSEC* (**Figure 3A**). Further scrutiny via the GEPIA database revealed a pronounced negative correlation between *GSEC* and *IGF2BP2* expression in PTC (P value = 5.4e-08; R = -0.24) (**Figure 3B**). Western blot analysis of 5 paired PTC samples and their adjacent nontumor tissues revealed significantly elevated expression of IGF2BP2 in tumor tissues (**Figure 3C**). We subsequently performed IHC staining on 5 pairs of samples and detected abnormal upregulation of IGF2BP2 in cancer tissues (**Figure 3D**). To corroborate this finding at the cellular level, we overexpressed *GSEC* and observed a substantial decrease in both the mRNA and protein levels of IGF2BP2 (**Figures 3E–F**). Collectively, these data suggest that the effects of lncRNA *GSEC* may involve targeting and suppressing IGF2BP2 expression.

IGF2BP2 drives cancer progression via GLUT1 upregulation

IGF2BP2 can increase GLUT1 expression by stabilizing its mRNA (16). Based on this mechanism, we hypothesized that lncRNA *GSEC* might influence glycolytic levels through an IGF2BP2-mediated GLUT1 regulatory mechanism. To investigate whether IGF2BP2 regulates *GLUT1* expression in PTC, we established *IGF2BP2* overexpression and knockdown models in TPC-1 cells combined with intervention with the GLUT1 inhibitor BAY-876. Western blot results (**Figure 4A**) revealed that *IGF2BP2* overexpression significantly upregulated GLUT1 protein levels, whereas *GLUT1* knockdown in the context of IGF2BP2 overexpression markedly reduced its expression, suggesting that IGF2BP2 positively regulates GLUT1. Further functional assays demonstrated that *IGF2BP2* overexpression significantly increased cell viability, whereas *GLUT1* knockdown or BAY-876 treatment reversed this effect (**Figure 4B**), indicating that IGF2BP2 critically influences cancer cell activity via GLUT1 regulation. In the migration and invasion assays, the Transwell results (**Figures 4C and D**) revealed that compared with control cells, oe-IGF2BP2 cells exhibited significantly increased invasive capacity. However, GLUT1 knockdown or BAY-876 treatment substantially suppressed this pro-invasive effect in oe-IGF2BP2 cells. A similar trend was observed in the wound-healing assay (**Figure 4E**), where IGF2BP2 promoted cell migration, whereas GLUT1 knockdown or inhibition significantly attenuated this effect. In summary, these findings demonstrate that IGF2BP2 enhances the proliferation, migration, and invasion of TPC-1 cells by upregulating GLUT1 expression, suggesting that the IGF2BP2/GLUT1 axis plays a critical role in PTC progression.

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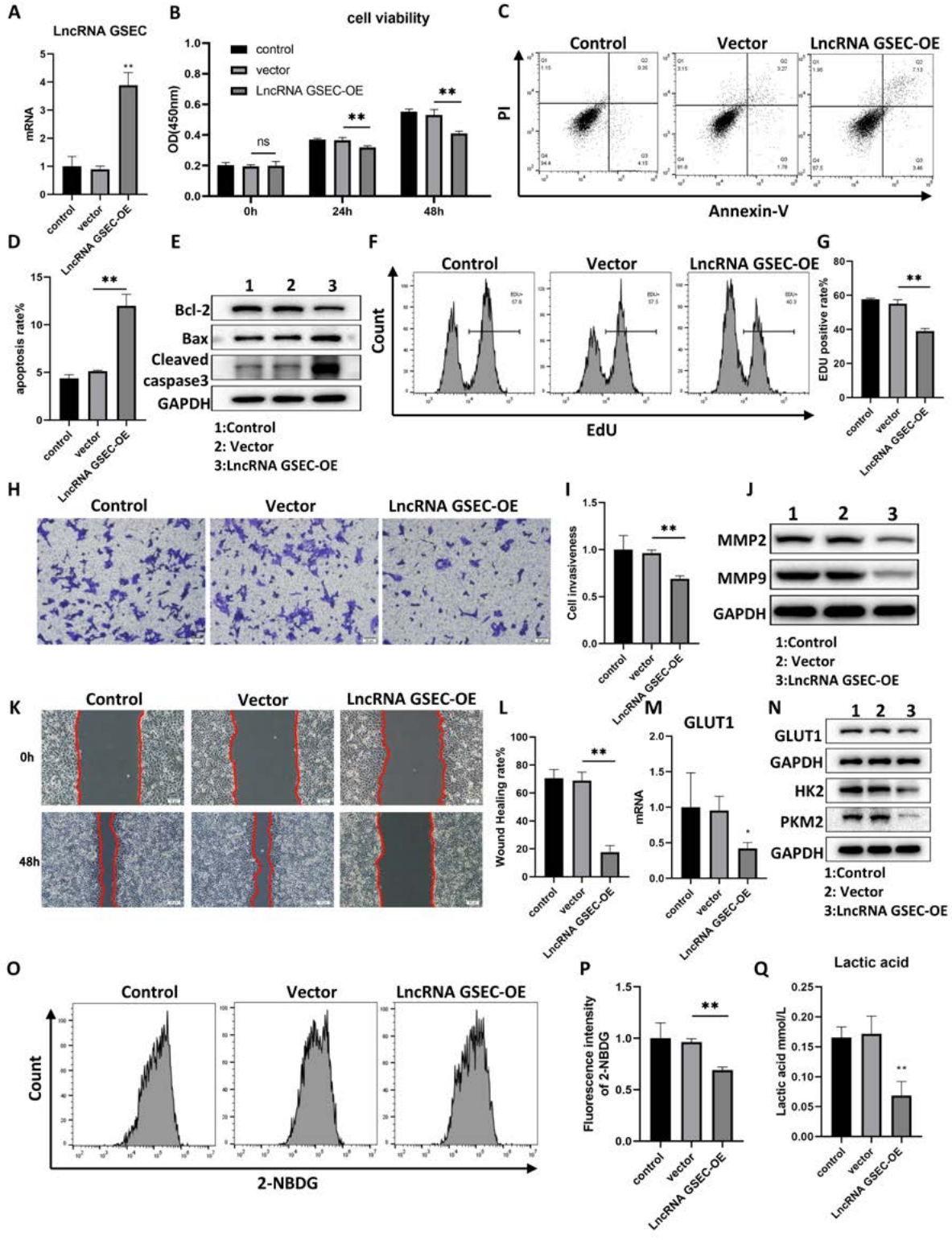


Figure 2. Overexpression of LncRNA *GSEC* inhibits PTC progression. (**A**) qRT-PCR analysis showed increased LncRNA *GSEC* mRNA levels in TPC-1 cells transfected with LncRNA *GSEC*-OE; (**B**) CCK-8 assay revealed decreased cell viability in TPC-1 cells at different time points; (**C-D**) Annexin-V/PI staining showed increased apoptosis levels in TPC-1 cells transfected with LncRNA *GSEC*-OE; (**E**) WB analysis showed changes in Bcl-2, Bax, and Cleaved caspase-3 protein levels in TPC-1 cells; (**F-G**) EdU staining revealed reduced proliferation in TPC-1 cells; (**H-I**) Transwell assay showed decreased invasion ability in TPC-1 cells; (**J**) WB analysis showed changes in MMP2 and MMP9 protein levels in TPC-1 cells; (**K-L**) Wound healing assay showed reduced migration ability in TPC-1 cells; (**M**) qRT-PCR analysis showed changes in *GLUT1* expression in TPC-1 cells transfected with LncRNA *GSEC*-OE; (**N**) WB analysis showed changes in GLUT1, HK, and PKM2 protein levels in TPC-1 cells; (**O-P**) Flow cytometry showed changes in glucose uptake ability in TPC-1 cells.; (**Q**) Lactate levels in the supernatant of TPC-1 cells were measured. **P* < 0.05; ***P* < 0.01.

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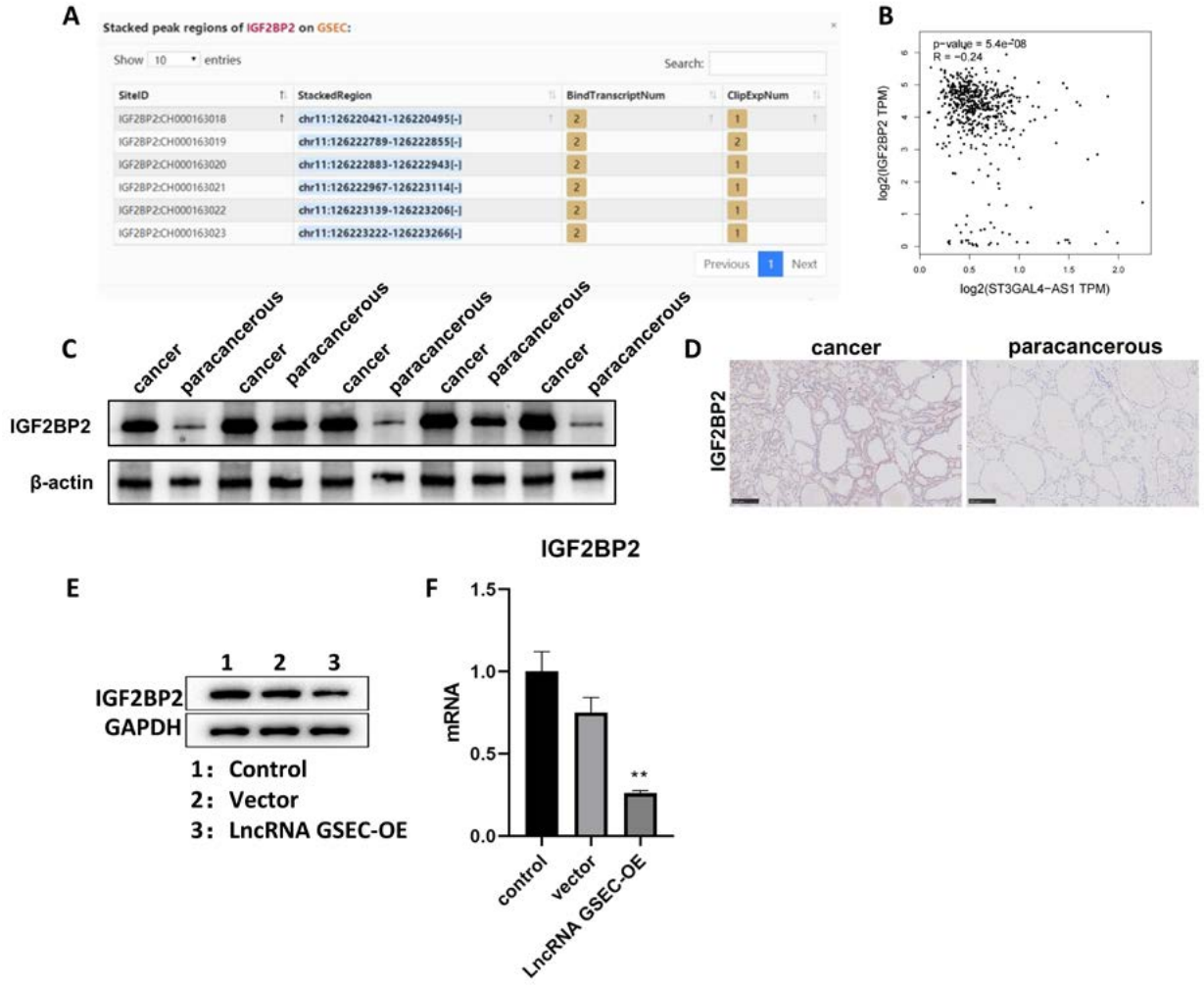


Figure 3. Overexpression of LncRNA *GSEC* downregulates IGF2BP2 expression. (A) Predicted target genes of LncRNA *GSEC* identified via Starbase; (B) Correlation analysis between LncRNA *GSEC* and *IGF2BP2* expression; (C-D) WB and IHC staining examined IGF2BP2 expression in tumor tissues and paired adjacent non-tumor tissues; (E-F) qRT-PCR and WB analysis revealed decreased *IGF2BP2* mRNA and protein levels in TPC-1 cells overexpressing *GSEC*. $^{**}P < 0.01$.

LncRNA *GSEC* regulates cancer cell glucose uptake via IGF2BP2

Having established that lncRNA *GSEC* upregulates IGF2BP2 expression and that IGF2BP2 promotes cancer progression through GLUT1, we further investigated whether *GSEC* exerts its regulatory effects on glycolytic metabolism and malignant phenotypes in an *IGF2BP2*-dependent manner. We established four cellular models—vector1, lncRNA *GSEC*-OE, lncRNA *GSEC*-OE+vector2, and lncRNA *GSEC*-OE+IGFB2BP2-OE—and measured the expression levels of relevant genes using qRT–PCR (Figures 5A–C). We found that *GSEC* overexpression significantly reduced the expression of IGFB2BP2 and GLUT1. However, when we transfected these cells with *IGFB2BP2*-OE, the expression of both *IGFB2BP2* and *GLUT1* was significantly restored (Figures 5B–C). This trend was also confirmed at the protein level. Western blot analysis revealed that the protein levels of HK2 and PKM2, as well as those of IGF2BP2 and GLUT1, were restored after *IGF2BP2*-OE reconstitution (Figure 5D). Systemic lactate production and glucose uptake were enhanced following *IGF2BP2*-OE transfection (Figures 5E–F). These results suggest that lncRNA *GSEC* inhibits glycolysis by downregulating the expression of IGF2BP2.

We evaluated the malignancy of cancer cells by examining their proliferation, invasion, and metastasis capabilities. The results of the CCK-8 assay revealed that compared with the lncRNA *GSEC*-OE + *IGFB2BP2*-OE group, the lncRNA *GSEC*-OE group had significantly lower cell viability. These findings

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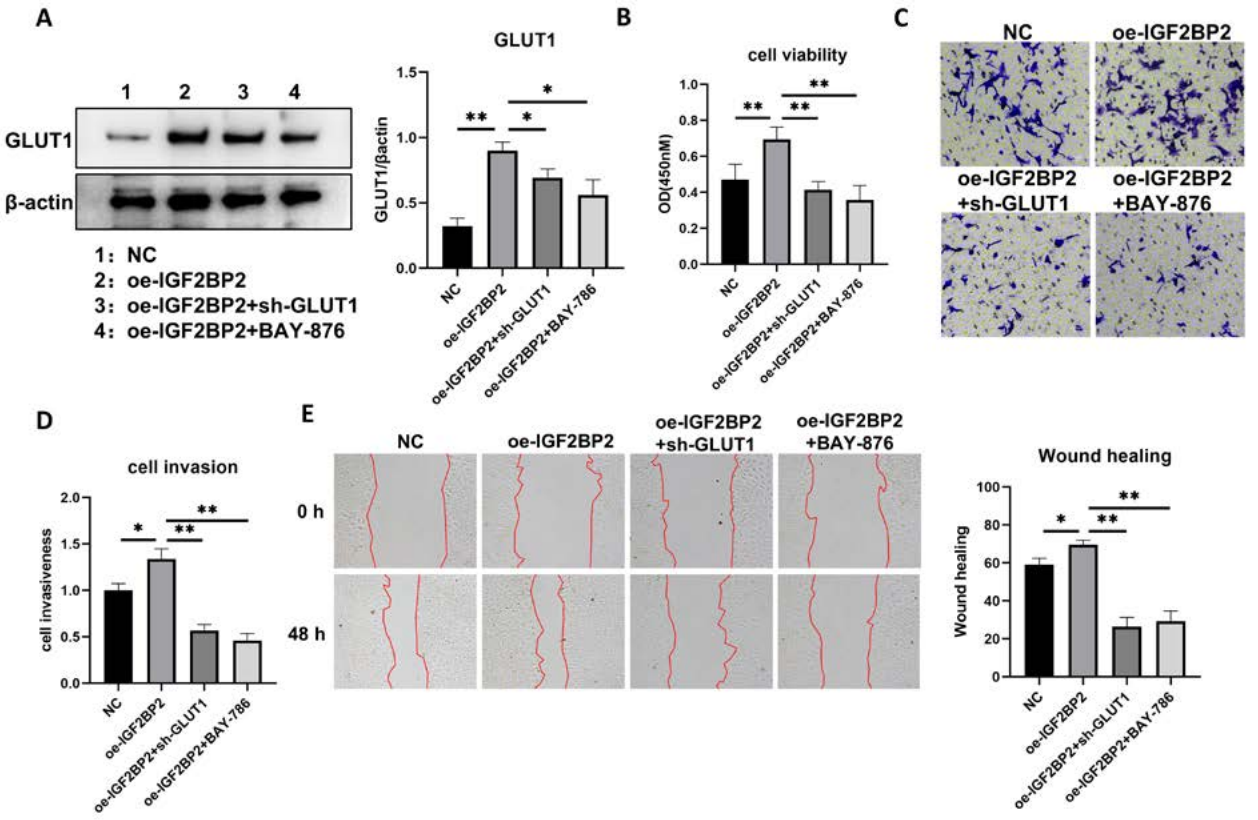


Figure 4. IGF2BP2 promotes cancer progression by upregulating GLUT1 expression. (**A**) WB analysis of GLUT1 protein expression in treated cells; (**B**) CCK-8 assay measuring cell viability of TPC-1 cells under different treatments; (**C, D**) Transwell assay evaluating cell invasive capability; (**E**) Wound-healing assay assessing cell migratory ability. **P* < 0.05; ***P* < 0.01.

suggest that *GSEC* expression inhibits cell proliferation, whereas IGFB2BP2 expression can counteract this effect (**Figure 5G**). The results of apoptosis assays further revealed that IGFB2BP2 expression weakened *GSEC*-induced apoptosis (**Figures 5H–I**). These findings highlight the potential therapeutic implications of targeting *GSEC* and IGFB2BP2 in cancer treatment. EdU staining also revealed a similar trend, with the proliferation rate of cells in the lncRNA *GSEC*-OE group being significantly lower than that in the lncRNA *GSEC*-OE + *IGFB2BP2*-OE group (**Figure 5J**). Consistent with these findings, invasion assays (**Figures 5K–L**) and wound-healing experiments (**Figure 5M**) revealed that the invasive and migratory abilities of the lncRNA *GSEC*-OE + *IGFB2BP2*-OE cells were significantly enhanced. These results collectively suggest that lncRNA *GSEC* can inhibit cancer progression by downregulating the expression of IGFB2BP2, thereby affecting glycolysis in cancer cells.

Overexpression of lncRNA *GSEC* suppresses PTC progression in nude mice

To assess the *in vivo* effects of *GSEC* on cancer progression, we established a mouse PTC xenograft model. Nude mice were randomly assigned to one of two groups, and TPC-1 cells transfected with either vector or lncRNA *GSEC*-OE were injected subcutaneously into their right flanks. Tumor growth was closely monitored throughout the experiment. As shown in **Figures 6A-D**, tumors in the control group grew rapidly and were significantly larger, whereas those in the lncRNA *GSEC*-OE group grew more slowly and were smaller at the end of the experiment. H&E staining of tumor tissues revealed that while the tumor architecture was intact in the vector group, the tumor architecture was noticeably necrotic in the lncRNA *GSEC*-OE group (**Figure 6E**). These findings were corroborated by TUNEL staining, which revealed a higher incidence of apoptosis in the lncRNA *GSEC*-OE group (**Figure 6E**), suggesting that *GSEC* overexpression

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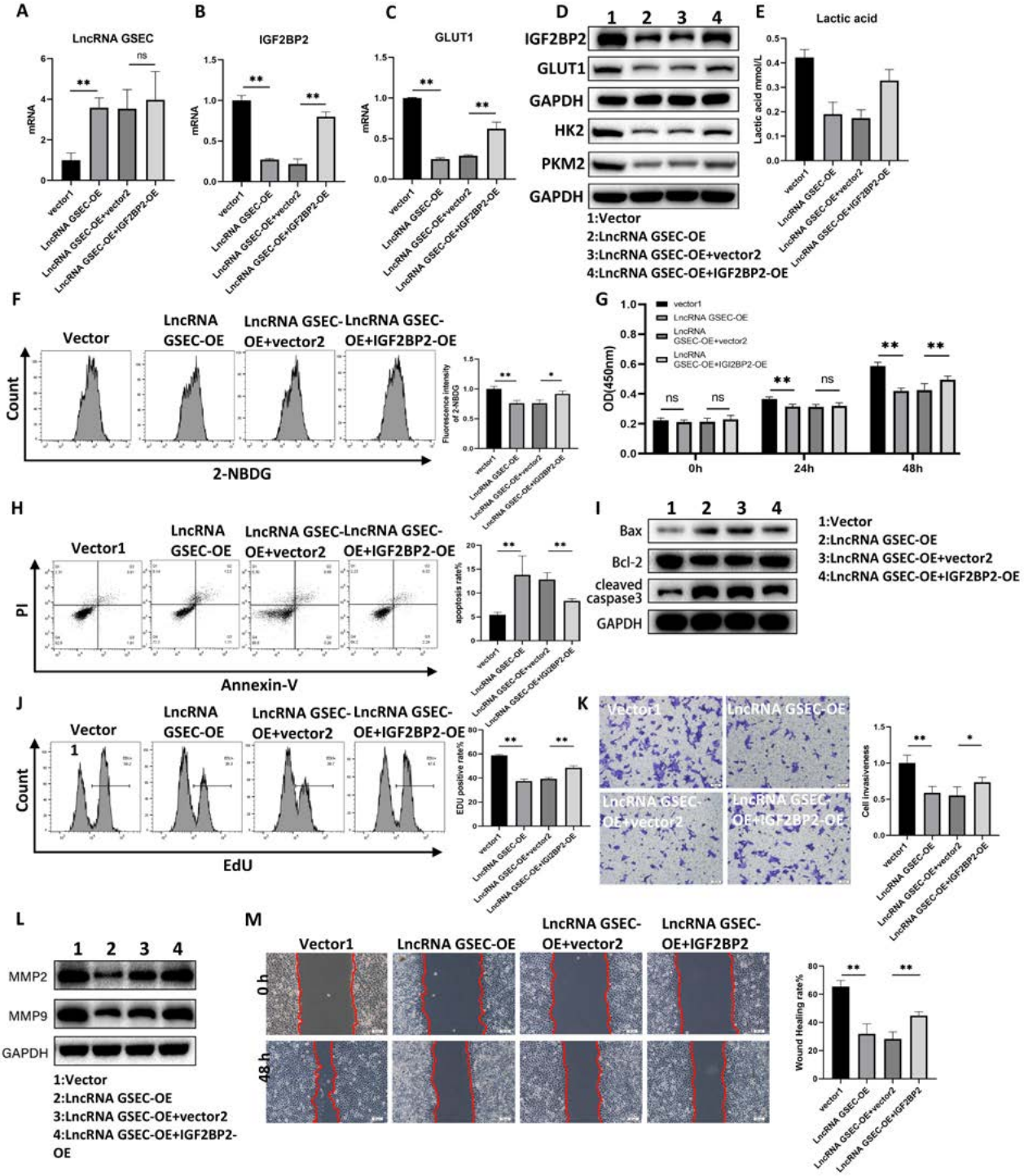


Figure 5. LncRNA *GSEC* regulates cancer cell glucose uptake via IGF2BP2. (A-C) qRT-PCR analysis of mRNA levels of *GSEC*, *IGF2BP2*, and *GLUT1* in TPC-1 cells; (D) WB analysis of protein expression levels of IGF2BP2, GLUT1, HK, and PKM2 in TPC-1 cells; (E) Lactate levels in the supernatant of TPC-1 cells; (F) Glucose uptake in TPC-1 cells assessed by flow cytometry; (G) Cell viability of TPC-1 cells at different time points assessed by CCK-8 assay; (H) Apoptosis levels in TPC-1 cells assessed by Annexin-V/PI staining; (I) WB analysis of Bcl-2, Bax, and Cleaved caspase-3 protein levels in TPC-1 cells; (J) Proliferation of TPC-1 cells assessed by EdU staining; (K) Invasion ability of TPC-1 cells assessed by Transwell assay; (L) WB analysis of MMP2 and MMP9 protein levels in TPC-1 cells; (M) Migration ability of TPC-1 cells assessed by wound healing assay. **P* < 0.05; ***P* < 0.01.

effectively inhibits PTC growth. Furthermore, *GSEC* mRNA expression was significantly upregulated in the lncRNA *GSEC*-OE group, verifying the successful overexpression of *GSEC* (Figure 6F). Moreover, the downregulation of IGF2BP2, GLUT1, HK2, and PKM2 expression in the lncRNA *GSEC*-OE group indicated

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that *GSEC* overexpression suppressed glycolysis in cancer cells (Figures 6G–I). To assess the impact of *GSEC* expression on cancer metastasis, we established an *in vivo* PTC metastasis model by intravenously injecting luciferase-labeled TPC-1 cells into mice. Live imaging revealed that the vector group exhibited robust lung metastasis, whereas the lncRNA *GSEC* group showed significantly reduced metastatic activity, as evidenced by lower avg counts (Figures 6J and K). These results indicate that *GSEC* overexpression can effectively diminish the metastatic potential of tumor cells *in vivo*.

DISCUSSION

In our study, we initially noted a pronounced downregulation of lncRNA *GSEC* in PTC. We found that *GSEC* expression can curb cancer cell progression and glycolysis. Using starBase, we identified IGF2BP2 as a

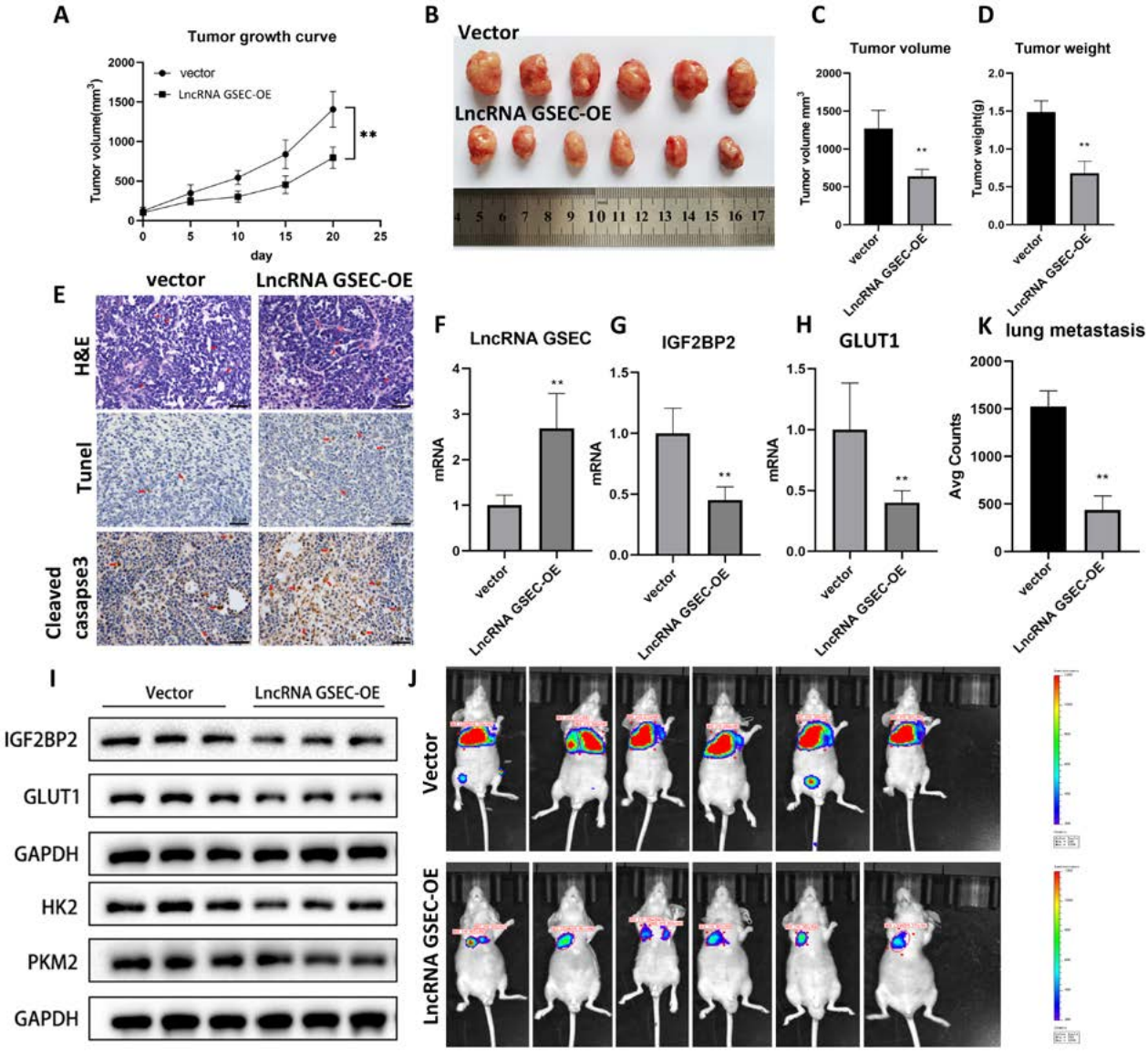


Figure 6. Overexpression of LncRNA *GSEC* suppresses TPC progression in nude mice. (**A**) Changes in tumor volume in mice during treatment; (**B**) Representative images of tumors excised from mice; (**C-D**) Tumor volume (**C**) and weight (**D**) at the end of treatment; (**E**) Histological staining of tumor tissues with H&E, TUNEL, and Cleaved caspase-3; (**F-H**) qRT-PCR analysis revealed mRNA expression levels of LncRNA *GSEC*, *IGF2BP2*, and *GLUT1* in tumor tissues; (**I**) WB analysis showed protein levels of IGF2BP2, GLUT1, HK2, and PKM2 in tumor tissues; (**J-K**) Representative bioluminescent images (**J**) and quantitative analysis of lung metastasis (**K**) in mice. **P* < 0.05; ***P* < 0.01.

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downstream target of *GSEC*. Our data show that *GSEC* could impede glycolytic reprogramming and malignant progression in cancer cells by inhibiting the IGF2BP2/GLUT1 axis. Our findings underscore the pivotal role of lncRNA *GSEC* in PTC progression and shed new light on the metabolic regulatory mechanisms in PTC.

LncRNA *GSEC*, also known as *DCPS-AS1* or *ST3GAL4-AS1*, is a long noncoding RNA containing a G-quadruplex structure that was first identified by Matsumura and cols. (13) in colon cancer. Previous studies have shown that *GSEC* is highly expressed in various types of tumors, such as colon cancer and lung adenocarcinoma, and can participate in tumor occurrence and progression through multiple molecular mechanisms (12,13). Among them, *GSEC* can regulate the availability of miRNAs through a competitive endogenous RNA (ceRNA) mechanism, thereby affecting the expression and function of downstream target genes (14). For example, in triple-negative breast cancer (TNBC), *GSEC* upregulates the expression of AXL by adsorbing miR-202-5p, thereby promoting the proliferation, migration, and invasion of tumor cells (17). In addition, increasing evidence suggests that *GSEC* is involved in regulating cellular metabolic reprogramming. For example, in sepsis models, *GSEC*s can promote inflammatory activation in neutrophils by regulating PFKFB3-mediated glycolytic metabolism (15). The results of this study suggest that *GSEC*-regulated glycolysis may be an important mechanism involved in disease progression. Notably, the above studies generally support the role of *GSEC* in promoting cancer or pathological progression in most diseases or tumors. However, an increasing number of studies have shown that the biological functions of lncRNAs often exhibit significant tissue specificity and tumor microenvironment dependence and that the same lncRNA may play vastly different regulatory roles in different tumor types (18). For example, the sialyltransferase ST3GAL4 can promote tumor progression by enhancing aerobic glycolysis (19). As the antisense transcript of *ST3GAL4*, lncRNA *GSEC* may interfere with the translation process of ST3GAL4 by forming a double-stranded RNA structure with its sense transcript (20-22), thereby inhibiting the activation effects of sialylation-related glucose metabolism. In this study, we observed a significantly low expression level of *GSEC* in PTC, and its overexpression significantly inhibited the proliferation, invasion, and migration abilities of cancer cells. Further mechanistic studies have shown that *GSEC* can weaken the glucose metabolism process in cancer cells by inhibiting the expression of IGF2BP2 and downregulating GLUT1 levels. These results indicate that the role of *GSEC* in PTC differs from its reported procancer function in other tumors, but that it exerts an anticancer effect by inhibiting the IGF2BP2/GLUT1 axis. This study not only reveals the unique biological function of *GSEC* in PTC but also provides a new perspective for understanding its functional differences in different tumor types and provides a potential molecular basis for metabolic targeted therapy for PTC.

IGF2BP2, an RNA-binding protein, is involved in multiple cellular processes (23). It binds to and stabilizes various mRNAs, thereby influencing gene expression at the posttranscriptional level and affecting essential cellular functions such as proliferation, differentiation, and metabolism (24). While IGF2BP2 is crucial for maintaining cellular homeostasis under normal conditions, its overexpression in cancer can drive tumor progression and malignancy. IGF2BP2 is often upregulated in various cancers and functions as an oncogene (25,26). In head and neck squamous cell carcinoma, it promotes tumor cell proliferation and growth through the miR-98-5p/PI3K/Akt signaling pathway. IGF2BP2 also appears to be a key regulator of glycolysis in cancer cells (27). In pancreatic ductal adenocarcinoma, IGF2BP2 plays a significant role in enhancing cancer cell metabolism, as reported by Huang and cols. (16). Specifically, IGF2BP2 binds to *GLUT1* mRNA and stabilizes it, leading to increased GLUT1 expression; this stabilization increases the degree of aerobic glycolysis in cells, which supplies them with the energy and metabolic precursors required for rapid proliferation; additionally, this mechanism alters the cellular metabolic microenvironment, driving tumor progression. A recent study by Wang and cols. (28) has shed light on the role of IGF2BP2 in PTC. Their analysis of PTC transcriptome data from the TCGA revealed that IGF2BP2 is significantly overexpressed in PTC tissues and that its overexpression

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is strongly correlated with disease-free survival and clinical outcomes in PTC patients. These findings from the two studies suggest that IGF2BP2 may increase glycolysis in cancer cells, potentially fuelling PTC progression. Our investigation further illuminated this area. In terms of the role of lncRNA *GSEC* in PTC, we observed that *GSEC* expression could decrease IGF2BP2 levels. Its downregulation, in turn, destabilized GLUT1, ultimately curbing the glucose uptake of PTC cells. Our findings not only elucidate how IGF2BP2 is regulated in PTC but also paves the way for new cancer therapies targeting IGF2BP2.

In summary, our research revealed the critical role of lncRNA *GSEC* in the progression of PTC. Our findings show that the expression of *GSEC* is markedly downregulated in PTC. The overexpression of *GSEC* inhibits the IGF2BP2/GLUT1 axis, effectively blocking glycolytic reprogramming and the subsequent malignancy of cancer cells. This discovery offers a fresh perspective on metabolic regulation in PTC and points to a potential molecular target for treatment. However, this study has several limitations. First, although we have shown through functional experiments and molecular-level evidence that lncRNA *GSEC* can reduce glucose uptake and lactate production by inhibiting the IGF2BP2/GLUT1 axis, thereby inhibiting cancer cell glucose metabolism reprogramming, there is currently a lack of direct metabolic flow evidence for its regulation of anaerobic glycolysis, which still needs to be further validated through metabolomics or isotope tracing experiments. In addition, the specific molecular mechanism through which lncRNA *GSEC* inhibits IGF2BP2 expression, whether it plays a role through RNA–protein interactions, posttranscriptional regulation, or other indirect channels, remains to be further studied. Finally, as multifunctional regulatory molecules, lncRNAs often have a wide spectrum of target genes. Although this study focused on the key role of the IGF2BP2/GLUT1 axis in PTC glucose metabolism reprogramming, we did not systematically evaluate the regulatory effects of *GSEC* on other metabolic pathways, nor did we explore its functional differences across different PTC cell subtypes or tumor microenvironments. These are the research directions that we need to improve upon in the future.

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Ethics approval and consent to participate: Experiments involving clinical samples were approved by the Medical Ethics Review Committee of The Affiliated Yixing Hospital of Jiangsu University, with the approval number 2025-111-02. Informed consent was obtained from all patients. Experiments involving animals were approved by the Lab of Animal Experimental Ethical Inspection of Dr. Can Biotechnology (Zhejiang) Co., Ltd., with the approval number: DRK20240226098.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

Association between vitamin D deficiency and type 2 diabetes in an Indigenous Brazilian population

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ABSTRACT

Objective: This study investigated the association between vitamin D levels and type 2 diabetes mellitus (T2DM) among Indigenous adults of the Tupiniquim and Guarani ethnicities residing on a reserve on the southeastern Brazilian coast. **Subjects and methods:** This cross-sectional study, conducted between 2020 and 2022, included 946 Indigenous individuals (aged ≥20 years, both sexes) from the Tupiniquim and Guarani ethnic groups. T2DM was defined by a self-reported diabetes diagnosis, the use of glucose-lowering medications, or, in the absence of such reports, a fasting plasma glucose level ≥126 mg/dL, a 2-hour plasma glucose level ≥200 mg/dL following an oral glucose tolerance test, or a glycated hemoglobin level ≥6.5%. Hypovitaminosis D was defined as a serum 25-hydroxyvitamin D [25(OH)D] level <30.0 ng/mL. Associations were estimated using multiple logistic regression models. **Results:** The mean age of the participants was 41.3 ± 14.9 years; 56.8% were women, and 90% belonged to the Tupiniquim ethnic group. After adjusting for confounders, serum 25(OH)D levels were inversely associated with T2DM. Each 1-ng/mL increase in 25(OH)D was associated with lower odds of T2DM (OR = 0.96; 95% CI: 0.94–0.98). Individuals with hypovitaminosis D had higher odds of T2DM than those with adequate vitamin D levels (OR = 1.77; 95% CI: 1.20–2.62). **Conclusion:** Vitamin D deficiency was independently associated with the presence of T2DM. The high prevalence of hypovitaminosis D underscores the importance of screening strategies and considering supplementation protocols, even among Indigenous populations with high solar exposure.

Keywords: Diabetes mellitus, type 2; vitamin D deficiency; Indigenous peoples

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic, multifactorial disease characterized by persistent hyperglycemia resulting from insulin resistance in peripheral tissues (1). Over the past decades, the global prevalence of T2DM has more than doubled, and it is estimated that by 2030, over 600 million adults aged 20–79 years will be living with the disease worldwide (2,3).

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In Brazil, the prevalence of T2DM has been estimated at 10.6% (4). Among the Brazilian Indigenous population, the prevalence of T2DM is remarkably high. A review of studies published between 2011 and 2022 reported that the prevalence of T2DM ranged from 3.0% to 24.9% (5). Consistent with these findings, the prevalence of this disease among Indigenous people living in the Aracruz reserve in Espírito Santo State (southeastern Brazil), increased from 4.3% in 2003 to 13.7% in 2020 (6). The main factors associated with the high incidence of T2DM include older age (7), lifestyle behaviors (8), family history of the disease (9), obesity (10), hypertension (11), dyslipidemia (12), low educational attainment (13), unfavorable socioeconomic conditions (8,13) and low circulating levels of vitamin D (14,15).

Vitamin D is a fat-soluble prohormone essential for regulating calcium metabolism; it plays multiple

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roles across various tissues and organs, influencing the expression of genes related to the development of metabolic, hormonal, and immunological diseases (16). Only 10–20% of the daily vitamin D requirement is obtained through diet, while 80–90% depends on endogenous synthesis, which requires skin exposure to ultraviolet sunlight (17). In recent years, hypovitaminosis D has emerged as a relevant risk factor for the development of T2DM. Studies have consistently demonstrated an inverse association between serum levels of 25-hydroxyvitamin D [25(OH)D] (a biomarker of vitamin D status) and the risk of developing T2DM (14,15). Research conducted among Australian and Brazilian Indigenous populations has shown that individuals with lower 25(OH)D concentrations have a higher likelihood of developing T2DM (18,19). However, studies investigating this association, specifically among Indigenous populations living in tropical regions of Brazil, remain scarce.

Considering the increasing prevalence of T2DM among Indigenous peoples in Brazil, the relevance of vitamin D as a potentially modifiable risk factor, and the scarcity of studies investigating this association in this population, we aimed to analyze the association between vitamin D levels and T2DM in Indigenous adults of the Tupiniquim and Guarani ethnicities living in a reserve on the southeastern Brazilian coast.

SUBJECTS AND METHODS

Study design and population

This cross-sectional study used data from the survey "Evaluation of the Prevalence and Gravity of Chronic Diseases in the Indigenous Population of Espírito Santo", conducted at the University Hospital (HUCAM) of the Federal University of Espírito Santo. The officially demarcated Indigenous lands (Comboios, Caieiras Velha II, and Tupiniquim), located in the municipality of Aracruz on the northern coast of Espírito Santo state, are home to Indigenous people distributed across 12 villages of the Tupiniquim and Guarani ethnicities. The Tupiniquim, who belong to the Tupi-Guarani linguistic family, predominantly use Portuguese in their daily lives as a result of historical colonization and interethnic contact, alongside contemporary initiatives for cultural revitalization. The Guarani, mostly from the Mbya subgroup, preserve the Guarani language as their native tongue; it is widely used in social, educational, and spiritual practices, allowing them to maintain strong community organization and a distinct cultural identity. Both groups, however, maintain close contact with the general population living outside the reserve, with unrestricted access to commercial establishments such as shops and supermarkets.

Data collection was carried out between September 2020 and July 2022. All adults residing in the Indigenous reserve (approximately 2,500 individuals) in February 2020 were invited to the HUCAM outpatient clinic to undergo clinical and laboratory testing for cardiovascular risk factors. A subsample of 1,084 individuals attended the hospital clinic. For the present analysis, we excluded 110 participants who self-identified as non-Indigenous, 13 from ethnicities other than Tupiniquim and Guarani, 5 due to current vitamin D supplementation, 4 due to missing data on vitamin D levels, and 6 diagnosed with type 1 diabetes mellitus. Thus, the final sample consisted of 946 individuals. A detailed description of the research design and the main characteristics of the study population has been previously published (6).

This study was approved by the Research Ethics Committee (CAAE no. 22563019.6.0000.5060, approval no. 3.655.623) of the Health Sciences Center at the Federal University of Espirito Santo and the Brazilian National Research Ethics Commission (CAAE no. 22563019.6.0000.5060, approval no. 3.828.655). All participants signed the informed consent form before data collection.

Questionnaire application

Sociodemographic and lifestyle variables were collected during interviews using a standardized questionnaire. The variables included: sex (male or female), age (in years), and ethnicity (Tupiniquim or Guarani). The level of education (low, medium, or high) was categorized based on years of schooling (≤8, 9–11, and ≥12 years, respectively). Smoking status (never, former, or current) and alcohol consumption (consumer or non-consumer) were also determined during the interview. Seasonality was classified based on the

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month of data collection: summer (December, January, and February); autumn (March, April, and May); winter (June, July, and August); and spring (September, October, and November).

Biochemical measurements

Fasting blood samples were collected via venipuncture after a 12- to 14-hour fasting period to measure glucose, glycated hemoglobin (HbA1c), 25(OH)D, total cholesterol, cholesterol fractions, and insulin. Participants without a previous diagnosis of diabetes mellitus underwent a standard oral glucose tolerance test, which consisted of ingesting a flavored solution containing 75 g of glucose. Two hours after ingestion, a second blood sample was collected to measure plasma glucose levels. The samples were centrifuged and aliquoted at the collection site and subsequently transported to the clinical analysis laboratory at HUCAM. Fasting glucose, triglycerides, and HDL-c were measured using an enzymatic colorimetric method; HbA1c was measured using liquid chromatography with boronate affinity; and LDL-c was calculated using the Friedewald formula. Plasma concentrations of 25(OH)D were determined using a commercial Architect Abbott kit based on a chemiluminescent immunoassay method.

The presence of diabetes mellitus was defined according to the following criteria: self-reported diagnosis of diabetes, self-reported use of glucose-lowering medications, or, in the absence of such reports, fasting plasma glucose ≥126 mg/dL, plasma glucose 2 hours after the oral glucose tolerance test ≥200 mg/dL, or HbA1c ≥6.5% (1). Dyslipidemia was defined as self-reported use of lipid-lowering medications (statins, fibrates, or ezetimibe) or the presence of abnormal lipid levels, specifically LDL-c ≥160 mg/dL, triglycerides ≥150 mg/dL, or HDL-c <40 mg/dL for men and <50 mg/dL for women, according to the Brazilian Guidelines for Dyslipidemia and Atherosclerosis Prevention (20).

In this study, vitamin D was analyzed both as a continuous and a categorical variable. For the categorical classification, the following cut-off points were adopted: deficiency for concentrations <20 ng/mL, insufficiency between 21–29 ng/mL, and sufficiency for values ≥30 ng/mL (21). For analytical purposes, vitamin D status was dichotomized into adequate (≥30 ng/mL) and hypovitaminosis D (<30 ng/mL).

Blood pressure measurements

Blood pressure was measured on the left arm after a rest period of at least 5 minutes in a quiet, temperature-controlled environment (20–24 °C) using a validated oscillometric device (Omron HEM 705CPINT). Three measurements were taken at approximately one-minute intervals, and the average of the last two readings was recorded (22). Hypertension was defined as blood pressure ≥140/90 mmHg or self-reported continuous use of antihypertensive medication, including diuretics (23).

Anthropometric evaluations

Body weight was measured while participants were fasting, barefoot, without accessories, after bladder emptying, and wearing a standard uniform (400–600 g) using an electronic scale (Toledo; https://www.toledobrasil.com/) with an accuracy of 0.1 kg. Height was measured using a fixed wall stadiometer (Seca; https://www.seca.com/pt_mz.html) with an accuracy of 0.1 cm. These measurements were used to calculate body mass index (BMI), defined as weight (kg) divided by height squared (m^2) (24).

Statistical analyses

Continuous variables were described using the mean and standard deviation or the median and interquartile range, as appropriate, while categorical variables were presented as absolute and relative frequencies. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Comparisons between groups for continuous variables were performed using the independent samples Student's *t*-test or, when normality was not met, the Mann-Whitney U test. Differences in categorical variables were assessed using the chi-square test. Multiple logistic regression was conducted to identify independent associations between 25(OH)D levels (ng/mL) or vitamin D status and the presence of diabetes. Covariates were selected based on biological plausibility and statistical significance observed in the bivariate analyses between groups with and without hypovitaminosis D, as well as between participants

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with and without T2DM. Therefore, the variables included in the model were: sex, age, level of educational, smoking, alcohol consumption, BMI, dyslipidemia, hypertension, and season of the year. Participants without diabetes were the reference group. All statistical analyses were performed using Stata software v. 18. A *p*-value <0.05 was considered statistically significant.

RESULTS

The general characteristics of the sample, stratified by sex, are summarized in Table 1. The frequencies of smoking and alcohol consumption were higher among men. Moreover, men were older and exhibited higher levels of 25(OH)D, fasting blood glucose, systolic blood pressure, and diastolic blood pressure. In contrast, the frequencies of diabetes, dyslipidemia, and obesity were higher among women, who also presented higher HDL cholesterol levels and BMI.

Table 2 presents an analysis of sociodemographic, clinical, and lifestyle characteristics according to vitamin D status. Participants with adequate 25(OH)D levels demonstrated a lower frequency of diabetes and obesity, alongside a higher proportion of smokers and individuals of Guarani ethnicity. Furthermore, this group presented significantly lower fasting blood glucose and HbA1c levels compared to those with hypovitaminosis D. The season of data collection was not significantly associated with vitamin D status.

Table 1. Sociodemographic, clinical, and lifestyle characteristics of the sample

Variables	Total (n = 946)	Men (n = 409)	Women (n = 537)	*p*-value
Age, years	41.3 ± 14.9	43.1 ± 15.1	39.9 ± 14.5	**<0.01**
Ethnicity, n (%)				
Tupiniquim	850 (90.0)	376 (92.2)	474 (88.4)	0.06
Guarani	94 (10.0)	32 (7.8)	62 (11.6)	
Level of education, n (%)				
Low	302 (31.9)	138 (33.7)	164 (30.6)	0.18
Medium	546 (57.8)	237 (58.0)	309 (57.6)	
High	97 (10.2)	34 (8.3)	63 (11.8)	
25(OH)D, ng/mL	32.8 ± 9.9	33.6 ± 10.6	32.1 ± 9.3	**0.02**
Hypovitaminosis D, n (%)	413 (43.7)	167 (40.8)	246 (45.8)	0.13
Fasting blood glucose, mg/dL	98 (91–108)	100 (94–110)	97 (89–107)	**<0.01**
2-Hour glucose, mg/dL	126 (103.5–157)	116 (99–145.5)	134 (108–163)	**<0.01**
HbA1c, %	6.0 ± 1.6	5.9 ± 1.4	6.1 ± 1.7	0.15
BMI, kg/m^2	29.3 ± 5.8	27.7 ± 4.7	30.5 ± 6.3	**<0.01**
SBP, mmHg	126.4 ± 17.7	130.5 ± 16.1	123.3 ± 18.2	**<0.01**
DBP, mmHg	75.5 ± 9.7	76.6 ± 9.7	74.6 ± 9.5	**<0.01**
HDL-cholesterol, mg/dL	45.4 ± 11.3	44.5 ± 11.9	46.1 ± 10.7	**0.03**
LDL-cholesterol, mg/dL	116.1 ± 36.3	118.3 ± 37.1	114.5 ± 35.7	0.11
Total cholesterol, mg/dL	190.9 ± 42.7	192.7 ± 43.2	189.6 ± 42.3	0.26
Triglycerides, mg/dL	124 (85–185)	128 (84–195)	122 (85–170)	0.19
Dyslipidemia, n (%)	671 (71.5)	258 (63.9)	413 (77.3)	**<0.01**
Hypertension, n (%)	326 (34.5)	146 (35.7)	180 (33.5)	0.48
T2DM, n (%)	180 (19.0)	64 (15.6)	116 (21.6)	**0.02**
Obesity, n (%)	369 (39.0)	109 (26.6)	260 (48.4)	**<0.01**
Smoking, n (%)	145 (15.3)	85 (20.8)	60 (11.2)	**<0.01**
Alcohol use, n (%)	390 (41.4)	191 (46.8)	199 (37.3)	**<0.01**

Data are expressed as mean ± standard deviation or median (interquartile range) or n (%). HbA1c: glycated Hb; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; T2DM: type 2 diabetes mellitus.

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Table 2. Sociodemographic, clinical, and lifestyle characteristics according vitamin D status of the sample.

Variables	Adequate vitamin D (n = 533)	Hypovitaminosis D (n = 413)	*p*-value
Age, years	39.9 ± 14.8	40.0 ± 14.2	0.90
Ethnicity, n (%)			
Tupiniquim	467 (87.8)	383 (93.0)	**0.01**
Guarani	65 (12.2)	29 (7.0)	
Level of education, n (%)			
Low	177 (33.2)	125 (30.3)	0.62
Medium	304 (57.0)	242 (58.7)	
High	52 (9.8)	45 (10.9)	
Fasting blood glucose (mg/dL)	97 (90–106)	98 (92–113)	**0.01**
2-Hour glucose, mg/dL	121 (100–154)	130 (108–160)	**0.01**
HbA1c, %	5.9 ± 1.4	6.1 ± 1.8	**0.04**
BMI, kg/m^2	28.5 ± 5.4	30.3 ± 6.2	**<0.01**
SBP, mmHg	126.6 ± 18.1	126.0 ± 17.1	0.58
DBP, mmHg	75.5 ± 9.8	75.5 ± 9.4	0.94
HDL-cholesterol, mg/dL	45.1 ± 11.5	45.8 ± 11.1	0.37
LDL-cholesterol, mg/dL	117.5 ± 37.5	114.3 ± 34.7	0.19
Total cholesterol, mg/dL	190.9 ± 43.8	190.1 ± 41.3	0.60
Triglycerides, mg/dL	123 (83–184)	127 (85–185)	0.38
Dyslipidemia, n (%)	383 (72.7)	288 (70.1)	0.38
Hypertension, n (%)	195 (36.6)	131 (31.7)	0.12
T2DM, n (%)	87 (16.3)	93 (22.5)	**0.02**
Obesity, n (%)	188 (35.3)	181 (43.8)	**0.01**
Smoking, n (%)	95 (17.8)	50 (12.1)	**0.04**
Alcohol use, n (%)	233 (43.9)	157 (38.3)	0.08
Seasons n (%)			
Spring/summer	280 (52.5)	211 (51.1)	0.66
Autumn/winter	253 (47.5)	202 (49.9)	

Data are expressed as mean ± standard deviation or median (interquartile range) or n (%). HbA1c: Glycated Hb; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; T2DM: type 2 diabetes mellitus.

Table 3 presents a detailed analysis of sociodemographic, clinical, and lifestyle characteristics according to diabetes status. As expected, participants with diabetes were older, had lower levels of education, and presented a less favorable lipid and hemodynamic profile. Additionally, the diabetic group showed lower 25(OH)D levels and higher frequencies of obesity and hypovitaminosis D. Conversely, the frequencies of alcohol consumption and smoking were lower among individuals with diabetes.

Univariate and multivariate logistic regression models were employed to examine the association between vitamin D and T2DM (Table 4). In the fully adjusted model, serum 25(OH)D concentration, treated as a continuous variable, was inversely associated with T2DM. Each 1 ng/mL increase in 25(OH)D corresponded to a 4% reduction in the odds of having diabetes. Furthermore, when vitamin D was categorized, individuals with hypovitaminosis D had 77% higher odds of having diabetes compared to those with adequate vitamin D levels.

DISCUSSION

Our findings revealed that individuals with T2DM had significantly lower serum vitamin D concentrations than those without the disease. The high prevalence of T2DM and hypovitaminosis D among the participants is also noteworthy. Studies have shown a wide

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Table 3. Sociodemographic, clinical, and lifestyle characteristics according to diabetes status of the sample

Variables	Without diabetes (n = 766)	With diabetes (n = 180)	*p*-value
Age, years	38.6 ± 13.9	52.9 ± 13.2	**<0.01**
Ethnicity, n (%)			
Tupiniquim	682 (89.3)	168 (93.3)	0.10
Guarani	82 (10.7)	12 (6.7)	
Level of education, n (%)			
Low	214 (28.0)	88 (48.9)	**<0.01**
Medium	464 (60.6)	82 (45.5)	
High	87 (11.4)	10 (5.6)	
25(OH)D, ng/dL	33.3 ± 10.0	30.6 ± 8.9	**<0.01**
Hypovitaminosis D, n (%)	320 (41.8)	93 (51.7)	**0.02**
BMI, kg/m^2	28.9 ± 5.8	31.0 ± 5.6	**<0.01**
SBP, mmHg	123.8 ± 15.9	137.3 ± 20.4	**<0.01**
DBP, mmHg	74.7 ± 9.3	79.0 ± 10.3	**<0.01**
HDL-cholesterol, mg/dL	45.9 ± 11.4	43.3 ± 10.6	**0.01**
LDL-cholesterol, mg/dL	115.0 ± 35.4	121.1 ± 39.8	**0.05**
Total cholesterol, mg/dL	187.9 ± 41.7	203.9 ± 44.6	**<0.01**
Triglycerides, mg/dL	116 (80–166)	176.5 (121.5–254.5)	**<0.01**
Dyslipidemia, n (%)	516 (68.1)	155 (86.1)	**<0.01**
Hypertension, n (%)	200 (26.1)	126 (70.0)	**<0.01**
Obesity, n (%)	273 (35.6)	96 (53.3)	**<0.01**
Smoking, n (%)	123 (16.1)	22 (12.2)	**<0.01**
Alcohol use, n (%)	331 (43.5)	59 (32.8)	**0.01**

Data are expressed as mean ± standard deviation or median (interquartile range) or n (%). HbA1c: Glycated Hb; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein.

Table 4. Association between vitamin D status/levels and diabetes mellitus of the sample

Vitamin D	Odds ratio	95% Confidence interval	p-value
Status			
Crude model			
Adequate vitamin D	1		
Hypovitaminosis D	1.49	1.07–2.06	**0.02**
Adjusted model			
Adequate vitamin D	1		
Hypovitaminosis D	1.77	1.20–2.62	**<0.01**
Level			
Crude model			
25(OH)D (ng/dL)	0.97	0.95–0.99	**<0.01**
Adjusted model			
25(OH)D (ng/dL)	0.96	0.94–0.98	**<0.01**

Adjusted model: sex, age, education, ethnicity, smoker, alcohol use, body mass index, dyslipidemia, hypertension, season.

variation in the prevalence of T2DM among different Indigenous groups in Brazil, with rates ranging from 4.5% to 28.2% (25). In our study, the prevalence was 19%, falling within this range but remaining higher than the estimated 10.6% prevalence for the general Brazilian population (4). These findings underscore

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the importance of investigating potential contributing factors (e.g., hypovitaminosis D), which may play a role in T2DM development within this population.

The prevalence of hypovitaminosis D in the assessed Indigenous population was 43.8%, with 36.0% classified as insufficient and 7.7% as deficient (data not shown). In comparison with the available literature, our findings highlighted relevant particularities when contrasted with data from the Xavante Indigenous population. In this context, Abrahão and cols. (18) reported rates of 65% for insufficiency and approximately 28% for vitamin D deficiency among Xavante Indigenous individuals living in a large reservation in the state of Mato Grosso, in the Central-West region of Brazil. The substantial difference between these two studies may be attributed to factors such as geographical variations and differences in cultural and dietary habits. Moreover, our results were similar to those reported among Australian Indigenous populations in terms of insufficiency (42%) but lower regarding deficiency, which reached 31% in that population (19). These findings suggest that although vitamin D insufficiency is a common issue among different Indigenous groups, the magnitude of deficiency seems to vary substantially depending on environmental and sociocultural contexts. Furthermore, when comparing our results to those of the general population, the total prevalence of hypovitaminosis D was lower than that reported by Borba and cols. (26), who identified 15.0% insufficiency and 50.7% deficiency. This contrast suggests that certain Indigenous groups may benefit from greater sun exposure compared to the general population.

Within the study population, relevant differences in serum vitamin D levels were also observed between the Guarani and Tupiniquim ethnic groups. A higher proportion of Guarani individuals presented adequate vitamin D levels, which may be associated with the lower prevalence of overweight and obesity in this group compared to the Tupiniquim (62.8% [95% CI 60.1–65.5] *vs.* 78.0% [95% CI 75.3–80.7]; data not shown). In addition, the Guarani tend to maintain more traditional lifestyles, whereas the Tupiniquim exhibit a more urbanized lifestyle, which may negatively affect sun exposure and, consequently, serum 25(OH)D levels (27). These findings highlighted the importance of considering cultural and behavioral specificities when interpreting vitamin D nutritional status among Indigenous populations.

The association between hypovitaminosis D and higher serum HbA1c levels has been widely discussed in the literature, suggesting that vitamin D deficiency may contribute to poorer glycemic control (28). In this study, individuals with hypovitaminosis D showed significantly higher mean HbA1c levels than those with adequate vitamin D levels. Similar findings were reported by Bhat and cols. (29), who identified significantly higher 25(OH)D concentrations among individuals with good glycemic control (<7%) than those with poor glycemic control. Felício and cols. (30) also demonstrated that individuals with HbA1c ≥7% (i.e., poorer glycemic control) had significantly lower 25(OH)D concentrations than those with adequate glycemic control (<7%). These findings further support the hypothesis that vitamin D deficiency is associated with poorer glycemic control, thus predisposing individuals to diabetes development.

Regarding T2DM, we observed an inverse association between serum 25(OH)D levels and the presence of the disease, even after adjusting for potential confounding factors. These results are consistent with literature, which have identified vitamin D deficiency as a potentially modifiable factor in the pathogenesis of T2DM (21,31,32). This association has also been reported in studies conducted among Indigenous populations. In Brazil, Abrahão and cols. (18) found that higher 25(OH)D levels were significantly associated with a lower prevalence of diabetes mellitus and glucose intolerance among the Xavante Indigenous population. Specifically, each 1 ng/mL increase in serum 25(OH)D was associated with a 3% reduction in the likelihood of developing diabetes. Similarly, a study conducted with Australian Indigenous participants reported that lower 25(OH)D levels were associated with a higher prevalence of diabetes mellitus. Individuals with hypovitaminosis D had an odds ratio of 2.15 for diabetes, even after adjusting for cardiometabolic risk factors (19).

Proposed mechanisms for this association are based on a physiological perspective, wherein

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vitamin D plays a fundamental role in the regulation of glucose metabolism. It actively participates in pancreatic beta-cell function by acting as a chemical messenger through interaction with receptors that regulate calcium flux within beta cells, a process essential for insulin secretion (33). Additionally, vitamin D stimulates the expression of insulin receptors and activates the peroxisome proliferator-activated receptor delta, thereby promoting peripheral glucose uptake and improving insulin sensitivity. Moreover, vitamin D has been implicated in modulating inflammatory mechanisms and regulating the renin-angiotensin system, whose hyperactivity contributes to insulin resistance and endothelial dysfunction, both relevant factors in the pathophysiology of T2DM (34).

Our findings do not support the recommendation of universal vitamin D supplementation for all Indigenous individuals, nor do they imply a homogeneous classification of this population as high-risk for T2DM based solely on serum 25(OH)D levels. As highlighted by Fuentes-Barría and cols. (35), the American Diabetes Association does not currently recommend routine supplementation to prevent or treat T2DM, except in cases of laboratory-confirmed deficiency.

Nevertheless, our results indicated that low vitamin D levels were associated with a higher probability of T2DM in this specific context, reinforcing their clinical relevance as an indirect marker of metabolic health. Accordingly, rather than a mandate for mass supplementation, these data underscore the need for targeted clinical screening and public health strategies. Such efforts should focus on identifying the most vulnerable groups within Indigenous communities and promoting lifestyle-based interventions, including the preservation of traditional diets and adequate sun exposure, aligned with an evidence-based and individualized care approach.

A key strength of this study lies in the inclusion of a sample composed of Indigenous individuals living in villages with close interaction with urban environments, a reality that reflects the situation of many Indigenous populations in other regions of Brazil. Another noteworthy strength is the rigorous methodological approach employed during data collection, which contributes to the robustness of the results. Nevertheless, these findings must be interpreted cautiously given the potential limitations. First, the sample is not representative of the entire Indigenous population living in villages across Brazil; therefore, generalizing the findings should be approached with caution. In addition, the absence of relevant variables for understanding vitamin D levels (e.g., duration of sun exposure, sunscreen use, and type of clothing) may have limited the explanatory power of the results. Lastly, the inherent limitations of the cross-sectional study design prevent the establishment of causal relationships between the variables analyzed.

In conclusion, our findings reinforced the hypothesis that vitamin D deficiency was independently associated with the presence of T2DM. Furthermore, a high prevalence of hypovitaminosis D was observed in the study population, which reinforces the importance of screening strategies, as well as the definition of supplementation protocols even in Indigenous groups presumably exposed to high solar radiation.

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Vitamin D status and its association with metabolic syndrome and cardiometabolic markers in postmenopausal women: a population-based study

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ABSTRACT

Objective: To investigate the association between serum vitamin D levels, metabolic syndrome (MetS), and cardiometabolic markers in postmenopausal women from a population-based sample in Brazil. **Subjects and methods:** This cross-sectional study included 351 women aged 40 to 64 years with at least 12 months of amenorrhea. Serum 25-hydroxyvitamin D was measured by chemiluminescence and categorized into quartiles. MetS was defined according to the Joint Interim Statement criteria. Anthropometric, blood pressure, lipid profile, insulin, calcium, and phosphorus measures were also assessed. **Results:** Hypovitaminosis D (vitamin D <30 ng/mL) was observed in 66.4% of participants. Women in the highest quartile (≥31.0 ng/mL) had significantly more favorable metabolic profiles than those in the lowest quartile (<21.0 ng/mL), including lower BMI, waist circumference, insulin, and triglycerides, and higher HDL cholesterol. Vitamin D levels <21.0 ng/mL were associated with a 2.27-fold increased risk of MetS (95% CI: 1.21–4.24; p = 0.010). **Conclusion:** Higher vitamin D levels are associated with more favorable metabolic profiles and a reduced risk of MetS in postmenopausal women. Adequate vitamin D status may play a preventive role in mitigating metabolic alterations during postmenopause.

Keywords: Vitamin D; postmenopausal women; metabolic syndrome; anthropometric parameters; biochemical markers

INTRODUCTION

Vitamin D (VitD) is a fat-soluble prohormone that is primarily synthesized in the skin upon exposure to ultraviolet B radiation and, to a lesser extent, obtained from dietary sources (1,2). The circulating form, 25-hydroxyvitamin D (25[OH]D)], reflects VitD status and serves as a precursor to the active metabolite, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which regulates calcium and phosphate balance and supports bone health (2,3).

Beyond its established role in skeletal health, VitD exerts pleiotropic effects on multiple tissues that express VitD receptors and hydroxylation enzymes, influencing immune, cardiovascular, and metabolic pathways (4–6). Hypovitaminosis D represents a significant global public health concern, largely attributable to factors such as reduced sun exposure, darker skin pigmentation, aging, and obesity (7,8). Although some interventional studies have reported limited or no effects of VitD supplementation on glucose or lipid metabolism (9,10), VitD deficiency has been associated with hypertension, insulin resistance, dyslipidemia, and metabolic syndrome (MetS) (4,6,11). Moreover, recent clinical and meta-analytic studies have explored the impact of dietary and VitD-related factors on cardiometabolic outcomes, highlighting potential links between nutritional interventions and metabolic health (12–14).

Among postmenopausal women, estrogen decline may decrease hepatic 25-hydroxylase activity and reduce cutaneous VitD synthesis (15,16). These hormonal and physiological alterations, combined with insufficient sun exposure and dietary inadequacy,

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render this group particularly susceptible to VitD deficiency (14). Indeed, a Brazilian study reported that approximately 60% of older women exhibit suboptimal VitD concentrations (17).

In addition to their susceptibility to hypovitaminosis D, postmenopausal women are at elevated risk for MetS — a cluster of cardiometabolic abnormalities that includes central obesity, hypertension, dyslipidemia, and insulin resistance — and consequently have a higher likelihood of developing type 2 diabetes and cardiovascular disease (15,18). The coexistence of hypovitaminosis D and MetS may have synergistic impact, as hormonal decline, increased visceral adiposity, and inflammatory changes during menopause can simultaneously impair VitD metabolism and exacerbate adverse cardiometabolic profiles (19). This intersection underscores the importance of investigating the relationship between VitD status and MetS in this population.

Therefore, this population-based study aimed to examine the association between serum VitD levels, the presence of MetS, and cardiometabolic risk markers in postmenopausal women from Brazil. We hypothesized that lower VitD concentrations would be associated with a higher prevalence of MetS and an unfavorable metabolic profile.

SUBJECTS AND METHODS

Study participants and data collection

A total of 377 participants were recruited based on practical considerations and availability during the study period. Eligible participants were women aged 40–64 years who had experienced at least 12 consecutive months of amenorrhea. Participants were randomly selected from a registry of users of the Brazilian Unified Health System in Ouro Preto (Minas Gerais State, Brazil). Women within the target age range were identified from this registry and invited to participate via telephone calls or home visits. Additionally, eligible women were approached and recruited by community health agents, healthcare professionals, and members of the research team. Women who reported the current use of VitD supplements or who declined to participate in the interviews or assessments were excluded.

Data collection was carried out at primary healthcare units and facilities at the Federal University of Ouro Preto. Structured interviews were administered using standardized questionnaires that captured sociodemographic, reproductive, and lifestyle variables. This study was approved by the Research Ethics Committee of the Federal University of Ouro Preto under protocol number 56312916.8.0000.5150. All participants provided written informed consent.

VitD and other laboratory tests

Blood samples were collected after participants had fasted for 12–14 hours, abstained from alcohol for 72 hours, and avoided physical activity for 24 hours. The data collection period spanned more than a year, covering all seasons, which helped minimize the potential impact of seasonal variations in sunlight exposure on serum VitD concentrations. Serum 25-hydroxyvitamin D [25(OH)D] and insulin levels were measured via chemiluminescence using the Access 2 Immunoassay System® (Beckman Coulter). The lipid profile (total cholesterol, HDL-c, LDL-c, and triglycerides), fasting glucose, calcium, and phosphorus levels were determined using the Cobas Integra® 400 Plus analyzer (Roche). Derived indices included non-HDL cholesterol, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) (20), and the quantitative insulin sensitivity check index (QUICKI) (21), calculated using **Equations 1–3:**

$$non - HDLc = TC\ (mg/dL) - HDLc\ (mg/dL) \quad (1)$$

$$HOMA - IR = (fasting\ blood\ glucose \times 0.0555) \times \frac{insulin}{22.5} \quad (2)$$

$$QUICK = \frac{1}{loglog\ Insulin\left(\frac{\mu UI}{mL}\right) + \left(\frac{mg}{dL}\right)} \quad (3)$$

MetS was defined based on the harmonized criteria established by the joint interim statement (22).

Blood pressure and anthropometric measurements

Blood pressure was measured using a Bioland®-3005 digital wrist monitor, following the manufacturer's instructions. Anthropometric measurements included weight, height, waist circumference, and body fat percentage. Weight and body fat were obtained using a Tanita® digital bioimpedance scale (Model 2204;

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precision = 100 g; capacity = 150 kg), with participants standing barefoot, and upright in the center of the scale. Height was measured using a wall-mounted stadiometer (precision = 0.1 cm; maximum = 2.0 m) with participants standing erect, feet together, arms resting at their sides, and the head positioned in the Frankfurt plane. Waist circumference was measured with a non-elastic measuring tape at the midpoint between the lowest rib and the iliac crest; when this landmark was difficult to locate, the measurement was taken at the umbilical level.

The following indices were then calculated (Equations 4–6):

$$BMI = \frac{Weight\ (kg)}{[Height\ (m)]^2} \quad (4)$$

$$WHtR = \frac{Waist\ circumference\ (cm)}{Altura\ (cm)} \quad (5)$$

$$Conicity\ index = \frac{Waist\ circumference\ (m)}{0.109\sqrt{\frac{Weight\ (kg)}{Height\ (m)}}} \quad (6)$$

Statistical analysis

Data were coded and double-entered using EpiData to ensure accuracy. Statistical analyses were performed using SPSS version 20.0 (IBM Corp.). Serum 25(OH)D concentrations were categorized into quartiles. Binary logistic regression was used to estimate unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) for MetS across VitD quartiles, using the highest quartile (Q4: ≥ 31.0 ng/mL) as the reference group.

Categorical variables were compared using Pearson's chi-square test. The Kolmogorov-Smirnov test was used to assess the normality of continuous variables. Because most variables were non-normally distributed, data were expressed as medians and interquartile ranges and compared using the Kruskal-Wallis test. Post hoc pairwise comparisons were performed with the Bonferroni correction to account for multiple testing. Effect sizes (η^2h) for the Kruskal-Wallis tests were calculated according to Tomczak and Tomczak (2014), using the formula $\eta^2h = (H - k + 1)/(n - k)$, where H is the Kruskal-Wallis test statistic, k is the number of groups, and n is the total sample (23). A post-hoc power analysis was performed using G*Power software (F tests, analysis of variance: fixed effects, omnibus, one-way) with a minimum power of 80% and a significance level of 0.05. Only participants with complete data and no missing values were included in the final analysis. A *p*-value < 0.05 was considered statistically significant.

RESULTS

A total of 377 postmenopausal women were initially recruited. After excluding 14 participants who reported current use of vitD supplements and 12 with missing data, 351 women were included in the final analysis. The mean serum 25(OH)D concentration in the sample was 26.5 ± 8.7 ng/mL, with a median of 26.0 ng/mL and values ranging from 2.4 to 59.0 ng/mL (Figure 1). The distribution of serum VitD levels was approximately normal, with most participants clustering between 20 and 30 ng/mL, reflecting the predominance of suboptimal VitD status in this population. Hypovitaminosis D, defined as a concentration below 30.0 ng/mL, was observed in 66.4% of participants (n = 233). For analysis, participants were categorized into quartiles based on their serum 25(OH)D levels: Q1 (< 21.0 ng/mL), Q2 (21.0–26.9 ng/mL), Q3 (27.0–30.9 ng/mL), and Q4 (≥ 31.0 ng/mL).

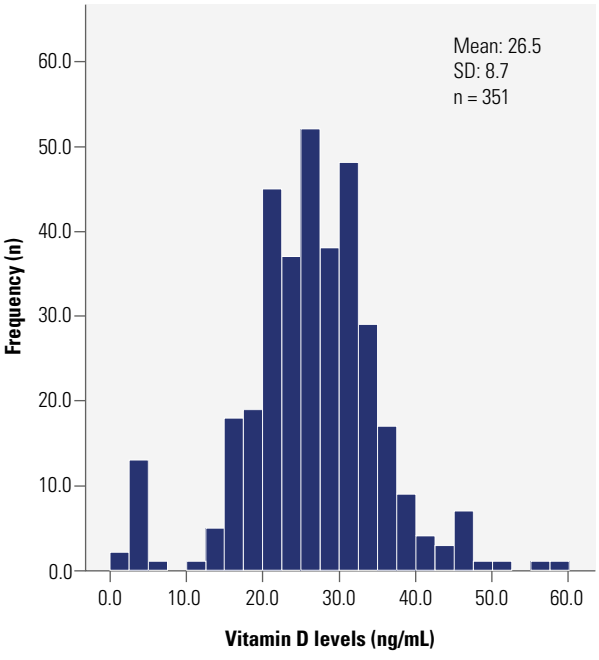


Figure 1. Distribution of serum 25(OH)D levels among postmenopausal participants (n = 351).

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Across all quartiles, most participants were between 50–60 years of age, were married or living with a partner, had a family income greater than twice the minimum wage, and reported current medication use. Smoking and alcohol consumption were infrequent, while 48.4% of the sample (n = 170) reported regular physical activity. The median age at menopause was 48 years, and the median time since menopause was six years. Hypertension and neuropsychiatric conditions were the most frequently reported comorbidities. There were no statistically significant differences in sociodemographic or behavioral variables between vitD quartiles (**Table 1**).

Anthropometric and metabolic parameters varied significantly according to vitD status (**Table 2**). Women in the highest quartile (Q4) showed more favorable anthropometric profiles than those in the lowest quartile (Q1). Specifically, they presented significantly lower values for weight (63.8 vs. 72.2 kg; $p = 0.009$), body mass index (25.6 vs. 28.5 kg/m^2; $p = 0.005$), body fat percentage (34% vs. 38%; $p = 0.013$), waist circumference (88 vs. 98 cm; $p = 0.002$), waist-to-height ratio (0.57 vs. 0.61; $p = 0.006$), and conicity index (1.27 vs. 1.31; $p = 0.027$). Significant differences between Q3 and Q1 were also observed for waist-to-height ratio (0.57 vs. 0.61; $p = 0.028$) and conicity index (1.27 vs. 1.31; $p = 0.040$).

Table 1. Sociodemographic and behavioral variables of the participants according to quartiles of vitamin D

Variable	Quartile of Vitamin D				Total (n=351)	p-value	χ^2
	Q1 (n=76) < 21.0 ng/mL	Q2 (n=108) 21.0–26.9 ng/mL	Q3 (n=76) 27.0 – 30.9 ng/mL	Q4 (n=91) ≥ 31.0 ng/mL			
	n (%) or median (1º and 3º quartiles)						
Age (years)	56.0	55.0	55.0	55.0	55.0	0.407	0.889
	(53.0–59.0)	(52.0–58.0)	(52.0–58.0)	(52.0–59.0)	(52.0–58.0)		
40-49	8 (10.5)	11 (10.2)	9 (11.8)	10 (11.0)	38 (10.8)	0.185	12.541
50-54	17 (39.0)	38 (35.2)	25 (32.9)	35 (38.5)	115 (32.8)		
55-60	38 (50.0)	43 (39.8)	34 (44.7)	26 (28.6)	141 (40.2)		
61-65	13 (17.1)	16 (14.8)	8 (10.5)	20 (22.0)	57 (16.2)		
Married/living with a partner	49 (64.5)	66 (61.1)	50 (65.8)	53 (58.2)	218 (62.1)	0.743	1.242
Family income (MW)*							
0,00 – 1,00	18 (23.7)	27 (25.0)	17 (22.4)	21 (23.1)	83 (23.6)	0.987	2.231
1,01 – 2,00	22 (28.9)	28 (25.9)	23 (30.3)	22 (24.2)	95 (27.1)		
> 2,00	32 (42.1)	43 (39.8)	30 (39.5)	41 (45.1)	146 (41.6)		
NA	4 (5.3)	10 (9.3)	6 (7.9)	7 (7.7)	27 (7.7)		
Use of medicines	62 (81.6)	75 (69.4)	50 (65.8)	69 (75.8)	256 (72.9)	0.117	5.894
Current smoke	9 (11.8)	13 (12.0)	9 (11.8)	14 (15.4)	45 (12.8)	0.867	0.725
Alcohol use	1 (1.3)	7 (6.5)	1 (1.3)	2 (2.2)	11 (3.1)	0.116	5.905
Regular physical activity	33 (43.4)	52 (48.1)	39 (51.3)	46 (50.5)	170 (48.4)	0.757	1.184
Age of menopause (years)	48.0	48.0	48.0	48.0	48.0	0.819	0.927
	(45.0–51.3)	(43.0–51.0)	(43.0–51.0)	(44.0–51.0)	(44.0–51.0)		
Time of menopause (years)	6.5	5.0	6.0	7.0	6.0	0.711	1.379
	(2.8–10.0)	(2.0–11.0)	(3.0–9.0)	(3.0–10.0)	(2.0–10.0)		
History of diseases							
Hypertension	37 (48.7)	35 (32.4)	23 (30.3)	41 (45.1)	136 (38.7)	0.032	8.821
Neuropsychiatric disease	22 (28.9)	27 (25.0)	22 (28.9)	36 (39.6)	107 (30.5)	0.155	5.240
Gastritis	22 (28.9)	23 (21.3)	19 (25.0)	22 (24.2)	86 (24.5)	0.699	1.427
Thyroid disorders	11 (14.5)	20 (18.5)	10 (13.2)	20 (22.0)	61 (17.4)	0.419	2.828

*The minimum wage (MW) during data collection varied from BRL 724.00 to BRL 1,100.00. NA = participant did not answer; the statistical analysis did not show significant differences among the quartiles.

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No statistically significant differences in blood pressure were found between the quartiles (Table 2). However, participants in Q4 exhibited significantly higher median HDL-C levels (59 vs. 49 mg/dL; $p < 0.001$) and QUICKI values (0.37 vs. 0.35; $p = 0.015$), along with lower triglyceride levels (109 vs. 138 mg/dL; $p = 0.002$), insulin concentrations (5.8 vs. 7.9 μIU/mL; $p = 0.021$), and HOMA-IR (1.31 vs. 1.68; $p = 0.015$) when compared with Q1. A significant difference in HDL-C was also found between Q2 and Q1 (56 vs. 49 mg/dL; $p = 0.016$).

Participants diagnosed with MetS had significantly lower serum VitD levels compared with those without the condition (25.4 ± 8.0 vs. 27.3 ± 9.1 ng/mL; $p = 0.041$). In the multivariate logistic regression analysis, women in the lowest VitD quartile (Q1) had a significantly higher likelihood of having MetS than those in the highest quartile (Q4), both in the unadjusted model (OR = 2.27; 95% CI: 1.21–4.24; $p = 0.010$) and after adjustment for age, smoking, and physical activity (OR = 2.15; 95% CI: 1.14–4.05; $p = 0.019$). In addition, a trend toward an increased MetS risk was also observed in quartiles Q2 and Q3, although these associations did not reach statistical significance (Table 3). The power analysis indicated that the minimum detectable effect size was Cohen's f = 0.177, corresponding to approximately 80% power to detect small-to-moderate differences across VitD quartiles.

Table 2. Anthropometric, blood pressure, and biochemical data of the participants according to quartiles of vitamin D

Variable	Quartile of Vitamin D				Statistics		
	Q1 (n=76) < 21.0 ng/mL	Q2 (n=108) 21.0-26.9 ng/mL	Q3 (n=76) 27.0-30.9 ng/mL	Q4 (n=91) ≥ 31.0 ng/mL	*p*-value	Test Statistics (H)	Effect size (η^2)
	Median (1º - 3º quartile)						
Anthropometric variables							
Weight (kg)	72.2 (63.6-79.8)	67.1 (59.5-77.5)	66.9 (62.2-74.5)	63.8 (58.4-75.9)[a]	**0.016**	10.363	0.021
BMI (kg/m²)	28.5 (25.8-31.7)	26.8 (24.4-31.3)	26.9 (24.3-30.2)	25.6 (23.1-28.8)[a]	**0.009**	11.481	0.024
Body fat (%)	38.0 (34.0-42.0)	36.0 (31.8-41.0)	36.0 (30.0-40.3)	34.0 (29.5-39.0)[a]	**0.023**	9.548	0.019
WC (cm)	98.0 (88.5-101.0)	91.0 (82.0-101.3)	90.5 (83.8-99.0)	88.0 (82.0-98.0)[a]	**0.003**	13.851	0.031
WHtR	0.61 (0.56-0.64)	0.58 (0.53-0.65)	0.57 (0.52-0.62)[a]	0.57 (0.51-0.62)[a]	**0.006**	12.502	0.027
Conicity index	1.31 (1.26-1.37)	1.28 (1.23-1.34)	1.27 (1.22-1.33)[a]	1.27 (1.22-1.34)[a]	**0.016**	10.340	0.021
Blood pressure							
Systolic	129.5 (120.0-140.0)	132.5 (120.8-145.3)	130.0 (121.0-142.3)	130.0 (122.0-144.5)	0.815	0.943	0.000
Diastolic	82.0 (78.0-90.0)	84.0 (78.0-90.3)	82.0 (77.0-88.3)	83.0 (77.5-89.5)	0.815	0.945	0.000
Biochemical variables							
Triglycerides (mg/dL)	137.5 (102.0-196.0)	115.0 (82.3-169.8)	116.0 (89.5-159.0)	109.0 (75.0-144.0)[a]	**0.004**	13.080	0.029
TC (mg/dL)	211.0 (186.8-246.3)	218.5 (192.5-244.5)[a]	214.0 (196.0-236.3)	207.0 (174.5-239.0)	0.285	3.789	0.002
HDLc (mg/dL)	48.5 (39.8-60.0)	55.5 (46.0-67.3)[a]	54.5 (45.8-65.0)	59.0 (48.5-67.5)[a]	**0.001**	17.471	0.042
LDLc (mg/dL)	128.6 (113.3-161.2)	137.6 (111.5-157.1)	129.0 (113.7-161.6)	124.6 (97.4-155.3)	0.456	2.606	0.000
Non-HDLc (mg/dL)	168 (138.0-190.0)	160.5 (133.0-182.3)	157.0 (135.8-180.3)	149.0 (116.5-179.0)	0.082	6.705	0.011
FBG (mg/dL)	94.0 (88.8-106.0)	93.0 (87.0-104.0)	92.5 (87.0-101.3)	89.0 (84.0-99.0)	0.152	5.285	0.007
Insulin (μUI/mL)	7.9 (4.8-11.9)	7.4 (4.6-10.6)	5.9 (4.4-10.1)	5.8 (4.2-7.9)[a]	**0.015**	10.516	0.022
HOMA-IR	1.68 (1.11-3.00)	1.64 (1.02-2.57)	1.41 (1.05-2.71)	1.31 (0.89-1.74)[a]	**0.015**	10.506	0.022
QUICKI	0.35 (0.32-0.38)	0.35 (0.33-0.38)	0.36 (0.33-0.38)	0.37 (0.35-0.39)[a]	**0.015**	10.503	0.022
Calcium (mg/dL)	9.7 (9.3-10.2)	9.6 (9.3-9.9)	9.7 (9.4-10.1)	9.7 (9.4-10.0)	0.622	1.769	0.000
Phosphorus (mg/dL)	3.8 (3.5-4.2)	3.8 (3.4-4.1)	3.8 (3.5-4.1)	3.9 (3.5-4.2)	0.133	5.588	0.007

[a] $p < 0.05$ compared with Q1. BMI: body mass index; WC: waist circumference; WHtR: waist-to-height ratio; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance; QUICKI: quantitative insulin sensitivity check index.

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Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for metabolic syndrome according to serum vitamin D quartiles, unadjusted and adjusted for age, smoking, and physical activity

Vitamin D quartile	OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
Q1 (< 21.0 ng/mL)	2.27	(1.21-4.24	0.010	**2.15**	**1.14-4.05**	**0.019**
Q2 (21.0 – 26.9 ng/mL)	1.55	0.87-2.76	0.137	1.55	0.86-2.79	0.143
Q3 (27.0 – 30.9 ng/mL)	1.74	0.93-3.26	0.082	1.81	0.96-3.43	0.068
Q4 (≥ 31.0 ng/mL)	1	-	-	1	-	-

*Adjusted for age, physical activity, and smoking.

DISCUSSION

We found that a high percentage (60%) of postmenopausal women from Ouro Preto (Minas Gerais State, Brazil) had serum VitD levels below 30.0 ng/mL. The high prevalence of hypovitaminosis D is a well-documented issue in various geographic regions, including Brazil (7,24). Because older adults spend more time indoors and have a reduced dermal capacity to synthesize VitD, they are at an increased risk of hypovitaminosis D (25). Additionally, hypoestrogenism, which is common after menopause, can contribute to a decline in VitD levels (26). Several studies have reported that VitD deficiency affects over 90% of individuals, depending on the studied population (7). A systematic review of 32 studies worldwide found that 77.4% (n = 16,440) of postmenopausal women had low serum VitD concentrations (<30 ng/mL), with the prevalence of hypovitaminosis D ranging from 29% in the United States to 99.4% in China (27). Consistent with our findings, another Brazilian survey reported a high prevalence of hypovitaminosis D among postmenopausal women (68%) (28).

Our study showed that all anthropometric variables improved across VitD quartiles. Studies have demonstrated that low VitD concentrations increase parathyroid hormone (PTH) levels (29). Elevated PTH levels increase calcium influx into adipocytes, leading to fat accumulation and weight gain (30). Additionally, significant increases in adipose tissue can sequester fat-soluble VitD from the bloodstream, subsequently reducing its circulating levels (31). Other studies have also found that higher VitD levels are negatively correlated with body mass index, body weight, and body fat percentage in postmenopausal women and other populations (28,30,32). Furthermore, some researchers have reported that VitD supplementation can improve these anthropometric parameters. A clinical trial involving 77 women with overweight or obesity concluded that 12 weeks of VitD supplementation was associated with a reduction in body fat mass (33). Although further research is needed, a systematic review and meta-analysis suggested that VitD supplementation offers a potential therapeutic option for weight loss (30).

Regarding lipid profiles, our study showed that parameters progressively improved from the first to the fourth quartile of VitD. Participants with VitD levels above 30 ng/mL (Q4) had significantly lower triglyceride and higher HDL-c levels than those with serum VitD concentrations below 21 ng/mL (Q1). Therefore, our results suggest that adequate VitD may be associated with a favorable lipid profile and a reduced risk of cardiovascular disease. VitD plays a crucial role in regulating calcium and PTH metabolism (29). Adequate serum VitD levels enhance the intestinal absorption of calcium, thereby reducing fatty acid absorption (34). Moreover, elevated VitD levels prevent the activation of PTH, which normally inhibits lipolysis, leading to increased lipolysis and reduced triglyceride levels (35,36). Additionally, VitD may affect lipoprotein metabolism by reducing triglyceride synthesis and secretion in the liver, culminating in decreased triglyceride and VLDL-c levels and an increased HDL-c levels (36). In a study involving Polish postmenopausal women, VitD was similarly associated with increased HDL-c and decreased total cholesterol and LDL-c (37). Furthermore, other studies have linked VitD deficiency to hyperlipidemia and MetS (28,36).

In this study, although the statistical analysis did not reveal significant differences, participants with higher VitD levels exhibited lower fasting blood glucose levels. VitD was also significantly associated with lower insulin levels and reduced insulin resistance. Several mechanisms have been proposed to explain the role of VitD in glucose metabolism: (1) direct

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stimulation of insulin secretion via the VitD receptor on pancreatic beta cells; (2) reduction of systemic inflammation, which subsequently mitigates insulin resistance; and (3) improvement of peripheral insulin sensitivity mediated by VitD receptors in the liver and skeletal muscles (9). Other studies have evaluated the relationship between VitD and glycemic levels in postmenopausal women as well. A review concluded that lower VitD levels correlated with higher glucose concentrations in participants aged 35–74 years (15). Moreover, Schmitt and colleagues (28) evaluated a cohort of Brazilian women and demonstrated that participants with hypovitaminosis D had higher levels of total cholesterol, triglycerides, insulin, and HOMA-IR than those with adequate VitD status. Conversely, another review concluded that there is insufficient evidence to support the use of VitD supplementation for managing insulin resistance and diabetes mellitus (10). Thus, further studies are needed to better understand how VitD influences carbohydrate metabolism in both the general population and postmenopausal women.

Some studies have reported that low VitD levels contribute to the inadequate activation of the renin-angiotensin system and increased blood pressure (38,39). These effects tend to be more prominent in postmenopausal women, as hypoestrogenism also increases blood pressure (18). However, in our study, we did not find significant differences in blood pressure according to the quartiles of VitD. The median systolic and diastolic blood pressure values of the participants were below or very close to the reference range, which may have hindered the detection of blood pressure variations across VitD levels. It is also pertinent to highlight that 38.7% of the women participating in this study reported having hypertension and using antihypertensive drugs. Because many participants had their blood pressure controlled by medication, this control may have masked potential associations. Similarly, a clinical trial did not find an association between VitD levels and blood pressure in American postmenopausal women (40). Despite this, we recommend further studies to identify the role of VitD in the blood pressure of middle-aged women.

MetS is a group of metabolic risk factors that includes abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. In perimenopausal and postmenopausal women, MetS is more prevalent because most of its components are adversely affected by reproductive aging. Several studies have shown an inverse relationship between serum VitD levels and MetS, diabetes, and insulin resistance in the general population (41). Nevertheless, data regarding postmenopausal women are limited and contradictory.

Recent meta-analyses of randomized controlled trials have reported conflicting results. One meta-analysis found that VitD supplementation improved glucose metabolism but had no significant effect on body composition or obesity parameters in postmenopausal women (42). Another review concluded that the effects of VitD supplementation on lipid parameters, specifically reductions in LDL-C and increases in HDL-C and total cholesterol, were clinically negligible (43). Together, these findings underscore the need for continued research and suggest that individualized VitD dosing strategies may be required, depending on the specific metabolic outcomes targeted in postmenopausal women.

In our study, we found that postmenopausal women with VitD levels below 21 ng/mL (Q1) had 2.27 times higher odds of having MetS compared with women with levels above 30 ng/mL (Q4). As demonstrated in our study and other surveys, VitD is associated with unfavorable changes in all parameters comprising MetS, which explains this result. A study evaluating 340 postmenopausal Thai women also showed that low VitD was associated with an increased frequency of MetS. Additionally, studies performed on postmenopausal Brazilian women showed that hypovitaminosis D was associated with an increased prevalence of MetS (28,44).

Researchers have recommended vitD levels between 30–60 ng/mL for specific groups, including patients aged >65 years, pregnant women, individuals with recurrent falls, fragility fractures, osteoporosis, secondary hyperparathyroidism, chronic kidney disease, or cancer, and individuals using medications that can potentially affect VitD metabolism (45). However, there is no specific reference range for vitD levels in postmenopausal women. Our study demonstrated better anthropometric and biochemical profile

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outcomes in postmenopausal women with VitD levels above 31 ng/mL. Similarly, we found the worst results in participants with VitD serum concentrations below 21 ng/mL. These findings suggest that maintaining serum VitD concentrations above 30 ng/mL may be beneficial for the metabolic health of postmenopausal women.

In this context, our results support the inclusion of serum VitD assessment in the routine health monitoring of postmenopausal women, particularly those with metabolic risk factors. Given the high prevalence of hypovitaminosis D and its association with adverse cardiometabolic profiles, strategies such as VitD supplementation, lifestyle modifications aimed at increasing sunlight exposure, and dietary guidance may serve as low-cost preventive measures (4,26). These interventions could help reduce the prevalence of MetS and improve overall metabolic health in this population.

This study has certain limitations. We did not assess dietary VitD intake or sun exposure, both of which are key determinants of serum VitD concentrations and might have contributed to individual variability in our findings. Although women who reported using VitD supplements were excluded, we did not control for the use of other medications that may affect VitD metabolism. Additionally, the cross-sectional design precludes causal inferences. Future longitudinal studies are needed to further investigate the potential protective role of VitD against MetS in postmenopausal women.

CONCLUSION

This population-based study demonstrated an inverse association between serum VitD concentrations and the presence of MetS in postmenopausal women. Women with VitD levels below 21 ng/mL had more than twice the risk of developing MetS compared to those with adequate levels. These findings highlight the potential role of VitD as a biomarker of metabolic health and underscore the importance of routine monitoring in postmenopausal populations as a low-cost preventive strategy. Future longitudinal and randomized controlled studies are warranted to confirm these associations and to determine whether improving VitD status can effectively reduce the risk of MetS and related cardiometabolic complications.

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Ethical statement: all procedures were performed in compliance with relevant laws and institutional guidelines and were approved by the appropriate institutional committee. Selected participants provided written informed consent. The Research Ethics Committee of the Federal University of Ouro Preto (CEP/UFOP) approved this study under protocol number 56312916.8.0000.5150.

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Data availability: all data generated or analyzed during this study were produced by the authors. Datasets are available from the corresponding author upon reasonable request.

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original

Characteristics of transgender health-related provider-to-provider consultations in Brazil's public primary health care system: A cross-sectional study from a Southern Brazilian telehealth service

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ABSTRACT

Objective: Transgender individuals often face structural barriers and adverse health outcomes. Provider-to-provider (P2P) consultations may improve the quality of care for this population. This study describes the characteristics, clinical questions, and care provided to transgender population in the Brazilian public primary health care (PHC) system, using data from a P2P telephone consultation service based in Rio Grande do Sul State. **Subjects and methods:** This cross-sectional study included all consultations classified as related to transgender health care conducted between TelessaúdeRS consultants and PHC professionals from 2015 to 2023. We extracted patient characteristics and clinical questions from each consultation. Continuous data are reported as means ± standard deviations or medians (interquartile ranges), and categorical data as absolute and relative frequencies. **Results:** Between 2015 and 2023, 752 of the 422,972 total consultations were classified as related to transgender health care. Regarding sex assigned at birth, 47.3% of patients were male, 43.4% were female, and 9.3% were unreported. The mean patient age was 24.5 years (±9.1). The most frequent consultation topics were laboratory monitoring (64.9%), hormone therapy (49.7%), and mental health (28.9%). Nonprescription hormone use was reported by 41.0% of patients. Referrals to specialized care were recommended in 68.4% of cases, and clinical guidance was provided in 71.4%. **Conclusion:** The growing number of P2P consultations reflects an increasing demand for support regarding transgender care within PHC. Frequently asked questions reveal knowledge gaps among PHC providers, and the high rate of unsupervised hormone use suggests persistent barriers to appropriate care. P2P consultations can support PHC professionals in providing safer, more timely transgender health care.

Keywords: Telemedicine; remote consultation; transgender persons; health services for transgender persons; primary health care

INTRODUCTION

The global prevalence of self-identified transgender individuals is estimated at approximately 0.6%, though estimates vary by region, age, and study method (1). In Brazil, recent estimates suggest a prevalence of 0.69% among adults, with relatively uniform distribution across regions (2). The transgender population faces significant barriers to accessing public health services, including stigma, discrimination, and geographic inequities (3). These barriers underscore the urgent need for inclusive and equitable public health policies.

The demand for comprehensive gender-affirming care for transgender individuals has increased significantly, while the average age at treatment initiation has decreased by approximately four years (4). Moreover, care-seeking among individuals assigned

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female at birth (AFAB) has increased substantially, now equaling or surpassing that of those assigned male at birth (AMAB), including transgender, nonbinary, and other gender-diverse individuals (4–6). Although these trends are encouraging and may reflect greater societal acceptance (4), they also present new challenges for healthcare systems that are often unprepared to meet this rising demand.

Despite being limited, existing literature indicates that specialized healthcare services and hospital-based procedures are concentrated in large urban centers and more socioeconomically developed regions (3,6–9). Limited access to care is frequently associated with nonprescribed hormone use and high rates of suicidal ideation prior to gender transition (6,10,11). As primary health care (PHC) often serves as the main entry point into the health system, transgender individuals frequently rely on these services (12). However, insufficient training in gender-affirming care, uncomfortable patient-provider interactions, and systemic discrimination within healthcare settings limit the effectiveness of care (7,10,13).

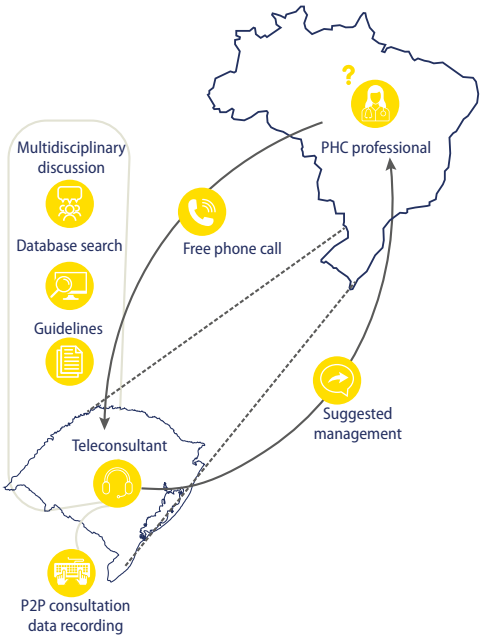
There is growing evidence supporting the utility of remote consultations, offered in both patient-directed and provider-to-provider (P2P) modalities (14–16). Such consultations have shown benefits in the management of stigmatized conditions, including syphilis in pregnancy, tuberculosis, and psychiatric disorder (17–20). Additional advantages include reduced travel time and lower costs (21). Thus, the use of telehealth to support transgender care represents a promising opportunity, particularly given the challenges PHC professionals face in this domain. This study aimed to describe the characteristics, clinical questions, and care provided to transgender individuals within Brazil's public PHC system, utilizing data from a large P2P telephone consultation service.

SUBJECTS AND METHODS

Study design and settings

This cross-sectional retrospective study analyzed records from a telehealth service and adhered to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines (22). Data were obtained from TelessaúdeRS, a toll-free P2P telephone consultation service supporting public PHC providers in Brazil, based in Rio Grande do Sul State. The service offers telehealth support during or after patient encounters, including consultation, diagnosis, gatekeeping, and continuing education for physicians, nurses, and dentists working in PHC nationwide. It operates on business days from 8:00 am to 6:00 pm, enabling real-time consultations between PHC professionals and specialist consultants (**Figure 1**).

Data were collected from 2015 to 2023 and extracted from the TelessaúdeRS database (Research Organization Registry [ROR]: https://ror.org/00g229b55). Each consultation was initiated by a PHC professional and recorded in a standardized report stored on a secure university server. Consultants reviewed the clinical question and available resources and provided evidence-based recommendations based on TelessaúdeRS clinical guidelines and national protocols (17). Although they did not have specific training in transgender health, consultants engaged in interdisciplinary discussions and followed the same evidence-based processes. Each consultation concluded with a proposed care plan and guidance on the appropriate level of care (i.e., PHC, specialized service, or emergency referral).



PHC: primary health care; P2P: provider-to-provider.

Figure 1. TelessaúdeRS synchronous provide-to-provider telephone consultation service.

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Participants

We selected consultations from January 2015 to December 2023 in which the primary topic was health care for the transgender population. Consultations focusing on clinical questions or guidance related to transgender health were included. Consultations that were misclassified (unrelated to transgender health), duplicated, or incomplete were excluded. Data were accessed for research purposes in August 2024 and analyzed between September and November 2024. For patients discussed in multiple consultations, the first call was defined as the index consultation, and subsequent calls were classified as follow-up discussions.

Variables

We reviewed each consultation was reviewed to extract patient, clinical, PHC professional, and consultant data. For patients, variables included age (years), sex assigned at birth (male or female), reported comorbidities, age of self-identification with gender diversity, history of hormone therapy (categorized as none, nonprescribed, or prescribed), type of hormones used in nonprescribed (classified as non- or guideline-recommended formulations), receipt of mental health support, type of mental health service accessed (psychologist, psychiatrist, or public health system), and presence of a support network, defined by the presence of a responsible adult during the consultation for patients under 16 years of age.

For PHC professionals and consultants, gender (male or female), professional category, and age were recorded. Patients were stratified into two age groups: < 16 and ≥ 16 years, based on the age criterion for hormone therapy initiation in Brazil. From the consultation records, we also extracted information regarding the type of consultation (specialist referral, clinical discussion, or both), mean age at referral, main clinical discussion topics, adherence to recommendations provided (categorized as yes, no, or partial), presence of vulnerability conditions, cancer screening status, and geographic location (state and municipality) of the consultation.

Not all variables were documented in every consultation, as certain data were not always pertinent to the clinical question. When this occurred, variables were recorded as unreported. These missing data did not compromise the descriptive nature of the analysis. The primary objective was to describe the characteristics of patients and their consultations; no *a priori* outcomes were defined.

Data analysis

Continuous variables were summarized as means with standard deviations (SD) (mean ± SD) or medians with interquartile ranges (median [$Quartile_{25-75}$]), while categorical variables were reported as absolute and relative frequencies. No comparative or inferential statistical analyses were planned. Data analysis was performed using Microsoft Excel and R software (version 4.3.1). A convenience sample was used, comprising all eligible telephone consultations during the study period.

Ethical approval

The study was approved by the Ethics Committees of *Hospital de Clínicas de Porto Alegre* (CAA no. 62602522.9.0000.5327) and AGHUse (protocol no. 2022-0371). As anonymized data from the TelessaúdeRS database were utilized, informed consent was waived.

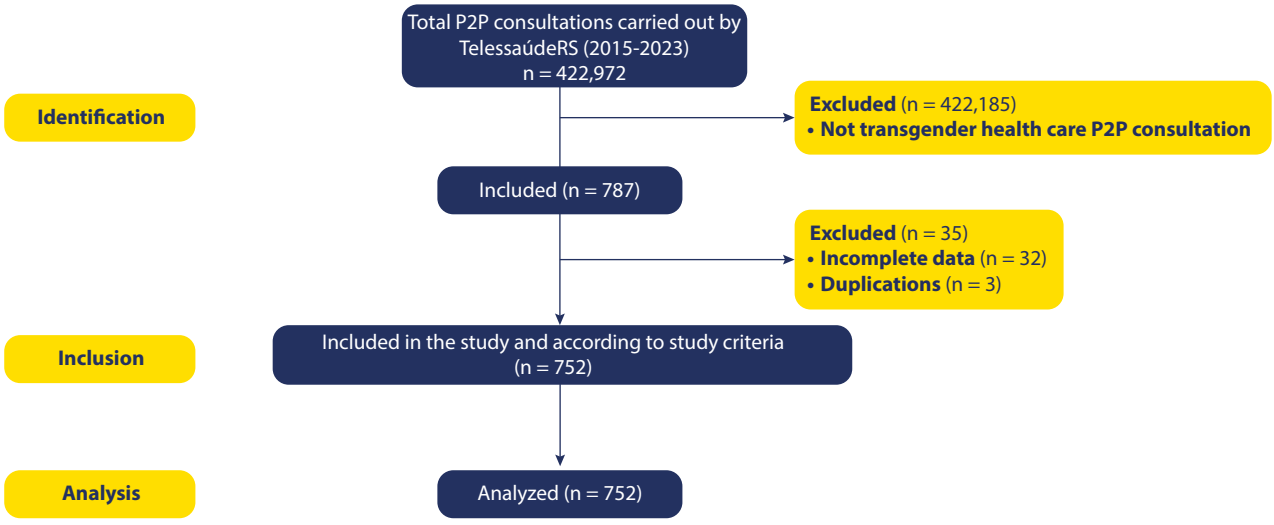
RESULTS

Of the 422,972 consultations conducted by TelessaúdeRS over the study period, 787 were classified as related to transgender health care. After excluding 35 consultations due to duplication or incomplete data, 752 were included in the analysis (**Figure 2**). Among these patients, 47.3% were AMAB and 43.4% AFAB, indicating a relatively balanced distribution. The mean age at consultation was 24.5 years (SD ± 9.1), with a median of 22 years (18; 28). **Table 1** summarizes the age distribution and additional patient characteristics.

Most individuals were receiving some form of hormone therapy, frequently through nonprescribed use. Among AMAB patients, non-guideline-recommended formulations were most commonly reported. In transgender men already undergoing appropriate hormonal monitoring, isolated progestins were used

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as a contraceptive method. For individuals who had not yet initiated testosterone therapy, combined oral contraceptives were frequently prescribed in PHC settings to suppress menstruation.



P2P: provider-to-provider.

Figure 2. Flowchart of provider-to-provider telephone consultations about transgender health care carried out by TelessaúdeRS (2015–2023).

Table 1. Characteristics of transgender patients in TelessaúdeRS provider-to-provider telephone consultations (2015–2023)

Clinical characteristic – Transgender patients		N (%) or mean ± standard deviation
Age at consultation (years) n = 702[a]	< 16 years ≥ 16 years	54 (7.7) 648 (92.3)
Sex assigned at birth n = 682[a]	Male Female	356 (52.2) 326 (47.8)
Reported comorbidities n = 179[a]	Depression Smoking General anxiety disorder HIV Hypertension	38 (21.2) 36 (20.1) 26 (14.5) 20 (11.2) 15 (8.4)
Age of self-identification as transgender n = 47[a]		12.6 ± 3.81
Hormone therapy history n = 471[a]	Without prior use Prescribed hormone use Non prescribed hormone use	157 (33.3) 118 (25.1) 196 (41.6)
Nonprescribed hormone use n = 196[a]	**AMAB (n = 109)**	
	Non-guideline recommended formulation Prioritized antiandrogenic formulations Prioritized estradiol formulations	90 (82.6) 36 (33) 25 (22.9)
	AFAB (n = 62)	
	Guideline recommended testosterone formulations Contraceptives/hormones to stop menstruation Non-guideline recommended testosterone formulations	53 (85.5) 10 (15.1) 1 (1.6)
Mental health support n = 166[a]	Psychologist follow-up Psychiatric follow-up Public health system mental health care	64 (38.5) 40 (24.1) 47 (28.3)

[a] Unreported data are not shown in the table. The sample size (n) for each variable is presented in the first column, where applicable. AMAB: assigned male at birth; AFAB: assigned female at birth; HIV: human immunodeficiency virus infection.

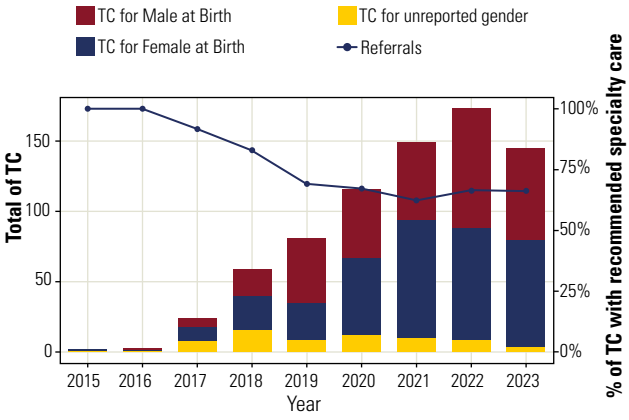
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The mean age of PHC professionals was 37.6 years (SD ± 10.7; n = 295), whereas that of consultants was 34.7 years (SD ± 4.2; n = 394). Female professionals accounted for 67.1% of PHC providers (n = 695) and 71.4% of consultants (n = 707). Among PHC professionals (n = 690), 95.6% of consultations were conducted by physicians, of whom 83.0% were general practitioners without residency training, 9.6% were family physicians, and 3.0% represented other specialties. Nurses and psychologists participated in a smaller proportion of consultations.

Most consultations (61.8%; n = 436) were answered by generalist medical professionals (e.g., family medicine or internal medicine), while 23.9% (n = 169) were handled by endocrinologists, and 10.8% (n = 76) by other specialists. In addition, 3.5% (n = 25) of consultations were conducted by nurses. Consultations were conducted across Brazil, with participation from 12 of the 27 states. Although the service is available nationwide, the vast majority (88%, n = 662) originated in Rio Grande do Sul, where the service is based.

The number of transgender-related case discussions increased steadily over the years. The first consultation occurred in 2015, two years after service initiation. The peak was in 2022, with 173 consultations, representing 23% of the study sample. **Figure 3** presents the annual distribution of consultations and referral rates. Overall, 68.4% of consultations resulted in referrals, and clinical guidance was provided in 71.4%. **Table 2** outlines the consultation topics and corresponding recommendations. Most consultations involved both clinical and referral-related questions. The most frequently addressed clinical issues were monitoring exams and hormone therapy. A substantial proportion also focused on broader aspects of patient evaluation, including clinical history and mental health concerns. In consultations with follow-up data, recommendations were generally followed. PHC professionals or consultants rarely assessed social vulnerability conditions.

Among patients under the age of 16 (n = 54), 57.4% of consultations documented a support network and the presence of an accompanying adult over 18 years old. Fourteen were accompanied by their mother, ten by both parents, two by their father, one by their grandmother, and four had no familial support network recorded. All individuals under 16 were referred to specialized reference centers for transgender health care, as required by Brazilian legislation.



TC: teleconsultation.

Figure 3. Annual number of provider-to-provider telephone consultations related to transgender care and proportion of recommended referrals, stratified by sex assigned at birth, in Brazil's primary health care system.

DISCUSSION

In our P2P consultation service, calls were evenly distributed between individuals AFAB and AMAB, with a noticeable increase in consultations over time. The most frequently addressed topics were hormone therapy management and laboratory evaluation. Our findings indicated that many transgender individuals were using hormones without medical supervision.

A similar rise in transgender-specific consultations has been reported in other countries, coinciding with the growing number of people identifying as gender diverse since the 1990s, once considered a rare phenomenon (4). In September 2022, TelessaúdeRS launched an e-book on transgender health to support PHC professionals (23). Developed in response to rising demand and the increasing need for follow-up care within primary care, this resource addresses the insufficient availability of specialized services to meet the needs of this population. The drop in consultations in 2023 may reflect improved access to updated national guidelines and the gradual expansion of transgender-specific health services in Brazil (7,8).

Most patients were young, consistent with recent findings showing a shift in the profile of transgender

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Table 2. Management characteristics of provider-to-provider consultations concerning transgender health provided by TelessaúdeRS

Clinical inquiries from PHC professionals		N (%)
P2P consulting recommendation	Clinical discussion and case referral	263 (35.0)
	Case referral	251 (33.4)
	Clinical discussion	238 (31.6)
Main clinical discussions topics[a] n= 501[b]	Monitoring exams	325 (64.9)
	Hormone therapy	249 (49.7)
	Clarification of clinical history necessity	186 (37.1)
	Mental health	145 (28.9)
	Gynecological conditions	18 (3.6)
	Cancer screening	14 (2.8)
	Other phenotypic interventions	12 (2.4)
	Cardiovascular risk prevention	7 (1.4)
Follow-up of the discussion n= 101[b]	Follow the suggested recommendation	76 (71.7)
	Did not follow the suggested recommendation	17 (16)
	Partially followed the suggested recommendation	8 (7.5)
Vulnerability conditions[a] n= 69[b]	Suicide attempt or ideation	25 (36.2)
	Self-harm/hetero aggression	18 (26.1)
	Domestic/sexual violence	12 (17.4)
	Difficult family acceptance	8 (11.6)
	Previous psychiatric hospitalization	6 (8.7)
	Sex worker (female trans)	5 (7.2)
	Homelessness	4 (5.8)
	Deprived of liberty	4 (5.8)
	Person with a disability	2 (2.9)
	Other phenotype interventions without medical supervision	2 (2.9)
Cancer screening[a] n= 14[b]	Cervix	10 (71.4)
	Breast	3 (21.4)
	Prostate	2 (14.3)

[a] Multiple-response variables.

[b] Unreported data are not shown in the table. The sample size (n) for each variable is displayed in the first column below each variable, where applicable.

PHC: primary health care; P2P: provider-to-provider.

individuals seeking care (4). There has been a steady rise in the number of AFAB individuals accessing services, now reaching parity with AMAB individuals, mirroring the balanced gender distribution in this study (4). The average age for initiating hormone therapy has also decreased over the past decade, now falling below 30 years (4,6).

A significant proportion of patients were already using hormone therapy at their initial consultation. Although hormone levels and dosages were not available, over 80% of AMAB individuals were using non-guideline-recommended feminizing therapies without medical supervision, whereas a similar proportion of AFAB individuals were using guideline-recommended testosterone formulations.

These patterns may reflect disparities in access to medications across gender identities (6,24). Contributing factors may include limited availability of free treatments and preferences for non-guideline-recommended hormones due to perceived faster phenotypic results (11,25,26). These findings underscore the importance of assessing history of (particularly nonprescribed) hormone use during P2P consultations. Mental health and addiction were among the most frequently reported concerns. Suicide attempts or ideation were documented in 3.3% of consultations, which is lower than that reported internationally among transgender individuals before transition, but higher than in the general population (3,27–29). This figure may be underestimated, as not all consultations addressed mental health due to their clinical focus. Only 22.1% of cases indicated that the patient was receiving mental health support. These findings highlight a gap in mental health assessment during

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P2P consultations and among PHC professionals, indicating a need for improved training. Systematically addressing mental health in future consultations could help identify unmet needs and improve care.

Common concerns among PHC professionals included laboratory monitoring, hormone therapy management, clinical history requirements, and mental health issues. With adequate training in gender-affirming care (e.g., mental health assessment and hormone therapy initiation) PHC providers can safely manage more cases without the need for specialist referral. Although international guidelines recommend multidisciplinary specialist follow-up for all transgender patients (23,30–32), P2P consultations can help prioritize referrals, ensuring in-person care is reserved for complex cases, such as individuals pursuing surgery or presenting with severe mental or clinical health conditions. This approach may improve care efficiency and reduce waiting times, which remain prolonged in many settings (9,33).

Regarding younger patients, the average age of self-identification was 12 years. Notably, over 40% of P2P consultations for individuals under 16 did not document family assistance, which is concerning. This may reflect two non–mutually exclusive possibilities: either insufficient attention to this issue by PHC professionals and consultants, or a genuine absence of family support. Family involvement is known to reduce suicidal ideation, lower depression rates, and improve overall quality of life among transgender individuals (27,34). Future remote consultation initiatives should systematically address this factor, particularly for younger populations.

Strengths

To our knowledge, this is the first study to examine how Brazil's PHC system addresses the needs of transgender individuals using real-world data. The large and diverse sample provides insight into service use across multiple regions, reflecting the broad reach of the TelessaúdeRS platform. In addition, the data are contemporary and mirror recent trends in transgender care delivery. Collectively, these elements represent a novel contribution, offering comprehensive and timely perspective on the specific demands and gaps faced by PHC professionals managing transgender health within the Brazilian public health system.

Limitations

First, the data were drawn exclusively from PHC professionals who voluntarily used the P2P service, representing a self-selected sample that may not fully reflect the routine practice of all Brazilian PHC providers. Nevertheless, while this limits generalizability at the national level, it provides a high-fidelity, "real-world" perspective on the clinical complexities and support needs of those actively engaged in transgender care. Additionally, the majority of consultations (88.6%) originated from Rio Grande do Sul, the state where the service is based; this requires caution when extrapolating findings nationally but offers a unique "sentinel" perspective on the clinical demands encountered by engaged providers within the public system. Finally, patient data were not stratified by race or skin color due to incomplete reporting, which constrained exploration of these specific social determinants in transgender care.

Furthermore, the extent of information collected varied by consultation, as some data were omitted when not directly relevant to the clinical inquiry. Future research should assess healthcare professionals' knowledge and preparedness to care for transgender individuals, as well as explore how transgender people perceive the quality, safety, and respectfulness of care received in primary health services. Studies evaluating and guiding improvement of public health policies within the Brazilian Unified Health System are likewise essential to ensure they effectively address the needs of this population. Such evidence is crucial for promoting more inclusive, competent, and equitable care in primary health settings.

In conclusion, this study highlights the increasing volume of clinical questions related to follow-up and hormone therapy for transgender individuals in PHC. It underscores the need to support PHC professionals, particularly regarding hormone therapy initiation and monitoring, mental health care, and support for youth. Ultimately, P2P consultations may enhance PHC professionals' capacity to deliver appropriate care to transgender patients and facilitate

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timely access within primary or specialized services. Although our findings reflect the experience of a major telehealth service in southern Brazil and thus have limited national generalizability due to selection bias and regional concentration, they offer a foundational real-world perspective on clinical demands within the Brazilian public health system. Consequently, these results provide a vital evidence base for informing the expansion of similar support strategies and inclusive health policies in other Brazilian regions.

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Impact of premature mortality attributable to obesity on years of life lost in Brazil

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ABSTRACT

Objective: To quantify the societal burden of obesity in Brazil by estimating years of life lost (YLL) due to premature mortality. **Subjects and methods:** Given the Brazilian life expectancy of 76.4 years, premature death was defined as occurring before the age of 60. A quantitative epidemiological approach was applied to estimate obesity-attributable YLL in Brazil from 2014 to 2023. Mortality data were obtained from the Mortality Information System, and demographic projections were sourced from the Brazilian Institute of Geography and Statistics. The YLL was calculated by multiplying the number of deaths by the remaining life expectancy (using 2021 Global Burden of Disease reference tables), stratified by age and sex. Statistical analyses were conducted using R software and Python, with results presented as rates per 100,000 population. **Results:** The total number of events exhibited an upward trend over the decade, with a pronounced peak after 2020, coinciding with the COVID-19 pandemic. The 50–59 and 60–69-year age groups consistently demonstrated the highest event frequencies. Sex-specific analyses revealed variations across age groups and years. Events were rare among those aged 1–14 but increased from age 15–19 onwards. Among older age groups (40–69 years), females exhibited higher rates than males, particularly in the 60–69-age bracket. **Conclusion:** This study demonstrates an increasing trend in obesity-related premature mortality in Brazil, disproportionately affecting older populations and necessitating targeted public health interventions.

Keywords: Obesity; years of life lost; premature mortality

INTRODUCTION

Obesity has emerged as one of the most pressing global public health challenges, with its prevalence rising dramatically in both high-income and low- and middle-income countries (1). Defined by excessive body fat accumulation, obesity is a major contributor to premature mortality through its association with cardiovascular diseases, type 2 diabetes mellitus, at least 13 types of cancer, respiratory diseases, musculoskeletal disorders, chronic kidney disease, liver disease, and numerous other conditions (2). In Brazil, obesity prevalence has increased over the past 50 years, affecting individuals across all age groups and socioeconomic strata (3). This escalating issue is compounded by regional disparities in healthcare access, socioeconomic inequalities, and the double burden of malnutrition (4). Quantifying the societal impact of obesity-related deaths requires robust epidemiological metrics, such as years of life lost (YLL), which estimate the gap between observed age at death and standard life expectancy (5).

YLL serves as a critical metric to contextualize the burden of premature mortality, emphasizing the societal cost that extends beyond traditional mortality

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statistics (6). Studies utilizing YLL calculations have revealed up to 40% higher disease impacts than conventional mortality analyses, underscoring the necessity for region-specific assessments (7). Socioeconomic determinants play a pivotal role in obesity outcomes in Brazil, with income inequality and limited healthcare access contributing to differential mortality rates (8). Individuals from lower socioeconomic backgrounds face higher risks of obesity-related complications and premature death, attributable to reduced access to healthy foods and limited opportunities for physical activity (9).

Temporal trends reveal concerning patterns, as national surveillance data indicate a 45% increase in obesity-related mortality between 2010 and 2020 (10). The COVID-19 pandemic significantly disrupted healthcare services, including limited access to bariatric surgery and clinical follow-up in both public and private healthcare systems, and may have exacerbated premature mortality among individuals with obesity, though findings are not entirely consistent across studies (11–13). Given this context, this study sought to quantify the societal burden of obesity in Brazil by estimating YLL due to premature mortality between 2014 and 2023, providing age-, sex-, and time-stratified estimates to inform evidence-based policy development aligned with Sustainable Development Goal target 3.4 (14).

SUBJECTS AND METHODS

This study utilized a quantitative epidemiological framework to estimate the burden of premature mortality attributable to obesity in Brazil. The core measure was YLL, which estimates the average years a person would have lived if not for premature death due to obesity-related causes.

Data collection

Mortality data were retrieved from the Mortality Information System maintained by the Brazilian Ministry of Health, covering 2014 to 2023. The dataset was disaggregated by age, sex, and cause of death. Selection criteria included records in which obesity was documented as either the underlying or a contributing cause of death, using International Classification of Diseases, Tenth Revision (ICD-10) codes: E66.0 (obesity due to excess calories), E66.1 (drug-induced obesity), E66.2 (extreme obesity with alveolar hypoventilation), E66.8 (other forms of obesity), and E66.9 (obesity, unspecified). Importantly, obesity-related mortality is likely underestimated on death certificates, as obesity itself is frequently omitted, while obesity-associated conditions (e.g., cardiovascular or metabolic diseases) are commonly listed as causes. The principal underlying causes associated with obesity included diseases of the circulatory system (I00–I99), particularly ischemic heart diseases (I20–I25) and cerebrovascular diseases (I60–I69); endocrine and metabolic disorders, especially type 2 diabetes mellitus (E11); malignant neoplasms (C00–C97); and diseases of the respiratory system (J00–J99). This refined case definition enhances the etiological specificity of mortality patterns attributable to obesity in Brazil. During the COVID-19 pandemic, inconsistent documentation of anthropometric measurements further limited the precise assessment of adiposity status. Demographic information, including population size and age-sex distribution, was obtained from the Brazilian Institute of Geography and Statistics to accurately characterize the population at risk.

Calculation of years of life lost

YLL was calculated by multiplying the number of deaths within specific age and sex strata by the corresponding remaining life expectancy at the age of death. Life expectancy references were adopted from the 2021 Global Burden of Disease (GBD) study (15), ensuring standardized and internationally comparable benchmarks. Age weighting and time discounting were not applied, in keeping with current ethical considerations and best practices.

Data analysis

Data processing and statistical analyses were performed using the open-source software PSPP and the Python programming language, with additional calculations in Excel. Mortality rates were standardized per 100,000 population and were further stratified by age and sex to assess trends and disparities over the study period.

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Contextual factors

Socioeconomic and healthcare variables such as income distribution, access to health services, and public health policies relevant to obesity prevention and management were incorporated from governmental databases and published literature, providing a comprehensive understanding of the social determinants influencing premature mortality related to obesity.

Ethical considerations

Given the exclusive use of aggregated, anonymized, publicly available data, this study was exempt from individual consent or institutional ethical review, in compliance with Brazilian regulations governing secondary data research.

RESULTS

Obesity-related premature mortality in Brazil (2014–2023)

Premature death was defined as occurring before age 60, in the context of a national life expectancy of 76.4 years. The YLL was calculated by multiplying deaths in each age–sex stratum by the remaining life expectancy from the GBD study (15). Results are reported as age-standardized rates per 100,000 population.

Temporal trends in obesity-attributable mortality

Obesity-related deaths were observed throughout the study period, with a marked acceleration following 2020, temporally coinciding with the onset of the COVID-19 pandemic. Annual deaths increased from 1,900 in 2014 to a peak of 3,488 in 2021, then declined modestly to 2,660 in 2023 (**Table 1**). This pattern suggests a pandemic-related disruption in chronic disease management and healthcare access that may have exacerbated underlying obesity-related pathologies. The analysis indicated that, while the pandemic amplified mortality, the long-term trend primarily reflects ongoing epidemiological transitions rather than an isolated infectious event.

Age distribution of mortality events

Mortality events were negligible among children and adolescents (ages 1–14), with fewer than five deaths annually in this group. Event frequency increased progressively from age 15 onward, with the highest burden concentrated in the 50–59 and 60–69 age groups. These two strata consistently accounted for over 50% of all obesity-attributable deaths each year, underscoring the disproportionate impact on middle-aged and older adults (**Figure 1**).

Sex-specific disparities

Sex-stratified analysis revealed notable differences in mortality patterns. Males had slightly higher death counts in younger adult groups (20–49 years), while females consistently surpassed males in the 50–69 age brackets **(Figure 2).** This disparity was most pronounced in the 60–69 group, where female deaths

Table 1. Annual distribution of obesity-attributable deaths by age group, Brazil

Year	Age range									Total
	01-04	05-09	10-14	15-19	20-29	30-39	40-49	50-59	60-69	
2014	1	2	3	11	102	287	439	548	507	1,900
2015	2	1	4	16	89	264	361	579	610	1,926
2016	0	2	4	12	102	309	489	625	541	2,084
2017	2	1	2	10	97	270	469	600	603	2,054
2018	2	3	2	10	101	307	531	616	658	2,230
2019	1	0	0	10	108	341	550	679	691	2,380
2020	1	0	1	17	136	447	671	779	838	2,890
2021	1	1	4	18	160	488	862	964	990	3,488
2022	2	5	5	23	132	369	617	744	781	2,678
2023	3	2	5	19	138	367	674	742	710	2,660

Source: TABNET – Brazilian Ministry of Health.

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exceeded male deaths every year, reaching a difference of 188 additional female deaths in 2021 (Table 2). These findings suggest a sex-based divergence in obesity-related disease progression, healthcare-seeking behavior, or biological susceptibility during later life stages.

Overall, this study demonstrated a sustained upward trend in obesity-attributable premature mortality across Brazil from 2014 to 2023, with the highest burden among middle-aged and older adults. A pronounced gender disparity was observed, as women exhibited consistently higher mortality rates than men in advanced age groups, suggesting sex-specific vulnerabilities in obesity-related health outcomes.

DISCUSSION

Our study demonstrated an escalation in obesity-driven premature mortality throughout Brazil, disproportionately affecting older demographics and revealing substantial sex-based vulnerabilities that necessitate urgent, evidence-based public health interventions.

Analysis of Mortality Information System data revealed that the main causes of death associated with obesity during the study period were predominantly cardiovascular diseases (including ischemic heart disease, stroke, and heart failure), type 2 diabetes mellitus and its complications, certain cancers (particularly those of the digestive system, breast, and reproductive organs), chronic respiratory diseases,

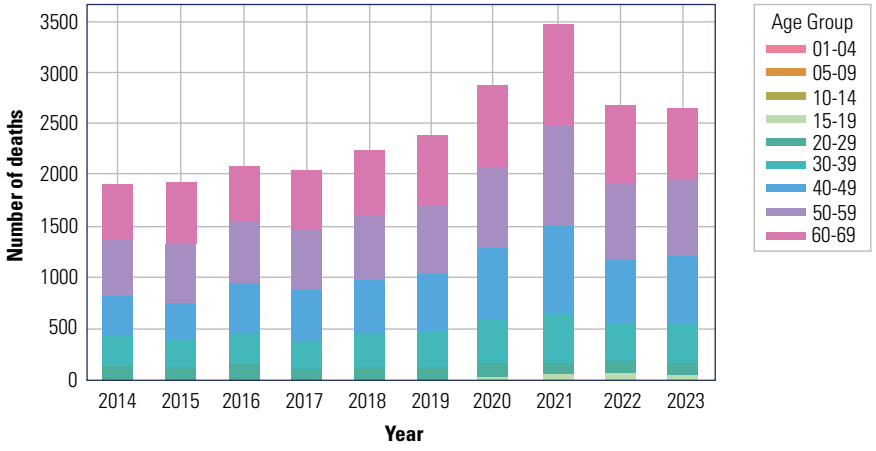


Figure 1. Trends in obesity-related premature mortality by age group.

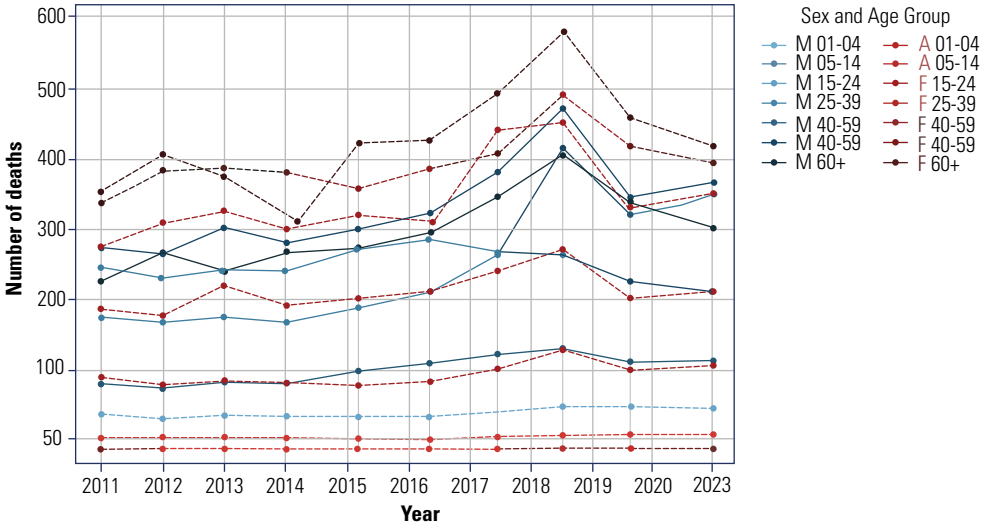


Figure 2. Sex-specific trends in obesity-attributable YLL across age groups

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Table 2. Sex-disaggregated counts of obesity-attributable deaths by age group and year

Age range	Male–female								
	01–04	05–09	10–14	15–19	20–29	30–39	40–49	50–59	60–69
2014	1–0	2–0	3–0	8–3	47–55	138–149	206–233	244–304	186–321
2015	0–2	1–0	2–2	9–7	43–46	127–137	188–273	222–357	231–379
2016	0–0	1–1	2–2	8–4	50–52	134–175	200–289	267–358	197–344
2017	1–1	1–0	2–0	4–6	49–48	125–145	200–269	245–355	231–272
2018	1–1	2–1	1–1	5–5	53–48	146–161	239–292	278–338	240–418
2019	1–0	0–0	0–0	5–5	64–44	170–171	259–291	304–375	268–423
2020	1–0	0–0	0–1	10–7	77–59	234–213	235–436	374–405	340–498
2021	1–0	1–0	2–2	7–11	80–80	241–247	411–451	474–490	401–589
2022	1–1	3–2	5–0	10–13	71–61	194–175	301–316	333–411	325–456
2023	1–2	1–1	2–3	8–11	73–65	184–183	336–338	353–389	287–423

Source: TABNET – Brazilian Ministry of Health.

and chronic kidney disease (16). These findings are consistent with established international evidence on obesity-related mortality, reinforcing that most premature deaths attributed to obesity occur through these well-characterized pathophysiological pathways rather than obesity itself being listed as the primary cause of death (17).

YLL offers a time-based metric that quantifies premature death by calculating the difference between age at death and standard life expectancy for that age, thereby avoiding arbitrary age thresholds and ensuring that all deaths contribute proportionally to burden estimates (18). For obesity, YLL calculations revealed significant variations across demographic strata, with younger adults experiencing greater life-year losses than older populations when facing comparable obesity severity (15). Methods such as those from the GBD calculate YLL by multiplying cause-specific deaths across age, sex, and location by the corresponding standard life expectancies from reference tables (19). These estimations have shown that severe obesity results in aggregate burdens exceeding ninety-five million years lost annually, with considerable contributions from certain demographic groups (20). Our study aligns with the established GBD (15) protocol, employing standardized life expectancy references and adapting operational thresholds to Brazilian demographic realities, thereby ensuring both international comparability and local epidemiological relevance.

The sustained upward trajectory in obesity-related mortality reflects broad epidemiological transitions: non-communicable chronic diseases (NCDs) have increasingly overshadowed infectious diseases as leading causes of death, particularly among aging populations burdened by cumulative metabolic dysfunction (21). Globally, obesity prevalence has tripled since 1975, with projections suggesting most adults will be overweight or obese by 2050, disproportionately impacting low- and middle-income countries (22). Our findings align with global trends linking rising obesity mortality to shifts favoring NCDs, while also revealing a distinct pandemic-associated acceleration, suggesting that disruptions in care during this period intensified pre-existing metabolic vulnerabilities, particularly in populations already facing systemic healthcare inequities. Thus, while international transitions favor chronic metabolic diseases over infectious causes, Brazil's trajectory demonstrates how health system disruptions can exacerbate established vulnerabilities in resource-constrained settings.

The disproportionate increase in obesity-related mortality observed during the COVID-19 period (2020–2021) warrants discussion. Multiple studies demonstrate that individuals with obesity faced higher risks of severe COVID-19 outcomes, including hospitalization, intensive care unit admission, mechanical ventilation, and death (11–13). The mechanisms underlying this vulnerability are multifactorial, including impaired immune response, chronic low-grade

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inflammation, reduced respiratory capacity due to chest wall restriction, and higher prevalence of comorbidities (e.g., diabetes and cardiovascular disease) (23). Additionally, the COVID-19 pandemic caused substantial disruptions to routine healthcare services, with postponements of elective bariatric surgeries, reduced access to nutritional counseling and weight management, and less physical activity due to lockdown measures (24). These factors likely contributed to the observed acceleration in obesity-attributable mortality during 2020–2021, representing both direct effects of SARS-CoV-2 infection in individuals with obesity and indirect effects from healthcare system disruptions.

Recent evidence underscores sex-specific vulnerabilities in obesity-related premature mortality, with men consistently exhibiting higher age-standardized YLL rates than women, potentially driven by differences in fat distribution, healthcare-seeking behaviors, and hormonal influences (25). Women, however, experience a disproportionate psychopathological burden and obesity-related malignancies, facing elevated mortality risks despite more frequent treatment-seeking compared to men (26). Our findings diverge from global patterns by revealing an unexpected female predominance in advanced age groups, rather than the typical male excess mortality. This inversion suggests distinctive Brazilian sociocultural factors, healthcare access barriers, or hormonal transitions uniquely amplifying women's vulnerability.

Analyzing trends in obesity-related mortality rates excluding the COVID-19 period (2020–2021) provides further insight. Between 2014 and 2019, deaths increased from 1,900 to 2,305, a 21.3% increase over five years, with an average annual growth rate of approximately 4.0%. Post-pandemic (2022–2023), mortality rates declined from the 2021 peak but remained higher than pre-pandemic levels (2,872 deaths in 2022 and 2,660 in 2023), suggesting only partial recovery. This indicates that while the COVID-19 pandemic exacerbated obesity-related mortality, an underlying upward trend was already established in pre-pandemic years. The persistence of elevated mortality rates in 2022–2023, compared to 2019, suggests potentially lasting negative effects on obesity outcomes, possibly due to delayed diagnoses, treatment interruptions, or worsening of metabolic health during lockdowns. This mirrors global findings where obesity-related mortality remained elevated post-pandemic, especially in high-income nations, suggesting systemic healthcare disruptions persisted beyond acute COVID-19 phases (27).

Limitations of this study include potential underascertainment of obesity-related deaths due to inconsistent coding practices on death certificates, where obesity is often omitted as a cause. Additionally, defining premature mortality as death before age 60, despite Brazil's life expectancy of 76.4 years, may underestimate YLL in older adults. Other limitations include reliance on secondary mortality data, introducing potential misclassification, particularly regarding the role of obesity as underlying versus contributing cause. Confounding variables including smoking, socioeconomic stratification, and comorbidity profiles remain unaccounted within aggregated datasets, potentially overestimating the direct attribution to obesity while obscuring complex causal pathways mediating YLL outcomes.

In summary, our study provides a decade-long quantification of obesity-attributable YLL in Brazil, revealing a concerning upward mortality trend, exacerbated post-2020. Notably, identifying paradoxical female predominance in advanced age groups challenges established international patterns, warranting further research into Brazil-specific sociocultural and healthcare determinants.

In conclusion, this study quantified the burden of obesity-attributable premature mortality in Brazil, revealing sustained temporal escalation with pandemic-era acceleration. Pronounced age-stratified patterns emerged, concentrating mortality among middle-aged and older individuals, with a paradoxical female predominance in advanced age brackets that challenges conventional epidemiological paradigms, requiring targeted interventions to address sex-specific vulnerabilities and reduce preventable years of life lost.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

Extrathyroidal extension is independently associated with cervical lymph node metastasis in pediatric papillary thyroid carcinoma

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ABSTRACT

Objective: To identify clinicopathologic predictors independently associated with cervical lymph node metastasis in pediatric papillary thyroid carcinoma. **Subjects and methods:** We performed a retrospective cohort including patients younger than 18 years who underwent total thyroidectomy for papillary thyroid carcinoma (PTC) (2013–2023) at a tertiary pediatric hospital in Brazil. Clinical and histopathologic variables were reviewed per World Health Organisation Classification 5th edition (2022) and the College of American Pathologists protocol (2023). Multivariable associations with cervical lymph node metastasis (LNM) were estimated using Firth's penalized logistic regression; discrimination was summarized by the area under the ROC curve (AUC) with 95% bootstrap confidence intervals. **Results:** Thirty-two patients were included (median age, 14 years; 81% female). Cervical LNM occurred in 78%. Extrathyroidal extension (ETE) was associated with cervical lymph node metastasis in bivariate analysis (100% vs 59%; p = 0.007) and remained independently associated with cervical lymph node metastasis after adjustment for tumor T stage and lymphatic invasion (OR, 14.29; 95% CI, 1.28–2028.82; p = 0.028). **Conclusion:** ETE was independently associated with cervical lymph node metastasis in pediatric PTC and may justify closer postoperative surveillance.

Keywords: Pediatric thyroid cancer; papillary thyroid carcinoma; extrathyroidal extension; cervical lymph node metastasis; risk stratification

INTRODUCTION

Although uncommon, papillary thyroid carcinoma (PTC) in children and adolescents has shown a rising incidence over recent decades, particularly among adolescent girls. This increase may reflect, at least in part, enhanced diagnostic surveillance with widespread use of imaging and biopsy, together with environmental and biological determinants that continue to be elucidated (1). While pediatric PTC shares features with adult disease, it exhibits distinctive clinical and biological behavior, including a higher frequency of extrathyroidal extension, elevated rates of cervical lymph node metastasis, and, in some cases, pulmonary metastasis at diagnosis (2).

Cervical lymph node metastases are present in approximately 70% of children with PTC (3). Although the impact on overall survival is limited, nodal involvement is a well-established prognostic factor for locoregional recurrence and directly influences follow-up strategy, the target level of TSH suppression, and

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the extent of surgery (2,4). Multiple clinicopathologic variables have been proposed as predictors of cervical nodal disease, including age, sex, tumor size, multifocality, extrathyroidal extension (ETE), capsular infiltration, positive surgical margins, and aggressive histologic variants such as the diffuse sclerosing subtype (5-7). However, published results are heterogeneous, partly due to small sample sizes and methodological differences across studies.

Within this context, individualized follow-up becomes particularly relevant. A proportion of patients evolve with indeterminate or biochemically incomplete response after initial therapy. According to the 2025 American Thyroid Association (ATA) management guidelines for adult differentiated thyroid cancer, such scenarios may reflect previously unrecognized cervical lymph-node metastases and often correspond to persistent or recurrent nodal disease (8). Similar risk-adapted principles have been increasingly considered in pediatric differentiated thyroid cancer because of its high initial nodal burden and excellent survival (2). Identifying, at baseline, the factors associated with cervical lymph node involvement can therefore refine risk stratification, guide individualized surveillance, and optimize clinical decision making. Hence, the purpose of this study is to identify independent predictors of cervical lymph node metastasis in pediatric PTC.

SUBJECTS AND METHODS

We conducted a retrospective cohort study based on medical record review of patients who underwent total thyroidectomy for PTC at a tertiary pediatric hospital in Northeastern Brazil between January 2013 and May 2023. The study was approved by the institutional Research Ethics Committee (CAAE 70588423.8.0000.5042; approval No. 6.299.762) and was conducted in accordance with the Declaration of Helsinki (2013 revision).

During the study period, 36 patients younger than eighteen years were evaluated. Thirty-two patients with PTC were included in the analysis, with 4 exclusions for not meeting the study scope. All histologic slides were reviewed by a single pathologist according to the 5th edition of the World Health Organization (WHO) Classification of Tumors (2022) and the College of American Pathologists (CAP) protocol, version 4.4.0 (2023) (9,10).

We collected clinical variables (age, sex) and histopathologic features (subtype, multifocality, extrathyroidal extension, lymphatic invasion, perineural invasion, surgical margins, mitotic index, and TNM staging per AJCC 8th edition). Microscopic extrathyroidal extension was defined as tumor extension into the strap muscles or perithyroidal soft tissues identified on histopathologic examination, without gross or intraoperative evidence of macroscopic invasion, in accordance with the College of American Pathologists protocol and the WHO Classification of Tumors. We also recorded the ATA 2015 recurrence-risk category and use of radioiodine therapy. The primary endpoint was the presence of cervical lymph node metastasis in the central and/or lateral compartments. A case was classified as positive if cervical lymph node metastasis was histologically confirmed either at the time of the initial thyroidectomy or neck dissection, or during any subsequent lymph node surgery performed for persistent or recurrent disease. For analysis, the primary outcome was categorized as metastasis present, defined as any histologically proven cervical nodal metastasis from diagnosis to last follow-up, or metastasis absent. Neck dissections were not performed prophylactically in this cohort. Lateral neck dissections (levels II–V) were undertaken only when lymph node metastasis was documented preoperatively by cytology consistent with papillary thyroid carcinoma. Central compartment dissections (level VI) were performed when there was clinical, imaging, cytologic, or intraoperative suspicion of nodal involvement. Follow-up time was counted from diagnosis to last contact or transition to adult care on reaching eighteen years of age.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, USA) and R version 4.5.0 (R Foundation, Austria). Categorical variables were summarized as counts and percentages. Continuous variables were tested for normality using

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the Shapiro–Wilk test and, due to nonparametric distribution, were reported as median and interquartile range. Bivariate comparisons were conducted using Fisher's exact test or the Mann–Whitney test, as appropriate. Variables with univariable $p < 0.20$ were entered into the multivariable model. Multivariable analysis was performed using Firth-penalized logistic regression, selected to account for small sample size, sparse categories, and potential quasi-separation. The model included three variables for 25 events, approximately eight events per variable. Odds ratios (ORs) with 95% confidence intervals (CIs) were derived from Firth's method. Model discrimination was evaluated using the area under the receiver operating characteristic (ROC) curve (AUC) based on predicted probabilities, with 95% CIs obtained by stratified bootstrap with 2,000 resamples. The optimal cutoff was defined by Youden's index, and corresponding sensitivity and specificity were reported. As a sensitivity analysis, AUC was also estimated using DeLong's method with concordant results; bootstrap CIs are reported.

RESULTS

A total of 32 pediatric patients with papillary thyroid carcinoma who underwent total thyroidectomy were included (Figure 1). The median age was 14 years [IQR, 10–15.7], ranging from 4 to 17 years, with a predominance of females (81%). Most tumors corresponded to the classic variant, followed by the encapsulated classic, diffuse sclerosing, solid/trabecular, infiltrative follicular, and oncocytic subtypes. Multifocality was present in 37.5% of cases, extrathyroidal extension in 47%, and lymphatic invasion in 59%. Mitotic activity was uniformly low across the cohort, and only one patient exhibited tumor necrosis on histologic review. Upon reassessment, this case showed combined morphologic features consistent with high-grade transformation and was reclassified as high-grade papillary thyroid carcinoma. Regarding T staging, T1b tumors predominated. Cervical lymph node metastasis confirmed by histopathology was observed in 78% of patients during the disease course, including cases identified at the initial surgery and during subsequent procedures throughout follow-up. According to the

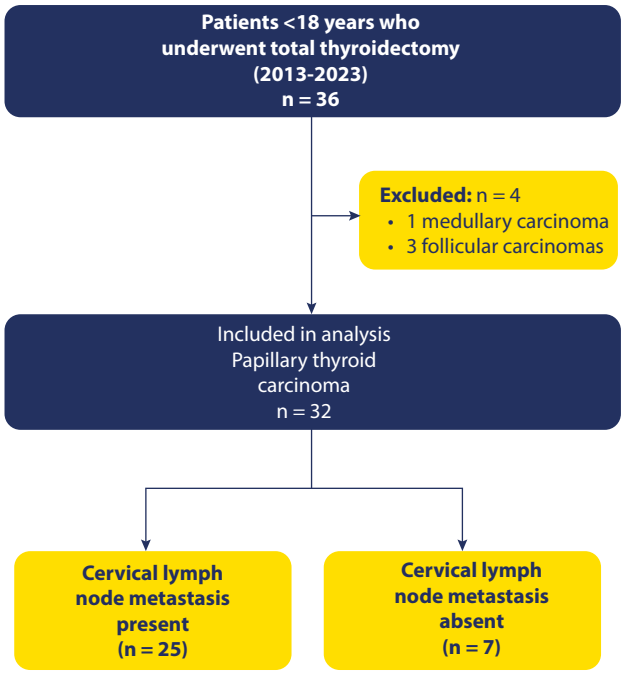


Figure 1. Study flow diagram of cohort selection and classification by cervical lymph node metastasis.

2015 ATA risk stratification, 34% were classified as low risk, 38% as intermediate risk, and 28% as high risk. Half of the patients (16/32) received adjuvant radioiodine therapy, and three (9.3%) had distant pulmonary metastases identified on whole-body radionuclide scans during follow-up. The median follow-up was 28 months [IQR, 17–44], and one patient was lost to follow-up.

In the bivariate analysis, age showed no association with the presence of lymph node metastasis, either as a continuous variable (median age 13 years in patients with metastasis vs 15 years in those without; p = 0.20) or when categorized as ≤12 years and >12 years (p > 0.999). Sex was also not significant (p = 0.29), although all six male patients presented cervical metastasis. Multifocality was not associated with metastasis (p = 0.68). Likewise, positive surgical margins (p = 0.30) and perineural invasion (p > 0.999) showed no significant relationship with cervical nodal metastasis. By contrast, extrathyroidal extension (100% vs 59%; p = 0.007), lymphatic invasion (95% vs 54%; p = 0.010), and advanced tumor stage (T1b–T3a: 91% vs T1a: 44%; p = 0.010) were associated with lymph node involvement (Table 1).

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Table 1. Clinicopathologic features and bivariate analysis by cervical lymph node metastasis in 32 pediatric patients with papillary thyroid carcinoma

Variable	Total (N = 32)*	Cervical lymph node metastasis		*p* value**
		Absent (n = 7)	Present (n = 25)	
Age (years)	14.0 [10.0–15.5]	15.0 [13.5–16.0]	13.0 [9.0–15.0]	0.20
Sex				0.29
Female	26 (81%)	7	19	
Male	6 (19%)	0	6	
Pathological subtype				-
Classic	19 (59.3)	5	14	
Encapsulated classic	3 (9.4)	2	1	
Diffuse sclerosing	4 (12.5)	0	4	
Infiltrative follicular	1 (3.1)	0	1	
Oncocytic	1 (3.1)	0	1	
Solid/trabecular	3 (9.4)	0	3	
High-grade papillary carcinoma	1 (3.1)	0	1	
Multifocality				0.68
Absent	20 (62.5)	5	15	
Present	12 (37.5)	2	10	
Margins				0.30
Free	25 (78.1)	7	18	
Involved	7 (21.9)	0	7	
Perineural invasion				> 0.999
Absent	30 (93.8)	7	23	
Present	2 (6.2)	0	2	
Extrathyroidal extension				0.007
Absent	17 (53.1)	7	10	
Present	15 (46.9)	0	15	
Lymphatic invasion				0.010
Absent	13 (40.6)	6	7	
Present	19 (59.4)	1	18	
Vascular invasion				-
Absent	32 (100)	7	25	
Mitotic index†				-
0	20 (62.5)	4	16	
1	6 (18.8)	1	5	
2	5 (15.6)	2	3	
3	1 (3.1)	0	1	
Necrosis				-
No	31 (97%)	7	24	
Yes	1 (3.1%)	0	1	
T stage(AJCC)††				0.010
T1a	9 (28.1)	5	4	
T1b	12 (37.5)	1	11	
T2	9 (28.1)	1	8	
T3a	2 (6.2)	0	2	

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Variable	Total (N = 32)*	Cervical lymph node metastasis		*p* value**
		Absent (n = 7)	Present (n = 25)	
N Stage				
N0	7 (22)	-	-	-
N1a	14 (44)	-	-	
N1b	11 (34)	-	-	
Cervical metastasis				-
Absent	7 (21.9)	-	-	
Present	25 (78.1)	-	-	
Distant metastasis				-
Absent	29 (90.7)	-	-	
Present	3 (9.3)	-	-	
Recurrence risk (ATA)				-
Low	11 (34.4%)	-	-	
Intermediate	12 (37.5%)	-	-	
High	9 (28.1%)	-	-	
Radioiodine therapy				-
Yes	16 (50%)			
No	16 (50%)			

* Categorical variables are reported as n (%); continuous variables as median [IQR]

** p values were obtained using Fisher's exact test for categorical variables and Mann–Whitney for continuous variables; two-sided $p < 0.05$ was considered statistically significant.

† Mitotic index is reported as mitoses per 2 mm² (WHO 2022).

†† T stage comparison: T1a vs T1b, T2, T3a (AJCC 8th edition).

After adjustment for covariates in a Firth-penalized logistic model, extrathyroidal extension remained independently associated with cervical lymph node metastasis (OR, 14.29; 95% CI, 1.28–2028.82; p = 0.028). Tumor stage showed a trend toward association (OR, 5.95; 95% CI, 0.49–119.98; p = 0.162), whereas lymphatic invasion did not reach statistical significance (OR, 2.97; 95% CI, 0.20–49.61; p = 0.407) (Table 2). The overall model was statistically significant (likelihood-ratio test: $\chi^2 = 14.30$; df = 3; p = 0.0025). The ROC curve yielded an AUC of 0.93 (95% CI by bootstrap, 0.84–1.00); Youden's index was 0.714, with sensitivity of 88% and specificity of 86%. The positive predictive value (PPV) and negative predictive value (NPV) at the optimal cutoff were 0.96 and 0.67, respectively (Figure 2).

Table 2. Firth's penalized logistic regression for clinicopathologic predictors of cervical lymph node metastasis in children and adolescents with papillary thyroid carcinoma

Variable	OR (95% CI)	p value*
Tumor stage (T)	5.95 (0.49 – 119.98)	0.162
Extrathyroidal extension	14.29 (1.28 – 2028.82)	0.028†
Lymphatic invasion	2.96 (0.20 – 49.61)	0.407

* Two-sided $p < 0.05$ was considered statistically significant

† Statistically significant.

OR: odds ratio; CI: confidence interval. Model fitted using Firth's penalized logistic regression.

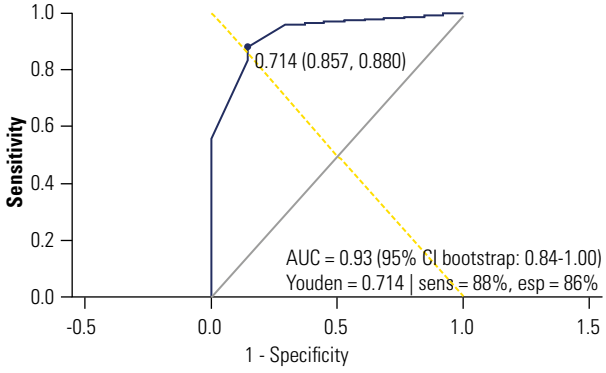


Figure 2. ROC curve for the multivariable Firth's penalized logistic regression model predicting cervical lymph node metastasis (variables included in the model: T stage, extrathyroidal extension, lymphatic invasion). The optimal cutoff by Youden's index was 0.714 (sensitivity 88%, specificity 86%). AUC = 0.93; 95% CI by bootstrap, 0.84–1.00.

DISCUSSION

In this single-center cohort of children and adolescents with papillary thyroid carcinoma, we observed a high burden of cervical lymph node metastasis and found that extrathyroidal extension was the only factor independently associated with nodal involvement after multivariable adjustment. The predictive model showed strong discrimination, supporting the use of extrathyroidal extension to refine risk stratification,

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inform surgical planning, and guide closer postoperative surveillance.

The increasingly widespread use of ultrasonography in clinical practice, together with a growing concern about thyroid cancer, has contributed to the rising diagnosis of thyroid nodules and PTC in children and adolescents, particularly among females. Although the overall prognosis is excellent, the disease imposes a meaningful burden on patients and families because it entails a chronic oncologic condition requiring long-term follow-up (11).

In our cohort, the age at diagnosis and the female predominance aligned with international pediatric series (12). The classic variant was most frequent, followed by the encapsulated classic variant, whereas the diffuse sclerosing variant, although less prevalent, deserves attention due to its association with more aggressive behavior reported in several studies (13,14).

Multifocality, extrathyroidal extension, and lymphatic invasion occurred at rates comparable to those described in other pediatric series (5,6). In accordance with the 5th edition of the WHO Classification (2022) and the CAP protocol (v4.4.0.0), vascular and lymphatic invasion were reported independently. No vascular invasion was identified, while lymphatic invasion was commonly observed, reinforcing the propensity of PTC to spread via lymphatic pathways (9,10). Cervical lymph node metastases were frequent during the disease course and fell within the range reported by pediatric cohorts (5,6). Pulmonary metastases were uncommon and within the frequency described in larger studies (10). Adjuvant radioiodine was used in about half of the cohort, guided primarily by stimulated thyroglobulin, cervical ultrasound, and post-therapy whole-body scans rather than strictly by ATA risk category (2,6).

We evaluated clinicopathologic factors associated with cervical lymph node metastasis, including both the central and lateral compartments, considering findings at initial diagnosis and throughout the disease course. Age was not associated with metastasis, either as a continuous variable ($p = 0.20$) or when categorized as ≤12 vs >12 years ($p > 0.99$), in accordance with the 2015 Pediatric ATA guideline recommendation to distinguish prepubertal and postpubertal periods (2). This result contrasts with previous series reporting greater aggressiveness and higher nodal burden in prepubertal children compared with adolescents (15,16). Similarly, sex was not associated with metastasis ($p = 0.29$), although all male patients presented lymph node involvement.

The diffuse sclerosing variant of papillary thyroid carcinoma accounted for 4 of 32 cases (12.5%). All presented with cervical metastasis at diagnosis; across the cohort, three patients had pulmonary metastases, including one within this subtype. Using the classic and encapsulated classic variants as the less aggressive reference group, the diffuse sclerosing subtype showed a higher frequency of cervical nodal involvement, although the difference was not statistically significant. Larger pediatric series have reported higher incidences of central and lateral nodal disease and a greater likelihood of pulmonary metastasis with this variant (14,17). Differences in study design, such as systematic dissection of central and lateral levels, as well as the small sample size, may account for discrepancies. Multifocality, positive surgical margins, and perineural invasion were not associated with cervical metastasis in our cohort. By contrast, extrathyroidal extension and lymphatic invasion were associated with nodal disease, and a more advanced tumor stage (T1b–T3a vs T1a) also showed association in bivariate analyses. These findings are consistent with pediatric literature identifying extrathyroidal extension, lymphatic invasion, and tumor size/T stage as the main predictors of cervical metastasis, whereas multifocality, margins, and perineural invasion yield inconsistent results (5,6,18,19). We did not analyze necrosis or mitotic index statistically because mitotic counts were uniformly low; the single tumor with necrosis was the case reclassified as high-grade papillary thyroid carcinoma according to WHO 2022, precluding meaningful comparisons (20).

Extrathyroidal extension plays a key role in the locoregional biology of papillary thyroid carcinoma in children and is the only independent predictor of nodal involvement with cervical lymph node metastasis in the central and lateral compartments (21-23). In our cohort, when present, extrathyroidal extension was microscopic. Although the AJCC 8th edition restricts

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T staging to gross invasion, even minimal microscopic extension may be biologically relevant in pediatrics (22,24). After adjustment for covariates in a Firth-penalized logistic model, ETE remained independently associated with nodal involvement, whereas tumor stage showed only a trend and lymphatic invasion did not retain significance. The model's ROC analysis demonstrated strong discriminative ability; however, given the single-center design and limited sample size, this performance should be interpreted as internally validated, and external validation in independent pediatric cohorts is warranted before broader clinical application. Taken together, these findings support using extrathyroidal extension to refine risk stratification and to plan closer postoperative cervical surveillance.

This study has some limitations. It was retrospective and conducted at a single tertiary center, which may limit generalizability, and the sample size was relatively small, reflecting the lower frequency of papillary thyroid carcinoma in the pediatric population. The number of outcome events was also limited, so some estimates should be interpreted with caution. Furthermore, near-complete separation was observed when conventional logistic regression was initially applied, which led us to adopt Firth's penalized logistic regression to obtain more stable estimates. In addition, although neck dissections were not performed prophylactically, the indication for central compartment dissection was not always based on preoperative cytologic confirmation and in some cases relied on intraoperative suspicion of nodal involvement, which may have introduced a degree of selection bias and increased the likelihood of detecting cervical lymph node metastases in this cohort. Histopathologic evaluation was performed by a single pathologist, which does not allow assessment of interobserver variability, and the absence of independent blinded review by multiple pathologists represents an additional limitation. Nonetheless, the study has important strengths: histologic slide evaluation followed the most recent WHO and CAP criteria, ensuring diagnostic consistency; standardized ATA pediatric risk stratification and AJCC 8th-edition staging were applied; and Firth's penalized logistic regression was used to reduce small-sample bias.

In conclusion, extrathyroidal extension was the only factor independently associated with cervical lymph node metastasis in children and adolescents with papillary thyroid carcinoma. These data support considering ETE as an informative variable within dynamic risk stratification to help individualize postoperative surveillance; however, prospective multicenter studies are needed to determine how best to integrate ETE into pediatric care.

Author contribution statement: all authors contributed substantially to the work. Conceptualization and study design: first and senior authors. Methodology and supervision: senior author and statistician. Formal analysis and visualization: statistician. Investigation and data curation: surgical and pathology team members. Writing – original draft: first author. Writing – review and editing: all authors. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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original

Predictive factors for remission in pediatric Graves' disease treated with antithyroid drugs: a retrospective study

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ABSTRACT

Objective: Pediatric Graves' disease (PGD) is a rare autoimmune disorder. Predictors of remission after antithyroid drug (ATD) therapy have been described, but their applicability in pediatric populations remains inconsistent. This study aimed to identify clinical and biochemical factors associated with remission in PGD. **Subjects and methods:** Retrospective observational study of children diagnosed with Graves' disease at a tertiary hospital in Portugal (2001–2021), all initially treated with ATD. Demographic, clinical, and biochemical data at diagnosis, as well as treatment characteristics, were analyzed. The primary outcome was "In Remission". Secondary outcomes included "Remission Experienced" and "Disease Not Active ≥1 Month". Comparative analyses, logistic regression, and receiver operating characteristic (ROC) curves were applied. **Results:** Among 45,000 pediatric patients, 36 had PGD (prevalence 0.08%). Median age at diagnosis was 13.7 years; 81% were female, and 78% were pubertal. Methimazole was prescribed in 81%. Mean initial treatment duration (ITD) was 35 months, and 47% of patients underwent definitive therapy. At data collection, 18% of patients were in remission. Longer ITD (>24 months) was associated with remission; the optimal cutoff ITD was 51 months. Smaller thyroid volume and TRAb levels at diagnosis correlated with "Disease Not Active ≥1 Month". Thyroid volume ≥2.5-fold the upper limit of normal (ULN) and TRAb ≥7.055-fold the ULN reduced the likelihood of disease inactivity. **Conclusion:** Prolonged ATD therapy, smaller thyroid volume, and lower TRAb levels at diagnosis were associated with favorable outcomes in PGD. The optimal ITD (≥51 months) supports extended ATD therapy. Larger multicenter studies are warranted to confirm these results.

Descriptors: Hyperthyroidism; Graves disease; pediatric endocrinology; antithyroid agents; remission

INTRODUCTION

Pediatric hyperthyroidism is rare, with an estimated incidence of 0.9 per 100,000 in children under the age of 15 years and a clear female predominance. Graves' disease (GD) accounts for over 95% of cases (1–4). Its pathogenesis involves genetic, environmental, and immune factors mediated by thyrotropin receptor antibodies (TRAb) that stimulate thyroid hormone overproduction and may coexist with other autoimmune conditions, such as type 1 diabetes or celiac disease (2,3,5). A positive family history in a first-degree relative is observed in ~15% of cases, and the risk of the disease is higher in individuals with Down syndrome or under immunomodulatory therapy (2,3).

Clinically, pediatric GD (PGD) presents with typical symptoms of hyperthyroidism, *i.e.*, tachycardia, palpitations, hyperphagia, tremors, diarrhea, heat intolerance, anxiety, and insomnia. Over 50% of patients have diffuse goiter, and mild ophthalmopathy may occur. Growth velocity and bone maturation can also be accelerated (1–3). The diagnosis relies on suppressed thyroid-stimulating hormone (TSH) levels, elevated

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free triiodothyronine (FT3) and free thyroxine (FT4) levels, and positive TRAb, which are highly specific for GD (1,2). Thyroid ultrasound, though not required for diagnosis, can aid prognostic assessment, while thyroid scintigraphy is not routinely indicated (1,2). Antithyroid drugs (ATDs) remain first-line therapy, with radioiodine (^{131}I) and thyroidectomy as second-line options (1,3,6,7). In children, however, the optimal therapeutic approach remains controversial (1,4–10).

Thionamides, such as methimazole, carbimazole, and propylthiouracil (PTU), inhibit thyroid hormone synthesis, but PTU is avoided in children due to hepatotoxicity (2,3,10). After 2 years of ATD therapy, remission rates are low (20%–30%), though they increase with longer treatment duration, reaching rates of 19.6%, 34.1%, 43.5%, and 50.6% after 3, 5, 7, and 10 years, respectively (1,3,4,11,12).

Since 2016, the American Thyroid Association (ATA) has acknowledged the potential benefit of extending ATD therapy for GD beyond 2 years (7). In contrast, the 2022 European Thyroid Association (ETA) guideline recommends an initial treatment duration (ITD) of at least 3 years (3). However, prolonged ATD therapy poses challenges, including the occurrence of adverse effects, requirement for frequent thyroid function monitoring, and poor adherence, especially among adolescents (4,5,10,13).

Adverse effects of ATDs, although generally uncommon, must be considered when evaluating prolonged therapy. Minor reactions such as rash, pruritus, or mild gastrointestinal intolerance occur in approximately 5%–25% of pediatric patients, while severe complications like agranulocytosis or hepatotoxicity are rare, with estimated incidences of 0.3%–0.7%. Definitive treatments remain controversial in children due to the potential oncogenic risk of ^{131}I, and higher surgical complication rates than in adults (1–4). These considerations highlight the importance of balancing the risks and benefits of prolonged medical therapy versus early definitive treatment, and of identifying reliable clinical and biochemical predictors of response to ATD that can guide individualized management in PGD (10,14).

While international studies have explored age, sex, TRAb levels, thyroid volume, and treatment duration as prognostic factors in PGD, results have been inconsistent (1,4,6,8–16). In Portugal, research is scarce, with only one multicenter study published to date (6). Further investigation is needed to clarify prognostic markers and align national practice with international evidence.

In this context, the present study aimed to evaluate clinical and biochemical factors associated with remission in PGD following ATD therapy in patients diagnosed and followed at a Portuguese tertiary hospital between 2001 and 2021.

SUBJECTS AND METHODS

The study protocol was approved by the Ethics Committee and the Data Protection Office of Braga Hospital. Ethical guidelines and good clinical practice standards were followed, and data confidentiality was maintained throughout the study period.

This retrospective, observational, analytical, longitudinal study included children and adolescents diagnosed with GD at Braga Hospital, a Portuguese tertiary care center, between January 2001 and December 2021. All patients received initial ATD treatment and were followed at the Pediatrics Department and, when applicable, the Pediatric Endocrinology Unit.

A non-random convenience sample was selected. The inclusion criteria were a first consultation within the study period, a GD diagnosis with ATD as the initial therapy, and a follow-up ≥ 6 months. The exclusion criteria included neonatal GD, age > 18 years at diagnosis, and loss to follow-up.

A GD diagnosis required suppressed serum TSH levels, elevated FT3 and/or FT4 levels, and positive TRAb. In some cases, FT3 measurement was unavailable at diagnosis; in these instances, the diagnosis was based on TSH and FT4 levels, along with TRAb results. Consequently, FT3 was excluded from statistical analyses due to missing data.

Treatment followed a dose-titration regimen aiming for euthyroidism (thyroid hormones within reference ranges, followed by TSH). ATD withdrawal was considered after ≥ 24 months of stable euthyroidism on minimal doses (as low as 2.5 mg of methimazole on alternate days) and TRAb levels near or below the upper limit of normal (ULN). Remission was defined as

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≥ 12 months of euthyroidism after ATD discontinuation, and relapse as recurrent biochemical hyperthyroidism with positive TRAb after remission.

Throughout the 20-year study period, patients were managed exclusively using a dose-titration regimen. A block-and-replace approach was not employed at any time. Despite changes in ATD preference over time — namely, a shift toward reduced PTU use and increased methimazole use — and modifications to laboratory assay methodologies, clinical decision-making regarding treatment adjustment and ATD withdrawal followed consistent criteria.

Body mass index (BMI) — calculated as weight divided by the square of height (kg/m²) and expressed as a standard deviation (SD) score (SDS) by age and sex — was classified as underweight, normal weight, overweight, or obese (17,18). According to the World Health Organization (WHO) growth reference, for children under 5 years of age, underweight was defined as a BMI-for-age < −2 SD, normal weight as −2 ≤ SD ≤ +2, overweight as +2 < SD ≤ +3, and obesity as SD > +3. For children aged 5 years or older, underweight was defined as BMI-for-age < −2 SD, normal weight as −2 ≤ SD ≤ +1, overweight as +1 < SD ≤ +2, and obesity as SD > +2. Pubertal status was assessed at diagnosis, based on Tanner staging documented at the first endocrinology evaluation, classifying patients as prepubertal or pubertal.

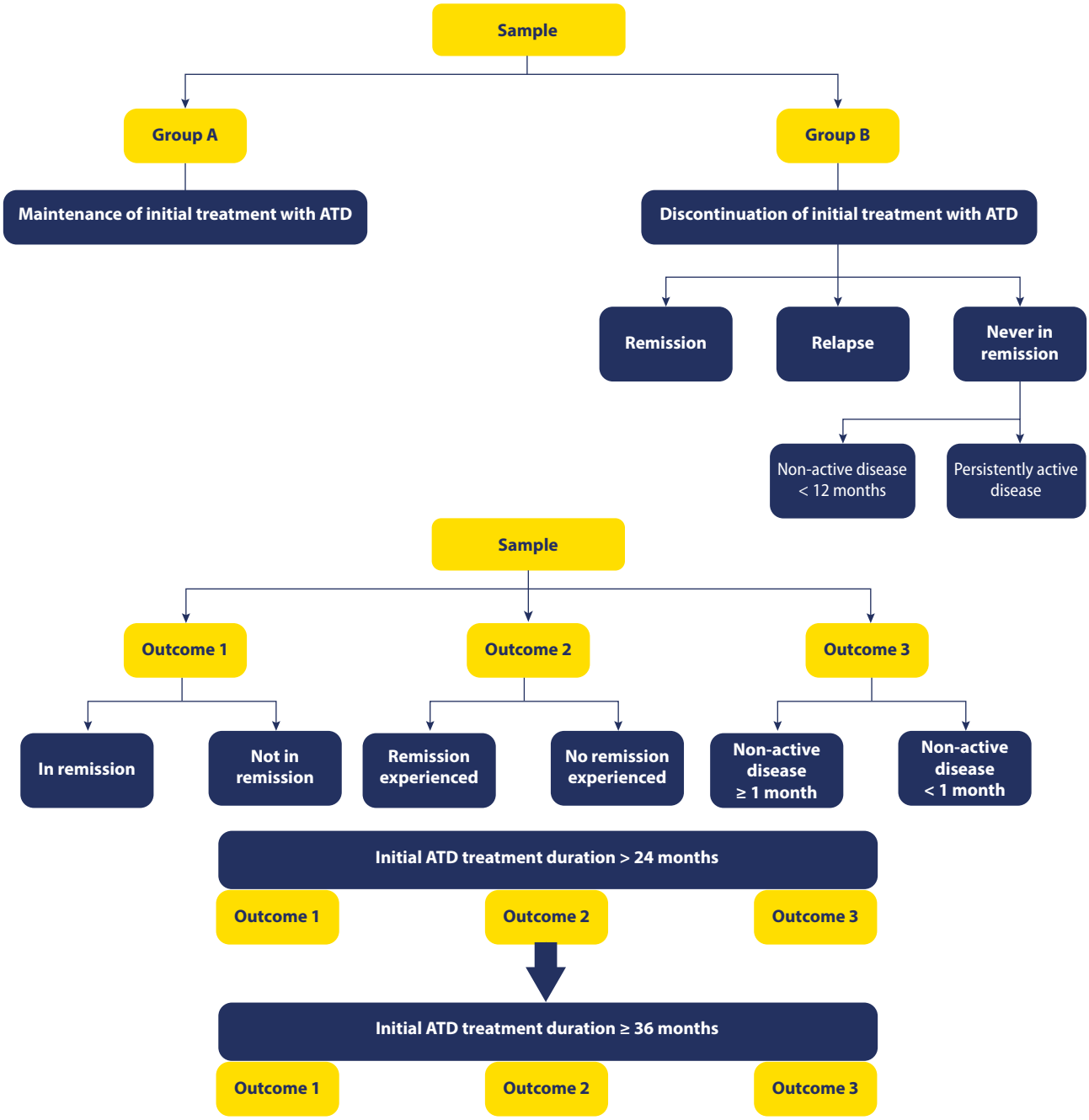
Puberty was defined as Tanner stage ≥ 2 for breast development in girls and Tanner stage ≥ 2 for genital development (testicular volume ≥ 4 mL) in boys (19). Thyroid volume was determined by ultrasound using the ellipsoid formula (width x length x depth x 0.52), standardized by the age-specific ULN, through the ratio of measured volume to ULN (7 years: 4.4; 8 years: 4.5; 9 years: 4.9; 10 years: 5.5; 11 years: 6.4; 12 years: 6.7; 13 years: 7.7; 14 years: 7.8). For children younger than 7 years and older than 14 years, ULN values of 4.4 and 7.8, respectively, were applied (20,21). Not all patients underwent thyroid ultrasound.

Serum FT4 and FT3 levels were measured using competitive chemiluminescent immunoassays, while TSH levels were measured by immunochemiluminescent assay (Atellica IM Analyzer, Siemens Medical Solutions Diagnostics, Erlangen, Germany) and TRAb by radioimmunoassay until 2017 and by chemiluminescent immunoassay from 2017 onwards (IMMULITE 2000 Systems Analyzers, Siemens Medical Solutions Diagnostics). Results were standardized by dividing each by its respective ULN. Reference values for FT4 were 0.9–2.3 ng/mL for ages 0–12 months, 0.8–1.8 ng/mL for ages 1–5 years, 1.0–2.1 ng/mL for ages 6–10 years, and 0.8–1.9 ng/mL for ages 11–18 years. Reference values for TRAb were < 1 U/L or < 9 U/L before 2017 and < 0.55 U/L thereafter (22).

Data were obtained from medical records. Collected variables included sex, the presence of other autoimmune diseases (AIDs), age at diagnosis, pubertal stage, BMI, thyroid volume, FT4 and TRAb levels, type and dose of initial ATD therapy, and outcome after the initial ATD treatment. The sample was subsequently divided into groups according to the classification schemes shown in **Figure 1**. Group A comprised patients who were still receiving their initial ATD regimen at the time of data collection, while Group B comprised patients who had already discontinued their initial ATD regimen before data collection. Within Group B, patients were further categorized into the following groups: "Remission" (euthyroidism maintained for ≥ 12 months after ATD discontinuation), "Relapse" (suppressed serum TSH levels and elevated FT3 and/or FT4 levels, along with positive TRAb, after achieving remission), and "Never in Remission" (disease inactive for < 12 months or persistently active). For analytical purposes, three outcomes were defined: Outcome 1 – "In Remission", which included patients in remission at the time of data collection, and "Not in Remission", which encompassed all remaining patients, including those who had never discontinued initial ATD therapy (Group A); Outcome 2 – "Remission Experienced", grouping patients who achieved remission at any time, and "No Remission Experienced", grouping those who never achieved remission or relapsed without attaining a subsequent remission; Outcome 3 – "Disease Not Active ≥ 1 Month", which included all patients from the "Remission Experienced" group plus those with inactive disease lasting ≥ 1 month but < 12 months after discontinuation of the initial ATD regimen, and those with "Disease Not Active < 1 month". The selected outcomes were defined to reflect clinically meaningful

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stages of PGD management. While sustained remission represents the primary therapeutic goal and was therefore defined as the primary outcome ("In Remission"), shorter periods of disease inactivity and remission experienced were included as secondary outcomes, as they are also clinically relevant and frequently inform decisions regarding ATD withdrawal, treatment continuation, and timing of definitive therapy.



ATD: antithyroid drugs.

Figure 1. Characterization of the sample according to maintenance or discontinuation of the initial treatment, by Outcomes 1, 2, and 3, and according to the duration of the initial treatment. **Group A:** initial antithyroid drug treatment ongoing at data collection. **Group B:** discontinuation of initial treatment with antithyroid drugs. "Remission": patients in remission at the time of data collection. "Relapse": patients who experienced relapse after remission (without achieving a second remission). "Never in Remission": patients with inactive disease for less than 12 months or with persistently active disease. **Outcome 1:** "In Remission" included patients who were in remission at the time of data collection; "Not in Remission" included all remaining patients, including those who had never discontinued initial antithyroid drug treatment **(Group A). Outcome 2:** "Remission Experienced" grouped patients who had either achieved remission or relapsed after remission (without reaching a new remission). **Outcome 3:** "Disease Not Active ≥ 1 Month" included the sum of patients from the "Remission Experienced" group and those who had inactive disease lasting at least 1 month and less than 12 months.

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Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 29.0 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as $p < 0.05$, and 95% confidence intervals (CIs) were used to support the interpretation of the results.

The minimum sample size considered representative of the Portuguese pediatric population was $n \geq 16$, calculated using an online sample size calculator (23), assuming a 95% CI and a 5% margin of error.

Normality of quantitative variables was assessed using the Shapiro–Wilk test, histogram inspection, and evaluation of skewness and kurtosis (24). Depending on the normality assumption, parametric or nonparametric tests were applied.

Normally distributed quantitative variables were summarized as mean and SD, while non-normally distributed variables were reported as median and interquartile range. Qualitative variables were expressed as absolute frequencies (n) and/or relative frequencies (%) (24).

Comparisons between groups were performed using statistical tests selected according to group dependence (paired or independent), variable type (quantitative or qualitative), and distribution pattern of quantitative variables, with parametric or nonparametric methods applied as appropriate (25-27).

Binary logistic regression (LR) models — univariate and multivariate — were used to identify potential predictors of outcomes (28). Variable selection considered statistical significance in group comparisons, sample size, and the number of variables analyzed. To reduce overfitting, the "1:10 rule" (one predictor per ten positive events) (29) was applied, although not strictly, for exploratory purposes, given conflicting evidence in the literature (30,31).

To minimize multicollinearity and improve robustness, only variables with $p < 0.20$ in comparative analyses were included in the univariate LR. Those with $p < 0.20$ in univariate LR were subsequently entered into multivariate LR (10). Results were reported as odds ratios (ORs) with 95% CIs. For significant predictors ($p < 0.05$), Nagelkerke R^2 was calculated to estimate the variance explained by the model (28).

Receiver operating characteristic (ROC) curve analysis was performed for continuous variables of potential clinical interest to assess discriminative ability. For variables with an area under the curve (AUC) > 0.5 — indicating discrimination above chance and improving as AUC approached 1 — the optimal cutoff was determined using the Youden Index (J = Sensitivity + Specificity – 1). A p value < 0.05 indicated statistically significant discriminative capacity (26,28,32).

RESULTS

A total of 45,000 children and adolescents were followed at the Pediatrics Department of Braga Hospital from January 2001 to December 2021, of whom 36 were diagnosed with PGD, corresponding to a prevalence of 0.08%. Two patients treated with PTU were subsequently excluded due to loss to follow-up, resulting in a final sample of 34 patients included in the analysis (**Figure 2**). Clinical and biochemical characteristics at diagnosis, along with details of initial ATD treatment and outcomes, are summarized in **Table 1**. Not all patients had complete laboratory or thyroid ultrasound data available due to missing information in the clinical records.

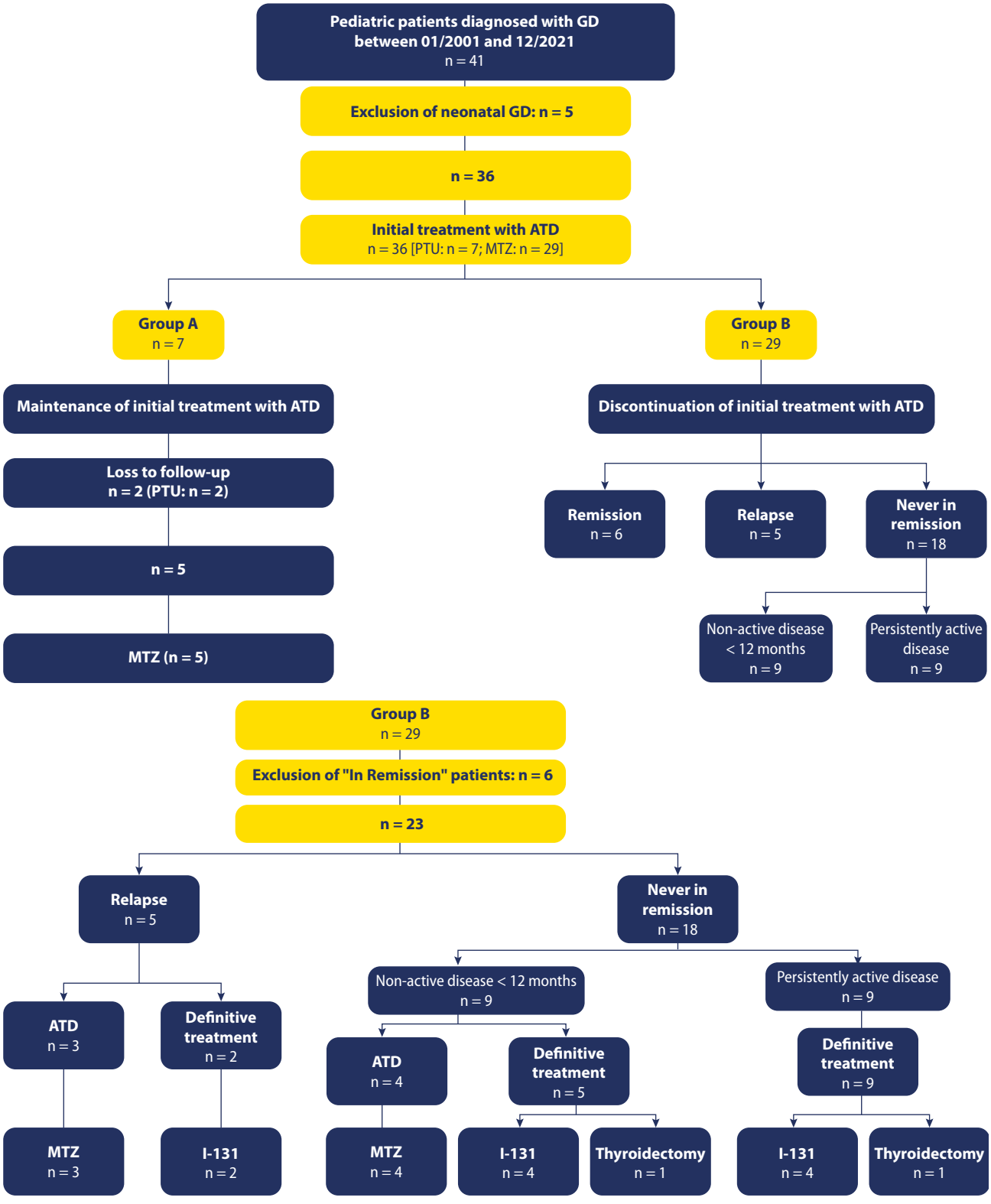
None of the patients was initially treated with definitive therapy. One patient, who developed PTU-induced toxic hepatitis, discontinued ATD after 4 months and underwent subsequent ^{131}I ablation.

In comparative analyses, longer ITD was significantly associated with remission outcomes. Among patients treated for more than 24 months, those "In Remission" had a mean ITD of 58 months compared to 41 months among those "Not in Remission" ($p = 0.049$). A similar trend was observed for "Remission Experienced" among patients treated for at least 36 months, where it was associated with longer ITD ($p = 0.002$) (**Table 2**).

Lower thyroid volume and TRAb levels at diagnosis correlated with "Disease Not Active ≥1 Month" ($p = 0.024$ and $p = 0.037$, respectively). For initial therapy longer than 24 months, standardized TRAb levels remained significantly lower in this group ($p = 0.035$) (**Table 2**).

Comparing the group of patients that maintained the initial ATD treatment with the group that discontinued it, only standardized TRAb levels were significantly higher: 31.64 (84.10) in Group A versus 6.57 (10.24) in Group B ($p = 0.008$).

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ATD: antithyroid drug; GD: Graves' disease; ^{131}I: radioiodine; MTZ: methimazole; PTU: propylthiouracil.

Figure 2. Selection of patients and disease progression according to initial treatment with antithyroid drugs; disease progression in patients who discontinued initial antithyroid drug therapy (Group B) and therapeutic choices made in cases of relapse or lack of remission (antithyroid drugs or definitive therapy with radioiodine or thyroidectomy). **Group A:** maintenance of initial treatment with antithyroid drugs. **Group B:** discontinuation of initial treatment with antithyroid drugs. "Remission": patients in remission at the time of data collection. "Relapse": patients who experienced relapse after remission (without achieving a second remission). "Never in Remission": patients with inactive disease for less than 12 months or with persistently active disease.

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Table 1. Descriptive analysis of clinical and biochemical characteristics at diagnosis, details of initial antithyroid treatment, and outcomes

Sex, n (%)	
Female	29 (80.56)
Male	7 (19.44)
Other autoimmune diseases*, n (%)	
Yes	7 (19.44)
Pubertal stage, n (%)	
Pubertal	28 (77.78)
Body mass index, n (%)†	
Underweight	1 (2.86)
Normal weight	27 (77.14)
Overweight	7 (20.00)
Obese	0 (0.00)
Initial treatment with antithyroid drugs, n (%)	
Propylthiouracil	7 (19.44)
Methimazole	29 (80.56)
Age (years), median (IQR)	13.67 (4.71)
Standardized thyroid gland volume (xN), median (IQR) (n = 31)‡	3.22 (1.94)
Standardized FT4 (xN), median (IQR) (n = 32)§	2.15 (1.24)
Standardized TRAb (xN), median (IQR) (n = 29)¶	7.11 (14.90)
Initial ATD dose (mg/kg/day), mean ± SD	
PTU (n=7)	4.50 ± 1.42
Methimazole (n=29)	0.36 ± 0.02
Duration of initial ATD treatment (months), median (IQR) (n = 35)	31.00 (23.00)
PTU, mean ± SD (n = 6)**	45.50 ± 14.45
Methimazole, mean ± SD (n = 29)	33.24 ± 2.48
Duration of initial ATD treatment > 24 months (n = 24)	
Mean ± SD	44 ± 3
Median (IQR)	40 (19)
Duration of initial ATD treatment ≥ 36 months (n = 16)	
Mean ± SD	51 ± 4
Median (IQR)	46 (12)
Duration of initial ATD treatment until definitive treatment†† (n = 16)	
Mean ± SD	34 ± 3
Median (IQR)	40 (22)
Duration of initial ATD treatment until I-131 ablation†† (n = 13)	
Mean ± SD	33 ± 4
Median (IQR)	39 (25)
Duration of initial ATD treatment until thyroidectomy†† (n = 3)	
Mean ± SD	35 ± 7
Median (IQR)	40 (.)
Outcomes, n (%)	
In Remission	6 (17.65)
Remission Experienced	11 (32.35)
Disease Not Active < 12 Months	9 (26.47)
Disease Not Active ≥ 1 Month	20 (58.82)
Relapse	5 (45.45)

Results are expressed as mean ± standard deviation, median (interquartile range), or number (%). The n value may vary due to missing data. Standardization: values divided by their respective upper limit of normal.

*Includes type 1 diabetes mellitus (n = 3), celiac disease (n = 3), juvenile idiopathic arthritis (n = 1); †n = 35 due to 1 missing result in the clinical records; ‡n = 31, due to 5 missing results in the clinical records; §n = 32, due to 4 missing results in the clinical records; ¶n = 29, due to 7 missing results in the clinical records; **n = 6, due to 1 patient having a follow-up duration < 6 months; ††Duration of initial antithyroid drug treatment, in months, for patients who underwent definitive therapy (radioiodine ablation or thyroidectomy). "In Remission" – patients in remission at the time of data collection; "Remission Experienced" – group of patients who achieved remission at any point (patients in remission at data collection plus patients who relapsed and did not achieve a subsequent remission); "Disease Not Active < 12 Months" – group of patients who had a period of inactive disease lasting < 12 months, without achieving remission; "Disease Not Active ≥ 1 Month" – total of patients who experienced remission at any point and those without signs of active disease for at least 1 month but less than 12 months; "Relapse" – patients who experienced remission at any point (n = 11) and subsequently relapsed (n = 5).

FT4: free thyroxine; IQR: interquartile range; SD: standard deviation; TRAb: thyrotropin receptor antibodies.

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Table 2. Variables with $p < 0.20$ in the comparative analyses for Outcome 1 ("In Remission" versus "Not in Remission"), Outcome 2 ("Remission Experienced" versus "No Remission Experienced"), and Outcome 3 ("Disease Not Active ≥ 1 Month" versus "Disease Not Active < 1 Month"), as well as for the comparisons on initial treatment durations of more than 24 months and at least 36 months

			Test statistic	*p*
Outcome 1 (n = 34)	**In remission (n = 6)**	**Not in remission (n = 28)**		
Standardized thyroid gland volume Mean ± SD (n = 29)	2.54 ± 0.78 (n = 5)	3.57 ± 1.50 (n = 24)	*t*(27) = -1.475	0.152
Duration of initial ATD treatment Median (IQR) (n = 34)	48.50 (43.00) (n = 6)	31.00 (19.00) (n = 28)	*U* = 51.500	0.145
Outcome 2 (n = 34)	**Remission experienced (n = 11)**	**No Remission experienced (n = 23)**		
Age Median (IQR) (n = 34)	14.92 (4.92) (n = 11)	13.00 (4.16) (n = 23)	*U* = 89.500	0.176
Standardized thyroid gland volume Mean ± SD (n = 29)	2.80 ± 1.20 (n = 8)	3.61 ± 1.49 (n = 21)	*t*(27) = -1.367	0.183
Outcome 3 (n = 34)	**Disease not active ≥1 month (n = 20)**	**Disease not active <1 month (n = 14)**		
Standardized thyroid gland volume Mean ± SD (n = 29)	2.89 ± 1.14 (n = 17)	4.10 ± 1.58 (n = 12)	*t*(27) = 2.400	**0.024**
Standardized TRAb Median (IQR) (n = 27)	6.46 (8.20) (n = 15)	13.84 (51.28) (n = 12)	*U*=133.00	**0.037**
Initial ATD treatment duration > 24 months				
Outcome 1 (n = 24)	**In remission (n = 4)**	**Not in remission (n = 20)**		
Standardized thyroid gland volume Mean ± SD (n = 19)	2.62 ± 1.09 (n = 3)	4.06 ± 1.48 (n = 16)	*t*(17) = -1.595	0.129
Duration of initial ATD treatment Mean ± SD (n = 24)	58.25 ± 12.61 (n = 4)	40.80 ± 15.70 (n = 20)	*t*(22) = 2.080	**0.049**
Outcome 2 (n = 24)	**Remission experienced (n=7)**	**No remission experienced (n = 17)**		
BMI SD, n (%) (n = 23) Underweight (n = 1) Normal (n = 18) Overweight (n = 4)	 (n = 0) 33.3 (n = 6) 0.0 (n = 0)	 100 (n = 1) 66.7 (n = 12) 100 (n = 4)	LR(2) =3.448	0.175
Duration of initial ATD treatment Median (IQR) (n=24)	58.43 ± 22.86 (n = 7)	37.65 ± 7.49 (n=17)	*t*(7) = 3.354	0.053
Outcome 3 (n=24)	**Disease not active ≥ 1 month (n=13)**	**Disease not active <1 month (n = 11)**		
BMI SD, n (%) (n=23) Underweight (n = 1) Normal (n = 18) Overweight (n = 4)	 (n = 0) 61.1 (n = 11) 25.0 (n = 1)	 100 (n = 1) 38.9 (n = 7) 75.0 (n = 3)	LR(2) = 3.286	0.193
Standardized TRAb Median (IQR) (n = 19)	6.84 (9.60) (n = 10)	15.56 (79.32) (n = 9)	*U* = 71.000	**0.035**
Duration of initial ATD treatment Mean ± SD (n = 24)	48.62 ± 20.42 (n = 13)	37.91 ± 6.99 (n = 11)	*t*(15) = 1.772	0.096
Initial ATD treatment duration ≥ 36 months				
Outcome 1 (n = 16)	**In remission (n = 4)**	**Not in remission (n = 12)**		
Standardized thyroid gland volume Mean ± SD (n = 12)	2.62 ± 1.09 (n = 3)	3.62 ± 0.92 (n = 9)	*t*(10) = -1.568	0.148
Duration of initial ATD treatment Median (IQR) (n = 16)	57.00 (24.00) (n = 4)	42.50 (10.0) (n = 12)	*U* = 8.500	0.058
Outcome 2 (n = 16)	**Remission experienced (n = 6)**	**No remission experienced (n = 10)**		
Standardized thyroid gland volume Mean ± SD (n = 12)	2.62 ± 1.09 (n = 3)	3.62 ± 0.92 (n = 9)	*t*(10) = -1.568	0.148
Duration of initial ATD treatment Median (IQR) (n = 16)	57.00 (30.00) (n = 6)	41.00 (8.00) (n = 10)	*U* = 3.000	**0.002**
Outcome 3 (n = 16)	**Disease not active ≥ 1 month (n = 10)**	**Disease not active <1 month (n = 6)**		
Standardized TRAb Median (IQR) (n = 11)	6.67 (8.20) (n = 7)	13.84 (76.94) (n = 4)	*U* = 24.000	0.073
Duration of initial ATD treatment Median (IQR) (n = 16)	50.50 (24.0) (n = 10)	41.50 (8.00) (n = 6)	*U* = 16.500	0.147

Results are expressed as mean ± standard deviation, median (interquartile range), or number (%); the n value may vary due to missing data. Standardization: values divided by their respective upper limit of normal. "In Remission": patients in remission at the time of data collection. "Remission Experienced": patients who experienced remission at some point (those in remission at the time of data collection, as well as those who relapsed without subsequently achieving a new remission). "Disease Not Active ≥ 1 Month": patients who experienced remission at some point, as well as those with inactive disease for a period of at least 1 month and less than 12 months.

ATD: antithyroid drug; BMI: body mass index; IQR: interquartile range; SD: standard deviation; TRAb: thyrotropin receptor antibodies.

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In univariate LR, ITD was a significant predictor of "Remission Experienced" in the group with initial therapy longer than 24 months (p = 0.041; OR = 1.14; 95% CI: 1.005–1.297), explaining 47% of outcome variability. For "Disease Not Active ≥ 1 Month", thyroid volume was initially significant (p = 0.042; OR = 0.49) but lost significance after adjustment for TRAb levels (Table 3). No further univariate or multivariate LR results reached significance.

An analysis of the ROC curve confirmed the discriminative ability of ITD for remission outcomes. For therapy longer than 24 months, ITD showed excellent performance (AUC = 0.894 for "In Remission" and 0.840 for "Remission Experienced"), with an optimal cutoff of 51 months. For treatment ≥ 36 months, the discriminative ability remained high (AUC = 0.823 and 0.950, respectively) (Figure 3).

Standardized TRAb levels demonstrated acceptable discrimination for "Disease Not Active ≥ 1 Month" (AUC = 0.739, p = 0.036), improving to excellent for treatment ≥ 36 months (AUC = 0.857, p = 0.059). The optimal TRAb cutoff point was 7.055-fold the ULN (Figure 3).

For standardized thyroid volume, discrimination was acceptable for "Disease Not Active ≥ 1 Month" (AUC = 0.752, p = 0.023), with an optimal cutoff point of 2.50 (Figure 3).

DISCUSSION

The optimal treatment for GD in children remains controversial due to the limited efficacy of available therapeutic options and the difficulty in predicting remission. Although ATDs are the recommended first-line therapy (3,7,9), long-term remission after 2 years

Table 3. Odds ratio of variables selected from the comparative analyses between groups (Table 2) for the occurrence of the events "In Remission", "Remission Experienced", and "Disease Not Active ≥ 1 Month"

	Variable	Unadjusted OR			Adjusted OR		
		OR	95% CI	*p*	OR	95% CI	*p*
Outcome 1	Standardized thyroid gland volume	0.462	0.156–1.362	0.162*	0.345	0.080–1.490	0.154
	Duration of initial ATD treatment	1.033	0.987–1.082	0.162*	1.078	0.977–1.191	0.136
Outcome 1 (>24 months)	Standardized thyroid gland volume	0.310	0.064–1.504	0.146*	0.063	0.000–8.311	0.267
	Duration of initial ATD treatment	1.058	0.990–1.132	0.099*	1.800	0.787–4.117	0.164
Outcome 1 (≥36 months)	Standardized thyroid gland volume	0.311	0.059–1.630	0.167*	-	-	-
	Duration of initial ATD treatment	1.038	0.967–1.115	0.297	-	-	-
Outcome 2	Age	1.090	0.869–1.367	0.456	-	-	-
	Standardized thyroid gland volume	0.602	0.283–1.280	0.188*	-	-	-
Outcome 2 (> 24 months)	BMI SD	0.376	0.035–4.037	0.420	-	-	-
	Duration of initial ATD treatment	1.142	1.005–1.297	**0.041***	-	-	-
Outcome 2 (≥36 months)	Standardized thyroid gland volume	0.311	0.059–1.630	0.167*	0.064	0.000–8.395	0.269
	Duration of initial ATD treatment	1.483	0.958–2.296	0.077*	1.793	0.778–4.129	0.170
Outcome 3	Standardized thyroid gland volume	0.492	0.249–0.975	**0.042***	0.519	0.217–1.244	0.142
	Standardized TRAb	0.943	0.871–1.020	0.143*	0.937	0.846–1.038	0.211
Outcome 3 (>24 months)	BMI SD	0.607	0.094–3.943	0.601	-	-	-
	Standardized TRAb	0.923	0.818–1.041	0.190*	0.923	0.811–1.053	0.226
	Duration of initial ATD treatment	1.060	0.981–1.146	0.141*	1.053	0.933–1.188	0.403
Outcome 3 (≥36 months)	Standardized TRAb	0.902	0.718–1.132	0.373	-	-	-
	Duration of initial ATD treatment	1.143	0.936–1.395	0.190*	-	-	-

Variables with $p < 0.20$ in the comparative analysis between groups were included in the univariate logistic regression (unadjusted odds ratio [OR]). Those with $p < 0.20$ in the univariate logistic regression (*) were included in the multivariate logistic regression (adjusted OR). Adjusted ORs with a $p < 0.05$ were considered statistically significant. Standardization: values divided by their respective upper limit of normal. "In Remission": patients in remission at the time of data collection. "Remission Experienced": patients who experienced remission at some point (patients in remission at the time of data collection, as well as those who relapsed without subsequently achieving a new remission). "Disease Not Active ≥ 1 Month": patients who experienced remission at some point, as well as those with inactive disease for a period of at least 1 month and less than 12 months.
ATD: antithyroid drug; BMI: body mass index; OR: odds ratio; SD: standard deviation; TRAb: thyrotropin receptor antibodies.

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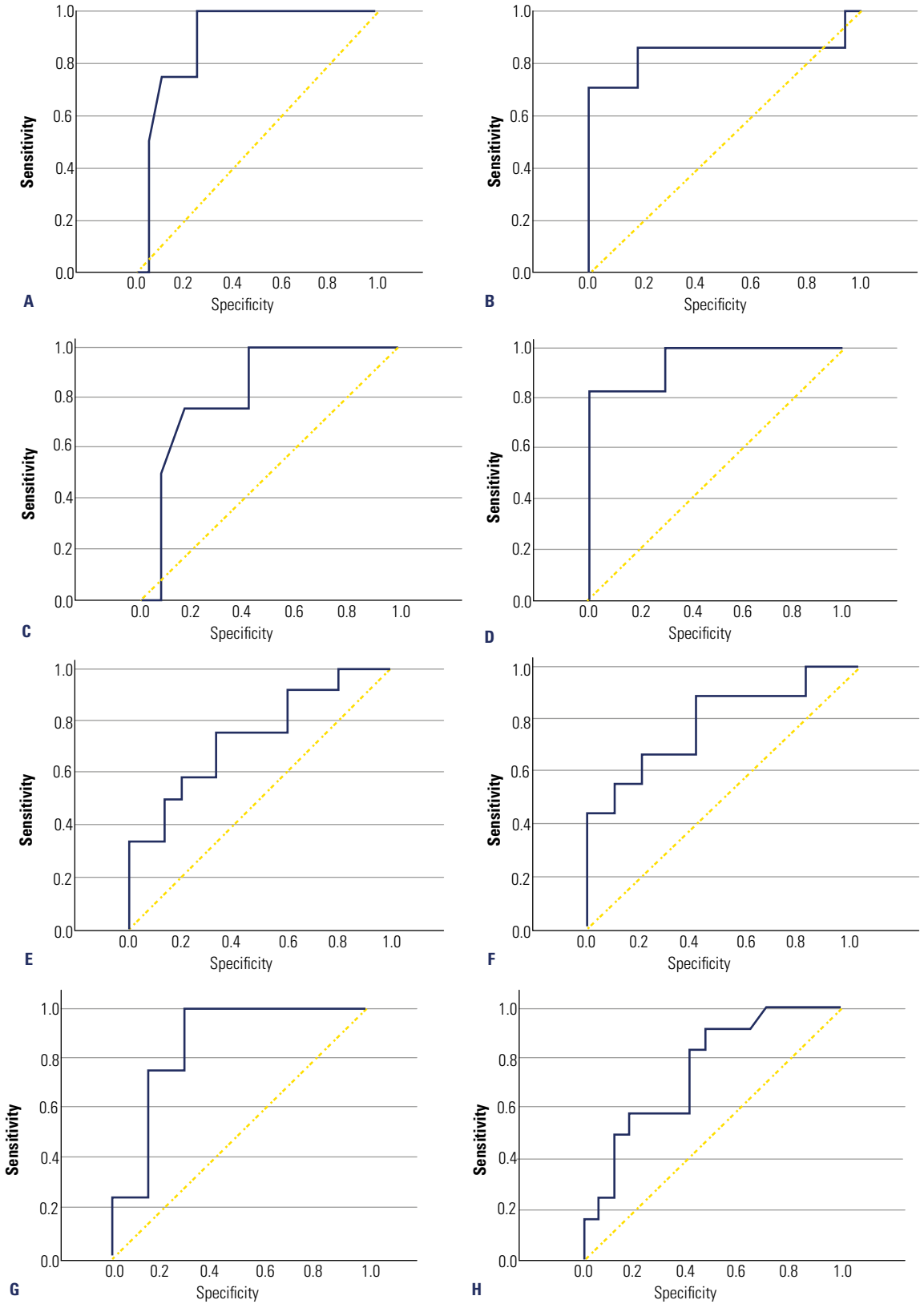


Figure 3. Receiver operating characteristic (ROC) curves. (**A**) ROC curve assessing the discriminative ability of the variable "Initial Treatment Duration" for the event "In Remission" (**Outcome 1**, treatment > 24 months). (**B**) ROC curve assessing the discriminative ability of the variable "Initial Treatment Duration" for the event "Remission Experienced" (**Outcome 2**, treatment > 24 months). (**C**) ROC curve assessing the discriminative ability of the variable "Initial Treatment Duration" for the event "In Remission" (**Outcome 1**, treatment ≥ 36 months). (**D**) ROC curve assessing the discriminative ability of the variable "Initial Treatment Duration" for the event "Remission Experienced" (**Outcome 2**, treatment ≥ 36 months). (**E**) ROC curve assessing the discriminative ability of the variable "Standardized TRAb" for the event "Disease Not Active ≥ 1 Month" (**Outcome 3**).

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is achieved in only 20%–30% of patients (1,3,13). The ETA currently advises at least 3 years of ATD treatment, extendable to 5 or more years depending on clinical and biochemical evolution (3). Identifying reliable prognostic markers at diagnosis could help individualize therapy and avoid unnecessary exposure to prolonged medication or premature definitive treatment.

Baseline characteristics in our sample — predominance of females, pubertal stage at diagnosis, and normal BMI — were consistent with previous studies (4–6,10,13,14). Notably, PTU was prescribed only before 2010, reflecting historical practice. Following INFARMED's (National Authority of Medicines and Health Products, I.P. in Portugal) 2010 recommendation highlighting the hepatotoxicity risk of PTU, its use in pediatric patients was gradually discontinued, and methimazole became the preferred first-line ATD in accordance with national and international guidelines.

Longer ITD was associated with remission outcomes, suggesting a potential benefit of extended therapy. Analysis of the ROC curve identified an optimal ITD of approximately 51 months, consistent with ETA recommendations and other reports showing increased remission rates with prolonged therapy (1,3,4). Studies by Léger and cols. and Ohye and cols. also observed higher remission rates after 4–6 years of ATD treatment (11,12). These findings support the rationale for maintaining ATD therapy for at least 3 years.

Smaller thyroid volume at diagnosis was associated with disease inactivity, in line with previous studies reporting poorer outcomes in patients with larger goiters (9). Analysis of the ROC curve in our sample suggested that a thyroid volume ≥ 2.5-fold the ULN reduced the likelihood of disease inactivity.

Lower TRAb levels at diagnosis were also associated with better outcomes. Similar trends have been reported by Gastaldi and cols. (8) and Rho and cols. (14), who showed that persistently high TRAb titers predicted relapse or failure to achieve remission. Our cutoff of 7.0-fold the ULN aligns with these results and supports the role of TRAb monitoring in guiding clinical decisions.

No statistically significant associations were found between remission outcomes and sex, pubertal stage, BMI, FT4 levels, or the type of initial ATD used (PTU versus methimazole), consistent with prior reports (8,14,33). Although the coexistence of other AIDs was more frequent among patients who experienced remission or relapse, this trend did not reach statistical significance and warrants further investigation. A similar observation was reported by Gatta and cols., who suggested that concurrent autoimmune conditions might modulate immune activity and favor GD remission. According to the GRAPHE study, the most reasonable hypothesis is that the immunological cascade involving multiple autoimmune diseases may contribute to shifts in TRAb profiles, particularly from stimulating to blocking antibodies, together with a lower overall immune response potentially related to differences in B-cell subsets (34).

Although our findings support the potential benefit of prolonged ATD therapy, the clinical implications of extended treatment must be interpreted with caution. This study did not systematically evaluate treatment-related adverse effects or adherence to long-term oral therapy, both of which are clinically relevant considerations, particularly in pediatric and adolescent populations. While serious adverse events associated with ATDs are uncommon, prolonged treatment may increase the cumulative burden of minor side effects and pose challenges to sustained adherence, potentially influencing real-world effectiveness. Therefore, decisions regarding extended ATD therapy should be individualized, balancing the potential benefits of remission against treatment tolerability, patient adherence, and family preferences.

Importantly, although several variables demonstrated significant associations with remission-related outcomes in univariate analyses, no independent predictors remained statistically significant after multivariate adjustment. This likely reflects limited statistical power due to the small sample size and the low number of outcome events. Therefore, the identified associations should be interpreted as exploratory and hypothesis-generating, rather than establishing independent predictive or causal relationships. Larger, adequately powered multicenter studies are required to confirm these findings and to determine whether these factors retain independent prognostic value in PGD.

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Limitations

This study has several limitations. The small sample size and retrospective single-center design limited statistical power, particularly for subgroup analyses, and did not allow standardized long-term follow-up. Missing clinical, laboratory, and imaging data further constrained the analyses. Inter-laboratory variability and suboptimal standardization methods over the long study period may have influenced biochemical measurements. In addition, the small number of outcome events reduces the reliability of multivariate models and ROC curve analyses; therefore, any derived cutoff points or predictive estimates should be considered exploratory rather than confirmatory. Larger prospective multicenter studies with standardized follow-up are needed to validate these findings and refine prognostic markers in PGD.

In conclusion, in this 20-year retrospective cohort study, prolonged ATD therapy was associated with higher remission rates, supporting treatment durations of at least 3 years and identifying an optimal cutoff of around 51 months. In addition, smaller thyroid volume and lower TRAb levels at diagnosis were associated with disease inactivity, suggesting a supportive role of these markers in clinical risk stratification. Given the small sample size and the retrospective single-center design, further multicenter studies are needed to validate these associations and refine prognostic tools for clinical practice.

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original

Association between autoimmune thyroiditis and clinicopathological features of papillary thyroid carcinoma: a prospective study

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ABSTRACT

Objective: This study aimed to evaluate the impact of coexisting autoimmune thyroiditis (HT) on the clinicopathological characteristics and prognosis of patients with papillary thyroid carcinoma (PTC). **Subjects and methods:** This prospective study included patients diagnosed with papillary thyroid carcinoma who underwent thyroid surgery. Patients were divided into two groups according to the presence or absence of histologically confirmed thyroiditis. Demographic data, tumor characteristics, pathological features, and postoperative outcomes were analyzed. Statistical comparisons were performed to assess differences between the two groups. **Results:** Thyroiditis was observed in a significant proportion of patients with PTC. Patients with associated thyroiditis showed smaller tumors and more favorable clinicopathological characteristics compared to those without thyroiditis. However, no significant differences were observed in major oncological outcomes. Recurrence rates were lower in the thyroiditis group. The presence of thyroiditis was associated with more favorable prognostic features. **Conclusion:** The coexistence of thyroiditis in patients with PTC was associated with more favorable clinicopathological features. However, no significant differences were observed in major oncological outcomes, and the short follow-up period limits the assessment of long-term prognosis. These findings suggest an association with less aggressive initial presentation rather than a definitive prognostic impact.

Keywords: Papillary thyroid carcinoma; autoimmune thyroid disease; Hashimoto's thyroiditis; anti-thyroglobulin antibodies

INTRODUCTION

Thyroid cancers account for approximately 1% of all malignancies and represent the most common endocrine cancers, of which 85–90% correspond to papillary thyroid carcinomas (PTCs). Their incidence has been steadily increasing in recent years, largely due to improved, more widely accessible diagnostic modalities, including cervical ultrasound and fine-needle aspiration cytology. Despite this rise in incidence, mortality remains low, with a 5-year survival rate exceeding 90% (1). In Algeria, thyroid cancer ranks as the third most common cancer among women (2).

Autoimmune thyroid disease is the most frequent autoimmune disorder and is represented by Hashimoto's thyroiditis and Graves' disease, with an estimated incidence of approximately 5% in the general population (3).

The association between PTC and autoimmune thyroiditis (HT) was first described in 1955 by Dailey (4). However, the relationship between these two conditions remains complex and not fully understood. Several mechanisms have been proposed, including chronic inflammation, immune-mediated effects, and the role of thyroid-stimulating hormone, although their exact contributions to tumor development and progression remain debated (5).

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The aim of this study was to investigate the associations between HT and clinicopathological features, as well as early treatment outcomes, in patients with PTC.

SUBJECTS AND METHODS

We conducted a prospective longitudinal study including 267 patients over the age of 18 years who were evaluated at a specialized referral center and diagnosed with PTC between January 2020 and December 2022.

The diagnosis of autoimmune thyroiditis was established on the basis of histopathological examination. After determining the prevalence of thyroiditis in the cohort patients were divided into two groups: those with thyroiditis and those without. The primary goal of the study was to assess the association between the presence of autoimmune thyroiditis and tumor size, which is a key clinicopathological feature.

Secondary outcomes included the evaluation of the association between thyroiditis and other prognostic factors, such as lymph node metastases, extrathyroidal extension, vascular invasion, multifocality, and risk stratification according to the TNM/AJCC 2017 (6) and ATA 2015 (7) classifications. Early treatment outcomes, including response to therapy and recurrence at 9 months, were also analyzed.

Given the increase in early diagnosis of PTC and the consequent reduction in tumor size, in addition to the classical prognostic threshold of 4 cm, a secondary stratification at 2 cm was applied to compare the two groups.

The exclusion criteria included patients with incomplete prior clinical data who had previously undergone thyroid surgery, or those who did not have papillary thyroid carcinoma. Potential sources of bias, including selection bias and the relatively short follow-up period, were considered when interpreting the results. A flow diagram of patient inclusion was constructed in accordance with the STROBE recommendations (**Figure 1**).

Statistical analyses were performed using SPSS version 27. Continuous variables were expressed as the means ± standard deviations, and categorical variables were expressed as frequencies and percentages.

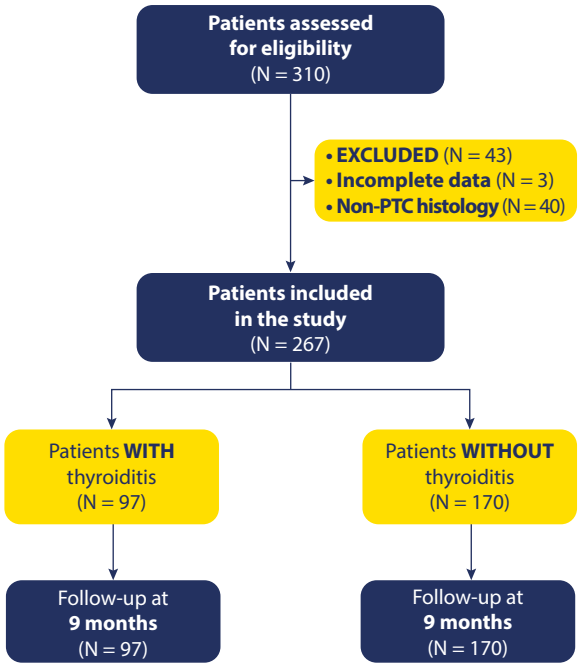


Figure 1. Flow diagram of patient selection and study design.

The normality of continuous variables was assessed prior to analysis. Depending on the data distribution, parametric or non-parametric tests were applied.

Comparisons between qualitative variables were performed using the chi-square test or Fisher's exact test when appropriate. Continuous variables were compared using Student's t test or non-parametric equivalents.

Variables with a p value < 0.2 in univariate analysis were included in a multivariate logistic regression model. The dependent variable in the multivariate analysis was the presence of thyroiditis. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Statistical significance was set at $p < 0.05$.

No adjustment for multiple comparisons was performed; therefore, and the results should be interpreted with caution.

The study was conducted in accordance with internationally accepted ethical principles, and written informed consent was obtained from all participants prior to inclusion.

RESULTS

A total of 267 patients with papillary thyroid carcinoma (PTC) were included. There was a marked female predominance, with 201 women (75.3%) and 66 men

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(24.7%). The mean age at diagnosis was 44.8 ± 0.8 years (range: 20–84 years). A family history of thyroid cancer was reported in 7 patients (2.6%).

The mean initial serum TSH concentration was 2.44 ± 0.15 IU/L (range: 0–27.49 IU/L). TSH values were within normal limits in 81.2%, low in 8.6%, and elevated in 10.2% of patients.

All patients underwent total thyroidectomy. Lymph node dissection was performed in 52.8% of cases, among whom 74.5% underwent prophylactic central dissections and 25.5% underwent therapeutic dissections including lateral compartments.

According to the 2017 TNM classification, 67% of tumors were staged T1 (including 31.8% microcarcinomas), 22.5% T2, and 10.2% T3–T4. One case of non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTPs) was identified (3.5 cm). Among patients who underwent lymph node dissection, 12% had lateral lymph node metastases, 7.1% had central metastases, and 33.7% had no metastatic involvement. Preoperatively, one patient presented with bone metastasis. Post-therapeutic scintigraphy revealed distant metastases in 5 patients (pulmonary, mediastinal, or clavicular), accounting for 2.24% of the cohort.

Based on the 2015 ATA recurrence risk stratification, 76% of the patients were categorized as low risk, 12.4% as intermediate risk, and 11.6% as high risk.

Radioiodine therapy was administered in 80.1% of cases. It was not indicated in 19.9%, predominantly for uni- or multifocal micro-PTCs with a cumulative tumor burden ≤ 1 cm and the absence of capsular invasion or vascular emboli.

At the nine-month follow-up, the treatment response, according to the ATA 2015 criteria, was as follows: 69.9% had an excellent response, 15.1% had an incomplete biochemical response, 7.5% had an incomplete structural response, and 7.5% had an indeterminate response.

Recurrence was documented in 22 patients (8.24%): 68.2% presented with cervical lymph node metastases, 9.1% developed distant metastases, and 22.7% demonstrated disease progression without identifiable lesions on ultrasound, CT, or FDG-PET. Management included surgery followed by radioiodine in 7 patients and a second radioiodine course in 15 patients. One patient was deemed inoperable due to local invasion and was not eligible for tyrosine kinase inhibitors. Another patient was classified as radioiodine refractory after a second treatment and initiated sorafenib treatment.

Histopathological features of chronic autoimmune thyroiditis were present in 97 patients (36.3%).

When comparing prognostic indicators between patients with and without thyroiditis, several statistically significant associations were identified. PTC occurring in the setting of thyroiditis was more common in women ($p < 0.001$) and in younger patients ($p = 0.015$). The mean tumor size was significantly smaller in the thyroiditis group (1.58 ± 1.1 cm vs. 1.78 ± 1.27 cm; $p < 0.001$), and multifocality was more common ($p = 0.043$).

Conversely, no significant differences were found for capsular invasion ($p = 0.79$), extrathyroidal extension, vascular emboli ($p = 0.20$), aggressive histologic variants ($p = 0.36$), lymph node metastases ($p = 0.49$), or distant metastases ($p = 0.67$). Likewise, the ATA 2015 ($p = 0.41$), TNM 2017 ($p = 0.38$), and AJCC staging ($p = 0.74$) did not significantly differ between the two groups.

Several ultrasound and biochemical parameters differed significantly. TSH levels were higher in patients with thyroiditis (3.21 ± 0.37 IU/L vs. 2.01 ± 0.48 IU/L; $p < 0.001$). The mean nodule size on ultrasound was smaller (1.82 ± 0.1 cm vs. 2.4 ± 0.1 cm; $p = 0.001$), and the EU-TIRADS 5 classification was more prevalent (71.1%; $p = 0.03$) in patients with thyroiditis. Thyroid volume also differed significantly ($p = 0.017$) (8). No difference was observed according to the 2017 Bethesda system ($p = 0.92$) (9).

Radioiodine indications ($p = 0.68$) and administered activities ($p = 0.10$) did not differ between the two groups. The mean thyroglobulin levels at ablation were significantly lower in the thyroiditis group (12.54 ± 8.04 ng/mL vs. 61.65 ± 38.04 ng/mL; $p < 0.001$), while anti-Tg antibody positivity showed no statistical difference ($p = 0.70$).

At the nine-month assessment, the response to therapy was significantly associated with the presence of thyroiditis ($p < 0.001$). Notably, 18% of

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patients with thyroiditis were classified as having an indeterminate response. However, the nine-month recurrence rates did not differ significantly between the groups (p = 0.92).

All outcomes are summarized in **Table 1**.

Multivariate binary logistic regression including, variables with p values < 0.2 in univariate analysis, revealed the following independent prognostic factors: age > 55 years, female sex, tumor size > 2 cm, and the presence of vascular emboli. The results are summarized in **Table 2**.

DISCUSSION

The association between papillary thyroid carcinoma (PTC) and autoimmune thyroiditis (HT) has been documented widely since its first description in 1955 (4). However, interpretation remains challenging due to substantial methodological heterogeneity among published studies, most of which are retrospective (10-12).

The relationship between HT and PTC remains complex and not fully understood. Elevated thyroid-stimulating hormone (TSH) levels, chronic inflammation, and cytokine-mediated pathways have been suggested to contribute to tumor development. However, their clinical implications remain debated, with recent studies highlighting the potential dual role of autoimmune processes in both tumor initiation and the modulation of tumor behavior (13,14).

In addition, the frequency of BRAFV600E mutations appears to be lower in PTC cases associated with HT. Even when present, autoimmune mechanisms may partially counterbalance their oncogenic effect, which could contribute to the more favorable clinicopathological features often observed in this subgroup (13).

Table 1. Clinicopathological characteristics according to the presence of autoimmune thyroiditis (HT)

Variable	HT (n = 97)	No HT (n = 170)	p value
Female sex, n (%)	85 (87.6)	116 (68.2)	**< 0.001**
Age, years (mean ± SD)	42.8 ± 1.1	46.2 ± 1.0	0.015
Tumor size, cm (mean ± SD)	1.58 ± 1.1	1.78 ± 1.27	**< 0.001**
Tumor size > 2 cm, n (%)	17 (18)	50 (29)	**0.03**
Multifocality, n (%)	34 (35)	40 (24)	**0.043**
Capsular invasion, n (%)	22 (22.7)	41 (24.1)	0.79
Vascular emboli, n (%)	21 (21.6)	49 (28.8)	0.20
Lymph node metastasis (yes), n (%)	18 (33)	33 (38)	0.49
TNM stage (advanced stage vs. low stage), n (%)	9 (9.2) 88 (90.8)	18 (10.7) 151 (89.3)	0.38
Aggressive histologic variants, n (%)	5 (5)	5 (3)	0.36
ATA high-risk category, n (%)	9 (9.3)	22 (12.9)	0.41
Radioiodine therapy (yes), n (%)	79 (81.4)	135 (79.4)	0.68
Recurrence at 9 months, n (%)	8 (9)	14 (9.3)	0.92

ATA: American Thyroid Association.

Low-stage tumors corresponded to T1–T2 tumors, and advanced-stage tumors corresponded to T3–T4 tumors according to the 2017 TNM classification. Lymph node metastasis analysis was restricted to patients who underwent lymph node dissection.

Table 2. Multivariate logistic regression analysis of factors associated with the presence of Hashimoto's thyroiditis

Variable	Adjusted OR	95% CI	p value
Female sex	3.59	1.43–8.98	0.006
Age >55 years	0.35	0.13–0.97	0.043
Tumor size >2 cm	0.38	0.15–0.98	0.046
Vascular emboli	0.39	0.16–0.94	0.036
Multifocality	—	—	0.11
Lymph node metastasis	—	—	0.60
Capsular invasion	—	—	0.60

"—" : not retained in the multivariate model.

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In line with previous series, our cohort demonstrated a marked female predominance, likely reflecting hormonal influences on immune modulation and tumorigenesis. Moreover, patients with HT were younger at diagnosis, which is a well-established favorable prognostic factor in contemporary staging systems (15).

Histopathologically, tumors were significantly smaller in the HT group, which is consistent with most published data (11,12). Multifocality was also more frequent, which may reflect a field-effect phenomenon rather than increased tumor aggressiveness (13,16).

An important explanation for the association between HT and smaller tumor size may be detection bias, as patients with HT are more likely to undergo clinical and ultrasound monitoring, leading to earlier diagnosis. However, this effect should be interpreted with caution, as a substantial proportion of patients in our cohort were euthyroid and did not undergo systematic surveillance.

We did not observe significant differences in vascular invasion, lymph node metastases, extrathyroidal extension, ATA risk stratification, or TNM staging, in agreement with several previous studies reporting inconsistent or non-significant associations (17-20). Recent large-scale analyses have also highlighted heterogeneous results regarding the relationship between thyroid autoantibodies and tumor aggressiveness (21).

Radioiodine use and administered activities were comparable between groups, consistent with findings from the previous studies (22-25).

Thyroglobulin levels at the time of ablation were significantly lower in patients with HT; however, these findings should be interpreted with caution due to the known interference of anti-thyroglobulin antibodies, which may lead to the underestimation of Tg levels (26). Recent prospective data further support the clinical relevance of thyroid autoantibodies in both diagnostic assessment and risk stratification in thyroid cancer (27).

At the 9-month follow-up, patients with HT were more likely to demonstrate an excellent response but were also more likely to be classified as having an indeterminate response. This pattern likely reflects the impact of autoimmune status on thyroglobulin and antibody kinetics rather than true differences in disease status. This ambiguity raises concerns regarding the applicability of the ATA indeterminate response category in this subgroup. Similar observations have been reported in the literature, with a high proportion of indeterminate responses subsequently reclassified as excellent responses during longer follow-up (28-30).

De Leo and cols. reported a higher frequency of incomplete biochemical response, likely related to residual thyroid tissue or abnormalities in thyroglobulin measurements, without necessarily affecting the structural aggressiveness of the cancer (31).

No significant association was observed between HT and early recurrence, consistent with findings from several previous studies (24,32,33). However, the short follow-up period limits the interpretation of long-term outcomes.

From a clinical perspective, the assessment of treatment response in patients with HT remains challenging because of the interference of anti-thyroglobulin antibodies. The higher rate of indeterminate responses observed in this subgroup may therefore reflect biochemical limitations rather than persistent disease. These findings suggest that early reassessment at 9 months, particularly when based on thyroglobulin levels, may lead to potential misclassification. In selected patients with positive anti-thyroglobulin antibodies, delaying assessment of response until anti-thyroglobulin antibodies become negative may help avoid misclassification and unnecessary therapeutic escalation, although this hypothesis requires confirmation in larger prospective studies.

This study has several limitations. Its single-center design may limit generalizability, although its prospective nature represents a strength, allowing for standardized data collection. The relatively short follow-up (9 months) does not allow for reliable assessment of long-term outcomes in this typically indolent disease. In addition, the association between HT and smaller tumor size may be partially influenced by detection bias, although many patients in our cohort were euthyroid and not routinely monitored. Finally,

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the interpretation of thyroglobulin levels remains limited by antibody interference, and residual confounding cannot be excluded given the study's observational design.

In conclusion, in this prospective study, autoimmune thyroiditis was associated with certain favorable clinicopathological features, including younger age at diagnosis and smaller tumor size. However, no significant differences were observed in key oncological outcomes, such as lymph node metastasis, extrathyroidal extension, staging, or recurrence, at 9 months.

These findings suggest that autoimmune thyroiditis may be associated with a less aggressive initial presentation of papillary thyroid carcinoma rather than a definitive prognostic impact.

Given the relatively short follow-up period and the potential influence of detection bias, these results should be interpreted with caution. Further prospective studies with longer follow-up periods are needed to better clarify the relationship between autoimmune thyroiditis and long-term outcomes in papillary thyroid carcinoma.

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original

Neurodevelopmental outcomes in children with transient hypothyroxinemia of prematurity assessed using the Denver Developmental Screening Test II (DDST-II)

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ABSTRACT

Objective: This study aimed to evaluate the impact of levothyroxine supplementation on neurodevelopmental outcomes in infants with transient hypothyroxinemia of prematurity (THOP). **Subjects and methods:** In this retrospective observational study, infants born at < 34 weeks of gestation were categorized into three groups: THOP treated with levothyroxine, untreated THOP, and non-hypothyroxinemic controls. Neurodevelopmental outcomes were assessed at 12–72 months of corrected age using the Denver Developmental Screening Test II (DDST-II). **Results:** Fifty-four infants (40.7% female) with a median gestational age (GA) of 31.0 (28.6–33.0) weeks and mean birth weight of 1414 ± 466 g were included. Infants treated for THOP had significantly lower GA compared with controls (p = 0.037) and lower initial FT4 levels (p < 0.001), while TSH levels were similar (p = 0.581). Prematurity-related morbidities were more frequent in the treated THOP group. No association was observed between DDST-II results and GA, birth weight, or prematurity-related morbidities. The DDST-II results did not differ significantly among treated THOP, untreated THOP, and controls (p = 0.484). **Conclusion:** Levothyroxine supplementation in infants with THOP was not associated with neurodevelopmental outcomes compared with untreated infants with THOP or non-hypothyroxinemic controls. In this cohort, DDST-II results were not significantly associated with GA, birth weight, or major prematurity-related morbidities, suggesting that routine levothyroxine supplementation may not confer a measurable neurodevelopmental benefit. Given the observational design and baseline clinical differences between groups, these findings should be interpreted cautiously. Further multicenter studies with larger cohorts and comprehensive follow-up are needed to clarify whether specific high-risk subgroups may benefit from treatment..

Keywords: Transient hypothyroxinemia of prematurity: preterm infants; levothyroxine supplementation; neurodevelopment; Denver Developmental Screening Test II (DDST-II)

INTRODUCTION

Thyroid hormones (TH) are essential for normal brain development, regulating neuronal migration, myelination, synaptogenesis, and axonal growth during fetal and early postnatal life. In early gestation, the fetus depends entirely on maternal TH supply, whereas from mid-gestation onward, the hypothalamic–pituitary–thyroid (HPT) axis gradually matures and begins to contribute to hormone production (1,2). In preterm infants, particularly those born before 30 weeks, the postnatal rise in TH levels is often delayed, and normalization may take several weeks (2).

Transient hypothyroxinemia of prematurity (THOP), characterized by low free thyroxine (FT4) levels with normal or low thyroid-stimulating hormone (TSH) levels, is the most common thyroid dysfunction

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in preterm infants, affecting up to 20% of those born ≤ 34 weeks. Its multifactorial etiology includes immaturity of the HPT axis, abrupt cessation of maternal TH transfer, limited thyroidal reserve, illness-related alterations in TH metabolism, and effects of medication such as dopamine and glucocorticoids (1–3). Risk factors include low birth weight, small for gestational age (GA), and major complications of prematurity such as respiratory distress syndrome (RDS), chronic lung disease (CLD), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), and sepsis (1–5).

The clinical significance of THOP remains debated. Some studies have reported an association between low neonatal TH levels and adverse neurodevelopmental outcomes (6–8), whereas others found no significant relationship (9–11). Randomized controlled trials of levothyroxine supplementation have likewise yielded conflicting results — with beneficial effects reported in extremely preterm infants (< 28 weeks) (12), neutral results in moderately preterm infants (13), and even potential harm in those born > 29 weeks (14). These controversies make the decision to treat THOP challenging, underscoring the need to distinguish infants who might benefit from supplementation from those in whom low FT4 represents a transient, adaptive phenomenon (15,16).

The Denver Developmental Screening Test II (DDST-II) is a practical screening tool for early detection of developmental delay in children up to 6 years of age and is widely applied in the follow-up of high-risk preterm infants (17). This study aimed to evaluate the neurodevelopmental outcomes of preterm infants with THOP, comparing levothyroxine-treated and untreated infants with non-hypothyroxinemic controls, while accounting for GA and prematurity-related morbidities.

SUBJECTS AND METHODS

Study design and participants

This single-center retrospective observational study included infants born between 24 and 34 weeks of gestation who were admitted to the neonatal intensive care unit of a tertiary care hospital between January 2014 and December 2019. Infants with chromosomal anomalies, major congenital malformations, or hypoxic–ischemic encephalopathy were excluded. Participants were categorized into three groups: (1) THOP treated with levothyroxine, (2) untreated THOP, and (3) controls without hypothyroxinemia. Neurodevelopmental outcomes were assessed at 12–72 months of corrected age using the DDST-II.

Data collection

Perinatal and neonatal data were retrieved from electronic medical records and included GA, birth weight, Apgar scores, mode of delivery, and major complications of prematurity such as RDS, CLD, IVH, NEC, PDA, and retinopathy of prematurity (ROP). Neurodevelopmental evaluation using DDST-II was performed at follow-up visits by trained clinicians as part of routine developmental screening in our center.

Thyroid function testing and definition of transient hypothyroxinemia of prematurity

Serum FT4 and TSH levels were measured routinely on postnatal days 7, 14, and 28 using an electrochemiluminescence immunoassay (Elecsys 2010 analyzer; Roche Diagnostics, Indianapolis, IN, USA). Because TH concentrations vary with GA in preterm infants, FT4 values were interpreted according to gestational age–specific percentile reference reported by Williams and cols. (18). The criteria for THOP included FT4 levels below the 10th percentile for GA with normal TSH values in at least two consecutive measurements. The decision to initiate levothyroxine therapy was made by the attending neonatologist and pediatric endocrinologist based on clinical judgment, considering FT4 levels, persistence of hypothyroxinemia, GA, and prematurity-related complications.

Denver developmental screening test

The DDST-II is a standardized tool designed to identify developmental issues in children aged 0–72 months, facilitating early intervention. During the study period, trained and experienced healthcare professionals administered the latest standardized version of DDST-II for Turkey to all children. The DDST-II comprises 134 developmental items categorized into four domains: personal-social, fine motor, language, and gross motor

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skills. Upon determining the child's chronological age, a developmental age line is established to assess developmental skill alignment. We evaluate test items based on their pass, fail, not applicable, or refusal status. We categorize test results as "normal", "abnormal", or "suspect". Passing all age-appropriate items or receiving at most one caution item is considered "normal". "Suspect" refers to receiving only one delay, two or more caution items without delay, or one delay with one or more caution items. Two or more delay items indicate an "abnormal" result (17).

Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA) and Jamovi version 2.3.21. Normality of continuous variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Normally distributed variables were expressed as mean ± standard deviation (SD) and compared among the three groups using one-way analysis of variance (ANOVA). Non-normally distributed variables were expressed as median (interquartile range [IQR]) and compared using the Kruskal–Wallis test. The DDST-II results were analyzed as categorical variables (normal, suspicious, abnormal). Categorical variables were presented as n (%) and compared using the chi-square test or Fisher's exact test when appropriate. When a significant difference was observed in the overall three-group comparison, descriptive pairwise comparisons were performed using the Dwass–Steel–Critchlow–Fligner test to identify which group(s) contributed most to the difference. Effect sizes for categorical variables were reported as Cramer's V correlations between DDST-II results, and clinical variables were assessed using Spearman's rank correlation coefficient. Secondary analyses were conducted to examine the association between GA, thyroid function tests, and prematurity-related morbidities (RDS, IVH, PDA, CLD) with abnormal DDST-II results. A two-sided p value < 0.05 was considered statistically significant.

RESULTS

A total of 54 preterm infants (22 females, 40.7%) were evaluated at a median corrected age of 2.3 years (IQR 1.8–3.4 years). Thirty-one infants had THOP, of whom 22 (71%) received levothyroxine supplementation. Twenty-three infants with normal thyroid function served as controls.

The median corrected ages were 3.2 (IQR 3.2–3.5) years in the treated THOP group, 2.2 (IQR 1.7–2.9) years in the untreated THOP group, and 1.9 (IQR 1.8–3.2) years in the control group. Additionally, GA was significantly lower in the treated THOP group compared with the non-hypothyroxinemic control group (30.4 [28.0–31.8] weeks vs. 32.3 [28.6–33.0] weeks, respectively, p = 0.037), whereas birth weight, sex distribution, and anthropometric parameters at follow-up were similar across groups (all p > 0.05). Initial TSH values did not differ (p = 0.581), but initial FT4 levels were lowest in the treated THOP group (0.77 [0.70–1.01] ng/dL), intermediate in untreated THOP (0.92 [0.74–1.07] ng/dL), and highest in controls (1.60 [1.44–1.72] ng/dL; p < 0.001). The median number of days to initiation of levothyroxine treatment was 20 (IQR 10–51). Detailed demographic, clinical, and laboratory findings are shown in **Table 1**. Infants treated with levothyroxine had lower GA and lower initial FT4 levels and exhibited a higher burden of prematurity-related morbidities compared with controls.

Prematurity-related morbidities differed significantly between groups. The treated THOP group had higher complication rates compared with controls: RDS (63.6% vs. 34.8%, p = 0.019), IVH (22.7% vs. 0%, p = 0.021), PDA (22.7% vs. 8.7%, p = 0.016), CLD (59.1% vs. 17.4%, p = 0.011), and ROP (18.2% vs. 8.7%, p = 0.012). Notably, NEC was rare and did not differ significantly among groups (p = 0.318).

The DDST-II results were comparable across groups (p = 0.484, Cramer's V = 0.179). Normal results were observed in 47.8% of controls, 31.8% of treated THOP, and 44.5% of untreated THOP infants; abnormal outcomes occurred in 34.8%, 59.1%, and 33.3% of these groups, respectively (**Table 2**). No correlation was observed between DDST-II results and perinatal or neonatal variables (p > 0.05) (**Table 3**).

When DDST-II results were stratified into normal, suspicious, and abnormal categories, no significant differences were observed in GA, birth weight, or the frequency of individual prematurity-related morbidities

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Table 1. Demographic, clinical, and laboratory characteristics of preterm infants with transient hypothyroxinemia of prematurity and controls

		Total (n=54)	Controls (n=23)	THOP Treated (n=22)	THOP Untreated (n=9)	*P* value
Age at follow-up (years)†		2.3 (1.8–3.4)	1.9 (1.8–3.2)	3.2 (3.2–3.5)	2.2 (1.7–2.9)	0.19*
Sex, n (%)	Female	22 (40.7)	11 (47.8)	8 (36.4)	3 (33.3)	0.65**
	Male	32 (59.3)	12 (52.2)	14 (63.6)	6 (66.7)	
Height SDS at follow-up††		-0.95 ± 1.20	-0.96 ± 1.19	-0.28 ± 1.79	-1.37 ± 0.61	0.11***
Weight SDS at follow-up††		-0.63 ± 1.72	-0.69 ± 1.58	-0.33 ± 2.15	-1.09 ± 1.72	0.92***
BMI SDS at follow-up††		0.12 ± 1.40	-0.14 ± 1.38	0.90 ± 1.61	0.17 ± 1.31	0.96***
Mode of delivery, n (%)	Vaginal	9 (16.7)	3 (13)	5 (22.7)	1 (11.1)	0.61**
	Cesarean	45 (83.3)	20 (87)	17 (77.3)	8 (88.9)	
Gestational age at birth (weeks)†		31.0 (28.6–33.0)	32.3 (28.6–33.0)	30.4 (28.0–31.8)	30.0 (28.0–31.8)	0.026* 0.037‡ (treated vs. controls)
Gestational age, n (%)						0.42**
24–27 weeks		11	3	6	2	
28–30 weeks		15	5	6	4	
31–34 weeks		28	15	10	3	
Birth weight (grams)††		1414 ± 466	1525 ± 511	1317 ± 358	1358 ± 553	0.31***
Initial serum TSH (mIU/L)†		4.38 (3.01–6.31)	4.46 (3.19–5.88)	5.05 (3.32–7.03)	3.66 (2.09–5.82)	0.58*
Initial serum FT4 (ng/dL)†		1.07 (0.77–1.55)	1.60 (1.44–1.72)	0.77 (0.70–1.01)	0.92 (0.74–1.07)	<0.001* <0.001‡ (treated vs. controls) <0.001‡ (untreated vs. controls)
Apgar scores at 1 minute†		4 (3–6)	6 (4–7)	4 (2.25–4.75)	4 (4–5)	0.010* 0.012‡ (treated vs. controls)
Apgar scores at 5 minutes†		7 (5–8)	7.5 (7–8)	5 (5–7)	6 (6–7)	0.007* 0.012‡ (treated vs. controls) 0.050‡ (untreated vs. controls)
IVH, n (%)		9 (16.7)	0 (0)	5 (22.7)	4 (44.4)	0.021**
RDS, n (%)		30 (55.6)	8 (34.8)	14 (63.6)	8 (88.9)	0.019**
PDA, n (%)		12 (22.2)	2 (8.7)	5 (22.7)	5 (55.6)	0.016**
ROP, n (%)		11 (20.4)	2 (8.7)	4 (18.2)	5 (55.6)	0.012**
CLD, n (%)		22 (40.7)	4 (17.4)	13 (59.1)	5 (55.6)	0.011**
NEC, n (%)		2 (3.7)	0 (0)	1 (4.5)	1 (11.1)	0.16**
Current serum FT4 (ng/dL)†		1.38 (1.29–1.48)	1.43 (1.30–1.50)	1.34 (1.33–1.40)	1.33 (1.24–1.43)	0.80*
Current serum TSH (mIU/L)†		2.40 (1.60–3.35)	2.19 (1.54–2.82)	2.14 (1.67–2.85)	2.42 (2.03–3.99)	0.60*

*Kruskal–Wallis test; **Fisher's exact test; ***One-way analysis of variance (ANOVA); ‡*post hoc* pairwise comparison (Dwass–Steel–Critchlow–Fligner test); †Values represent median (25th–75th percentile); ††Values represent mean ± standard deviation.

THOP: transient hypothyroxinemia of prematurity; SDS: standard deviation scores; BMI: body mass index; TSH: thyroid-stimulating hormone; FT4: free thyroxine; IVH: intraventricular hemorrhage; RDS: respiratory distress syndrome; PDA: patent ductus arteriosus; ROP: retinopathy of prematurity; CLD: chronic lung disease; NEC: necrotizing enterocolitis.

— including IVH, RDS, PDA, ROP, CLD, and NEC — across the groups (all p > 0.05) (Table 4). This finding indicates that abnormal DDST-II outcomes were not associated with neonatal characteristics or individual prematurity-related complications in our cohort.

DISCUSSION

In this study, we evaluated whether levothyroxine supplementation in preterm infants with THOP influences neurodevelopmental outcomes, assessed using the DDST-II between 12 and 72 months of corrected age. Our main finding was that levothyroxine treatment of infants with THOP was not associated with neurodevelopmental outcomes compared with untreated infants with THOP or non-hypothyroxinemic controls. More specifically, levothyroxine treatment was not associated with improved neurodevelopmental outcomes. Importantly, infants who

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Table 2. Denver Developmental Screening Test II (DDST-II) results in infants with transient hypothyroxinemia of prematurity and controls

	Total (n=54)	Controls (n=23)	THOP Treated (n=22)	THOP Untreated (n=9)	*P* value
Personal-social, n (%)					
Normal	34 (63)	17 (73.9)	12 (54.6)	5 (55.6)	0.65*
Suspicious	11 (20.4)	4 (17.4)	5 (22.7)	2 (22.2)	
Abnormal	9 (16.6)	2 (8.7)	5 (22.7)	2 (22.2)	
Fine motor-adaptive, n (%)					
Normal	36 (66.6)	17 (73.9)	15 (68.2)	4 (44.5)	0.33*
Suspicious	9 (16.7)	4 (17.4)	2 (9.1)	3 (33.3)	
Abnormal	9 (16.7)	2 (8.7)	5 (22.7)	2 (22.2)	
Language, n (%)					
Normal	28 (51.9)	14 (60.9)	8 (36.4)	6 (66.7)	0.18*
Suspicious	9 (16.6)	4 (17.4)	3 (13.6)	2 (22.2)	
Abnormal	17 (31.5)	5 (21.7)	11 (50.0)	1 (11.1)	
Gross motor, n (%)					
Normal	40 (74.1)	20 (87.0)	13 (59.1)	7 (77.8)	0.31*
Suspicious	9 (16.7)	2 (8.7)	6 (27.3)	1 (11.1)	
Abnormal	5 (9.2)	1 (4.3)	3 (13.6)	1 (11.1)	
Result, n (%)					
Normal	22 (40.7)	11 (47.8)	7 (31.8)	4 (44.5)	0.48*
Suspicious	8 (14.8)	4 (17.4)	2 (9.1)	2 (22.2)	
Abnormal	24 (44.5)	8 (34.8)	13 (59.1)	3 (33.3)	

*Chi-square test or Fisher's exact test, when appropriate.

THOP: transient hypothyroxinemia of prematurity.

Table 3. Correlation between Denver Developmental Screening Test II (DDST-II) results and perinatal/neonatal variables

	rho	*P* value
Age at follow-up (years)	-0.103	0.55
Gestational age at birth (weeks)	0.170	0.22
Birth weight (grams)	0.127	0.36
Apgar scores at 1 minute	0.292	0.19
Apgar scores at 5 minutes	0.376	0.09
Initial serum TSH (mIU/L)	0.092	0.51
Initial serum FT4 (ng/dL)	0.084	0.24
IVH	-0.177	0.20
RDS	0.015	0.92
PDA	-0.109	0.43
ROP	0.047	0.78
CLD	-0.045	0.75
NEC	-0.206	0.14

TSH: thyroid-stimulating hormone; FT4: free thyroxine; IVH: intraventricular hemorrhage; RDS: respiratory distress syndrome; PDA: patent ductus arteriosus; ROP: retinopathy of prematurity; CLD: chronic lung disease; NEC: necrotizing enterocolitis.

received treatment represented a clinically more vulnerable subgroup, with lower GA, lower FT4 levels, and a higher burden of prematurity-related morbidities at baseline. Therefore, treatment status in this retrospective cohort likely reflects underlying illness severity rather than a causal effect of levothyroxine therapy on neurodevelopment. These results are consistent with recent evidence indicating no significant benefit of levothyroxine replacement in moderately preterm infants (9–11,19,20).

Previous studies have reported conflicting results regarding the association between THOP and neurodevelopmental outcomes. Some investigators observed that lower neonatal TH levels were associated with impaired cognitive and motor development (6,8), whereas others found no such relationship (9–11). In line with our findings, Chung and cols. (10) and Dilli and cols. (9) reported that THOP was not associated with adverse outcomes when assessed using the Bayley Scales of Infant Development Second Edition (BSID II), whereas Hollanders and cols. (11) demonstrated no long-term association between THOP and neurodevelopment in young adulthood.

Although the overall comparison did not reach statistical significance, the proportion of abnormal DDST-II results was numerically higher in the treated

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Table 4. Comparison of perinatal characteristics and prematurity-related morbidities according to Denver Developmental Screening Test II (DDST-II) results

	Total (n=54)	Normal (n=22)	Suspicious (n=8)	Abnormal (n=24)	*P* value
Gestational age (weeks)†	31.0 (28.6–33.0)	31.4 (28.1–33.0)	32.4 (30.7–34.4)	30.5 (28.4–32.0)	0.41*
Birth weight (grams)††	1359±569	1540±575	1140±546	1266±544	0.16**
IVH, n (%)	9 (16.7)	2 (9.1)	1 (12.5)	6 (25)	0.38***
RDS, n (%)	30 (55.6)	10 (45.5)	6 (75)	14 (58.3)	0.17***
PDA, n (%)	12 (22.2)	3 (13.6)	2 (25)	7 (29.2)	0.45***
ROP, n (%)	11 (20.4)	5 (22.7)	1 (12.5)	5 (20.8)	1.00***
CLD, n (%)	22 (40.7)	6 (27.3)	5 (62.5)	11 (45.8)	0.16***
NEC, n (%)	2 (3.7)	0	0	2 (8.3)	-

*Kruskal–Wallis test; **One-way analysis of variance (ANOVA); ***Fisher's exact test.
IVH: intraventricular hemorrhage; RDS: respiratory distress syndrome; PDA: patent ductus arteriosus; ROP: retinopathy of prematurity; CLD: chronic lung disease; NEC: necrotizing enterocolitis.

THOP group. However, this pattern likely reflects baseline clinical differences rather than a detrimental effect of levothyroxine therapy. Infants who received treatment had lower GA, lower FT4 levels, and a higher burden of prematurity-related morbidities, suggesting greater clinical vulnerability in this group. Because both illness severity and prematurity-related complications may independently influence neurodevelopment, the slightly higher proportion of abnormal DDST-II results observed in treated infants is more likely to reflect underlying illness severity rather than the effect of levothyroxine therapy itself. This interpretation is further supported by the absence of a significant association between DDST-II outcomes and TH levels or individual prematurity-related morbidities in our secondary analyses.

Our results demonstrate no association between DDST-II outcomes and GA, birth weight, or individual prematurity-related morbidities, suggesting that in our cohort, neurodevelopmental outcomes were not primarily determined by these neonatal factors or thyroid status. Notably, THOP is increasingly regarded as a consequence of immaturity and clinical instability rather than a true abnormality of the HPT axis, and it often resolves spontaneously without treatment (10). Some authors have even suggested that hypothyroxinemia in the context of non-thyroidal illness may represent an adaptive neuroprotective mechanism that reduces protein catabolism and oxygen consumption (15,21), raising concerns that indiscriminate levothyroxine supplementation could interfere with such adaptive processes. In contrast to some earlier studies, our secondary analyses did not demonstrate significant associations between DDST-II results and GA, birth weight, or prematurity-related morbidities such as RDS, IVH, PDA, ROP, CLD, or NEC. This may reflect limited statistical power and the use of a screening rather than a diagnostic neurodevelopmental tool. While some studies, such as the one by Lin and cols. (22), reported that prematurity-related morbidities were stronger predictors of adverse outcomes than thyroid function, our findings align with those by Aygün and cols. (19), who also found no neurodevelopmental benefit from levothyroxine replacement in moderately preterm infants, despite higher morbidity rates in treated cases. Moreover, some studies even suggest that elevated FT4 levels may correlate with worse neurodevelopment (15,23).

Our findings are consistent with several previous studies reporting no clear neurodevelopmental benefit from routine levothyroxine supplementation in moderately preterm infants. Management of THOP remains controversial, and no consensus exists regarding routine levothyroxine supplementation (1,2). Early randomized trials reported heterogeneous results. Doses ranging from 4–20 μg/kg/day administered for 2–6 weeks showed no consistent benefit on neurodevelopment (13,24–26). However, van Wassenaer and cols. (27) and Briët and cols. (14) demonstrated that infants born before 27 weeks' gestation benefited from supplementation, showing higher Bayley scores at 24 months, while those born at or after 27 weeks showed no benefit or even worse outcomes. Long-term follow-up studies further highlighted gestational

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age–specific effects. Van Wassenaer and cols. (16,28) observed improved neuromotor outcomes up to 10.5 years in infants < 27 weeks who received levothyroxine. Our cohort consisted of infants with THOP characterized by low FT4 levels with normal TSH values according to gestational age–specific references. Therefore, infants with delayed or mild TSH elevation were not included in the study population. Neurodevelopmental outcomes and potential benefits of levothyroxine therapy may differ in preterm infants with elevated TSH levels; therefore, our findings should not be generalized to this subgroup.

More recent large-scale randomized controlled trials have yielded less encouraging results. La Gamma and cols. (29) reported shorter ventilation and reduced retinopathy rates with supplementation in < 28-week infants, but subsequent analyses from the same cohort found no difference in neurodevelopmental outcomes at 36 months (13). The multicenter TIPIT trial also failed to demonstrate improvements in growth or early neurodevelopment (30), although a later follow-up suggested possible cognitive benefits at 42 months (12). Taken together, these findings indicate that levothyroxine supplementation may confer benefit primarily in the most immature infants (< 27–28 weeks), but routine treatment of all preterm infants with THOP is not supported.

Importantly, levothyroxine supplementation is not without risk. Potential adverse effects include iatrogenic hyperthyroidism and late-onset circulatory collapse in very low birth weight infants (31). These risks reinforce the need for careful candidate selection rather than universal treatment.

Strengths of our study include the inclusion of both treated and untreated THOP infants, along with controls, and the integration of prematurity-related morbidities into the analysis. This design allowed us to examine the potential impact of levothyroxine supplementation while accounting for important neonatal clinical factors.

Several methodological limitations should be considered when interpreting the findings of this study. First, the retrospective design limits the ability to establish causal relationships and may introduce selection bias. In addition, treatment decisions were based on clinical judgment rather than randomization, which introduces the possibility of confounding by indication. Second, the relatively small sample size, particularly in the untreated THOP group, may have limited the statistical power to detect small but clinically meaningful differences in neurodevelopmental outcomes. Third, the higher median age at follow-up in the treated THOP group may have increased the likelihood of detecting developmental delay compared with the younger control group. Because developmental screening tools are age-dependent, this difference may have influenced the detection of developmental delays and should be considered when interpreting group comparisons. Finally, neurodevelopment was assessed using the DDST-II, which is a screening tool rather than a comprehensive diagnostic assessment. Neurodevelopmental assessment was performed over a relatively wide age range (12–72 months), which may have introduced heterogeneity and potentially obscured subtle developmental differences. Nevertheless, previous studies have reported that DDST-II demonstrates good sensitivity for detecting developmental problems in preterm infants when applied after the corrected age of 12 months (32). More comprehensive neurodevelopmental assessments, such as standardized cognitive or intelligence tests, may be required to detect subtle developmental differences that screening tools might miss.

In conclusion, levothyroxine supplementation in moderately preterm infants with THOP was not associated with neurodevelopmental outcomes in this cohort. Infants who received treatment had lower GA, lower FT4 levels, and a higher burden of prematurity-related complications, which may partly explain the lower proportion of normal DDST-II results observed in this group. Therefore, the observed differences likely reflect underlying clinical severity rather than a direct effect of levothyroxine therapy. These findings suggest that routine levothyroxine supplementation for moderately preterm infants with THOP may not provide measurable neurodevelopmental benefit.

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Evliyaoğlu performed the surgical and medical practices. Yavuz Özer, Olcay Evliyaoğlu analyzed and interpreted the data. Yavuz Özer, Gizem Yılmaz, and Olcay Evliyaoğlu collected and processed the data. Yavuz Özer and Olcay Evliyaoğlu conducted the literature search. Yavuz Özer and Olcay Evliyaoğlu wrote the manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Informed consent: due to the retrospective nature of the study, the requirement for written informed consent was waived by the ethics committee.

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Data availability: the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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original

Malignancy risk score in thyroid nodules with atypia of undetermined significance cytology: an approach based on cytological subgrouping and ultrasonographic data

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ABSTRACT

Objective: Thyroid nodules diagnosed as atypia of undetermined significance (AUS) are one of the most challenging categories in thyroid cytopathology due to histopathological ambiguity and complexities in clinical management. Therefore, this study aimed to develop and evaluate the diagnostic accuracy of a novel malignancy risk scoring system integrating cytological subclassification and key ultrasonographic features. **Subjects and methods:** A retrospective analysis was conducted on 357 patients who underwent thyroidectomy following an AUS cytology diagnosis. Cytological samples were subclassified as AUS-N or AUS-O, based on the presence of nuclear atypia, and fundamental sonographic parameters were recorded. Independent predictors of malignancy were identified using multivariable logistic regression, and the AUS-malignancy risk score (AUS-MRS) was developed accordingly. Diagnostic performance was compared with existing classification systems: ACR-TIRADS, EU-TIRADS, and K-TIRADS. **Results:** The AUS-MRS demonstrated the highest diagnostic performance, with a sensitivity of 84.6%, specificity of 79.6%, and a diagnostic odds ratio (DOR) of 21.8. Among the conventional systems, ACR-TIRADS showed the highest sensitivity (89.1%) but had lower specificity (69.7%) and a DOR of 18.4. EU-TIRADS and K-TIRADS showed more balanced metrics, with sensitivities of 82.7% and 81.4%, specificities of 73.6% and 74.1%, and DORs of 18.2 and 16.7, respectively. The inclusion of nuclear atypia increased specificity across TIRADS models (>85%); however, this was accompanied by reduced sensitivity and did not yield substantial gains in diagnostic performance. **Conclusion:** The AUS-MRS may offer improved diagnostic utility compared to existing ultrasound-based classification systems for assessing malignancy risk in AUS nodules.

Keywords: Atypia of undetermined significance; thyroid nodule; ultrasonography; nuclear atypia; AUS-malignancy risk score

INTRODUCTION

Fine-needle aspiration biopsy (FNAB) remains the primary diagnostic tool for assessing malignancy risk in thyroid nodules (1). The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), most recently revised in 2023, standardizes thyroid cytology reporting into six diagnostic categories to guide clinical management (2). Among these, atypia of undetermined significance (AUS) poses particular diagnostic challenges due to histopathological heterogeneity and the complexity of subsequent clinical decision-making. Recommended management options for AUS include repeat FNAB, molecular testing, or diagnostic thyroidectomy; however, the high costs of molecular tests and the invasive nature of surgery often limit their routine application (1,2). In this context, ultrasonography (US) plays a central role in both the initial evaluation and ongoing management of thyroid nodules.

Previous studies have demonstrated that various US features may assist in malignancy risk stratification for nodules diagnosed as AUS, although no single sonographic feature is sufficiently reliable in isolation (3,4). This limitation led to the development of

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ultrasound-based risk stratification systems, most notably, the Thyroid Imaging Reporting and Data System (TIRADS), which integrate multiple sonographic features into composite risk scores and are implemented in widely used frameworks, including the American College of Radiology (ACR-TIRADS), the European Thyroid Association system (EU-TIRADS), and the Korean TIRADS (K-TIRADS) (5–8). Despite widespread clinical adoption, the diagnostic performance of these systems in AUS nodules remains moderate, as highlighted in prior studies and meta-analyses (9–12).

The heterogeneity of the AUS category, coupled with widely variable reported malignancy rates ranging from 17 to 83%, has prompted efforts to improve risk stratification through cytological subclassification (13,14). Various studies have shown that nuclear atypia is associated with a higher malignancy risk compared with architectural atypia (15–18). Accordingly, the 2023 third edition of TBSRTC introduced the subclassification of AUS with nuclear atypia (AUS-N) and AUS with other atypia (AUS-O) to refine risk assessment (19). Nevertheless, cytological subclassification or sonographic assessment alone may be insufficient for accurate malignancy prediction.

Therefore, integrating cytological subgroups with key ultrasonographic features may offer a more comprehensive approach to malignancy risk stratification for AUS-diagnosed nodules. Accordingly, we aimed to develop and validate the AUS-Malignancy Risk Score (AUS-MRS), a novel malignancy risk scoring system that combines cytological subclassification with sonographic parameters. In addition, the diagnostic performance of AUS-MRS was compared with three widely used TIRADS-based systems: ACR-TIRADS, EU-TIRADS, and K-TIRADS.

SUBJECTS AND METHODS

Study design and patient selection

This retrospective observational study was conducted through collaboration between the Division of Endocrinology and Metabolism and the Department of Pathology at Necmettin Erbakan University Faculty of Medicine (Turkey) from 2016 to 2025. The study protocol was approved by the institution's ethics committee (decision no. 2024/5399; date: 20/12/2024). Patients included in the analysis had an initial FNAB diagnosis of AUS based on the TBSRTC and subsequently underwent thyroidectomy. Demographic data (e.g., age and sex), US characteristics, cytologic results, and final histopathological findings were retrospectively retrieved via the institutional hospital information system.

All AUS cytology slides were re-evaluated according to the updated 2023 TBSRTC criteria by an experienced pathologist blinded to the final histopathological diagnosis. Cases not confirmed as AUS upon re-evaluation were excluded. Confirmed AUS cases were subclassified into two groups: AUS-N and AUS-O. Patients were also excluded if their US reports lacked sufficient detail about nodule features, or if there was a mismatch in nodule localization between US and surgical pathology reports. Ultimately, 357 patients who met all eligibility criteria were included.

Ultrasound evaluation and classification

Prior to FNAB, ultrasound images were obtained by endocrinologists with at least 10 years of experience. A 12L3 transducer (3.6–12.9 MHz) was used with a Siemens Healthineers Acuson Juniper ultrasound system (Berlin, Germany). Each nodule was evaluated for size, composition, echogenicity, shape, margin characteristics, and echogenic foci. Nodule composition was classified as cystic, spongiform, mixed cystic-solid, or solid. Echogenicity was defined as anechoic, isoechoic, hyperechoic, mildly hypoechoic, or markedly hypoechoic, relative to the surrounding thyroid parenchyma. Shape was classified as wider-than-tall or taller-than-wide. Margins were categorized as smooth, lobulated, irregular, or showing extrathyroidal extension. Echogenic foci were characterized as comet-tail artifacts, macrocalcifications, peripheral calcifications, or microcalcifications. Based on previous research and the study design, solid composition, mild or marked hypoechogenicity, a taller-than-wide shape, irregular margins or extrathyroidal extension, and the presence of peripheral or microcalcifications were considered suspicious for malignancy. Each nodule was scored using the criteria of the ACR-TIRADS (1–5), EU-TIRADS (2–5), and K-TIRADS (2–5) classification systems, and corresponding risk categories were documented.

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Fine-needle aspiration and histopathological assessment

FNAB specimens were prepared using the conventional smear technique. After aspiration, approximately 1–2 drops of material were expelled onto a clean glass slide. A second slide was then placed over the first at an angle of approximately 45–90° and gently drawn across to produce a thin, even smear, similar to the peripheral blood smear technique. According to the staining protocol, smears were air-dried or immediately fixed. To ensure adequate sampling, at least two aspirations were performed for each lesion. Histopathological diagnoses were made in accordance with the 2022 World Health Organization Classification of Thyroid Neoplasms (20). Nodules were categorized as benign or malignant. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features, due to its indolent biological behavior, was classified as benign.

Development of the AUS-MRS

Based on the results of the multivariate logistic regression analyses, the novel malignancy risk scoring system (i.e., AUS-MRS) was developed. This system integrated cytological subclassification and key ultrasonographic parameters identified as independent predictors of malignancy.

Statistical analyses

All statistical analyses were performed using jamovi (v. 2.6.4) and R (v. 4.5.0). Continuous variables were presented as mean ± standard deviation or median and interquartile range; categorical variables were summarized as frequencies and percentages. Comparisons between benign and malignant cases were performed using the chi-square or Fisher's exact test for categorical variables, and the independent samples t-test or Mann-Whitney U test for continuous variables, as appropriate for data distribution. Initially, univariate analyses identified candidate predictors of malignancy. Variables with *p*-values < 0.10 were included into a multivariable binomial logistic regression model with backward elimination to identify independent predictors. Collinearity was assessed via variance inflation factors, and model performance was evaluated using Nagelkerke's R^2. The AUS-MRS was developed from the final regression model using normalized β coefficients. Diagnostic performance of AUS-MRS and other classification systems (i.e., ACR-TIRADS, EU-TIRADS, and K-TIRADS) was assessed with receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC), 95% confidence intervals, and optimal cut-off points (determined by the Youden index) were reported. Pairwise comparison of AUCs utilized the DeLong test for correlated ROC curves. Internal validation was conducted using a split-sample approach, allocating 70% of the dataset for model development and 30% for validation. Sensitivity, specificity, positive and negative likelihood ratios (LR+ and LR−), and diagnostic odds ratios (DOR) were calculated for each scoring system. Pairwise agreement among classification systems was assessed using Cohen's kappa coefficient (κ), interpreted per Landis and Koch criteria. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

A total of 357 patients with a prior cytological diagnosis of AUS who subsequently underwent thyroidectomy were included (**Table 1**). The cohort was predominantly female (n = 293, 82.1%), with a mean age of 46.6 ± 13.8 years (range: 17–87). Final histopathological analysis confirmed malignancy in 156 cases (43.7%) and benign findings in 201 (56.3%). In univariate analysis (**Table 2**), nuclear atypia was significantly more frequent in malignant cases (84.6%) compared with benign cases (49.3%) ($p < 0.001$). Malignant nodules more commonly exhibited high-risk ultrasonographic features, including solid composition (89.1% vs. 58.2%), hypoechogenicity (73.1% vs. 23.4%), irregular margins (45.5% vs. 6.0%), suspicious echogenic foci (54.5% vs. 15.9%), and a taller-than-wide shape (17.9% vs. 0.5%) (all $p < 0.001$). Nodules smaller than 10 mm were significantly more frequent in malignant cases (42.3%) than benign ones (12.9%) ($p < 0.001$). Additionally, a history of repeat biopsy was more common among malignant cases (75.6% vs. 65.2%; $p = 0.033$). All three risk stratification systems (ACR-TIRADS, EU-TIRADS, and K-TIRADS) were significantly associated with malignancy ($p < 0.001$).

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Table 1. Baseline characteristics of the study population

Variable	Cohort (n = 357)
Sex, n (%)	
Female	293 (82.1)
Male	64 (17.9)
Age, mean ± SD (min–max)	46.6 ± 13.8 (17–87)
Surgical diagnosis, n (%)	
Benign	201 (56.3)
Malignant	156 (43.7)
History of re-FNAB, n (%)	249 (69.7)
Cytology	
AUS-N	231 (64.7)
AUS-O	126 (35.3)
Ultrasound features of the nodule	
Size (mm), n (%)	
<10	92 (25.8)
10–20	140 (39.2)
20–40	90 (25.2)
>40	35 (9.8)
Composition, n (%)	
Solid	256 (71.7)
Mixed (solid-cystic)	97 (27.2)
Spongiform	3 (0.8)
Completely cystic	1 (0.3)
Echogenicity, n (%)	
Anechoic	1 (0.3)
Iso-hyperechoic	195 (54.6)
Slightly hypoechoic	136 (38.1)
Markedly hypoechoic	25 (7.0)
Margin, n (%)	
Regular	274 (76.8)
Irregular	83 (23.2)
Extrathyroidal extension, n (%)	3(0.8)
Echogenic focus, n (%)	
None/comet-tail artifact	240 (67.2)
Macrocalcifications	29 (8.1)
Peripheral calcifications	7 (2.0)
Microcalcifications	81 (22.7)
Shape, n (%)	
Wider-than-tall	328 (91.9)
Taller-than-wide	29 (8.1)
Classifications	
ACR-TIRADS, n (%)	
ACR-TIRADS 1	4 (1.1)
ACR-TIRADS 2	64 (17.9)
ACR-TIRADS 3	89 (24.9)
ACR-TIRADS 4	111 (31.1)
ACR-TIRADS 5	89 (24.9)
EU-TIRADS, n (%)	
EU-TIRADS 2	4 (1.1)
EU-TIRADS 3	165 (46.2)
EU-TIRADS 4	53 (14.8)
EU-TIRADS 5	135 (37.8)
K-TIRADS, n (%)	
K-TIRADS 2	5 (1.4)
K-TIRADS 3	167 (46.8)
K-TIRADS 4	86 (24.1)
K-TIRADS 5	99 (27.7)

SD: Standard deviation; re-FNAB: repeat fine-needle aspiration biopsy; AUS-N: atypia of undetermined significance - nuclear atypia; AUS-O: atypia of undetermined significance - other atypia; ACR-TIRADS: American College of Radiology Thyroid Imaging Reporting and Data System; EU-TIRADS: European Thyroid Association Thyroid Imaging Reporting and Data System; K-TIRADS: Korean Thyroid Imaging Reporting and Data System.

Regression analyses

A binomial logistic regression model was developed to identify independent predictors of malignancy (**Table 3**). The overall model was statistically significant ($p < 0.001$) and demonstrated strong discriminative capacity (Nagelkerke $R^2 = 0.607$). No evidence of multicollinearity was observed; all variance inflation factors were below 1.25. After adjustment for other variables, the strongest independent predictor of malignancy was a taller-than-wide shape (OR = 17.95, 95% CI: 2.11–152.46; $p = 0.008$). Presence of suspicious echogenic foci (OR = 9.72, 95% CI: 4.34–21.75; $p < 0.001$) and irregular or extrathyroidal margins (OR = 8.43, 95% CI: 3.70–19.20; $p < 0.001$) were also strongly associated with malignancy. Nuclear atypia (OR = 4.52, 95% CI: 2.29–8.92; $p < 0.001$), solid composition (OR = 2.44, 95% CI: 1.12–5.31; $p = 0.024$), and hypoechogenicity (OR = 2.46, 95% CI: 1.27–4.75; $p = 0.008$) remained significant predictors. Nodule size was not independently associated with malignancy.

Development of the new classification system

A point-based AUS-MRS was developed using β coefficients from the final multivariable logistic regression model (**Table 4**). Each variable's coefficient was divided by the smallest statistically significant β (composition:

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0.89) to calculate a point ratio, which was rounded to assign whole-number weights. The final scoring system included six predictors: taller-than-wide shape (6 points), high-risk echogenic foci (5 points), irregular or extrathyroidal margins (5 points), nuclear atypia (3 points), hypoechogenicity (2 points), and solid composition (2 points). Total risk scores ranged from 0 to 23 points.

Table 2. Univariate analysis of clinical and ultrasound predictors of malignancy in patients with AUS cytology

Variable	Benign (n = 201)	Malignant (n = 156)	p-value
Female sex, n (%)	168 (83.6)	125 (80.1)	0.399
Age (years), mean ± SD	47.6 ± 13.1	45.3 ± 14.6	0.119
History of re-FNAB, n (%)	131 (65.2)	118 (75.6)	0.033
AUS-N, n (%)	99 (49.3)	132 (84.6)	<0.001
Ultrasound features			
Nodule size <10 mm, n (%)	26 (12.9)	66 (42.3)	<0.001
Solid composition, n (%)	117 (58.2)	134 (89.1)	<0.001
Hypoechogenicity, n (%)	47 (23.4)	114 (73.1)	<0.001
Irregular margin, n (%)	12 (6.0)	71 (45.5)	<0.001
Suspicious echogenic foci, n (%)	32 (15.9)	85 (54.5)	<0.001
Taller-than-wide shape, n (%)	1 (0.5)	28 (17.9)	<0.001
ACR-TIRADS, n (%)			
ACR-TIRADS 1	4 (2.0)	0	<0.001
ACR-TIRADS 2	59 (29.4)	5 (3.2)	
ACR-TIRADS 3	77 (38.3)	12 (7.7)	
ACR-TIRADS 4	56 (27.9)	55 (35.3)	
ACR-TIRADS 5	5 (2.5)	84 (53.8)	
EU-TIRADS, n (%)			
EU-TIRADS 2	4 (2.0)	0	<0.001
EU-TIRADS 3	144 (71.6)	21 (13.5)	
EU-TIRADS 4	36 (17.9)	17 (10.9)	
EU-TIRADS 5	17 (8.5)	118 (75.6)	
K-TIRADS, n (%)			
K-TIRADS 2	5 (2.5)	0	<0.001
K-TIRADS 3	144 (71.6)	23 (14.7)	
K-TIRADS 4	40 (19.9)	46 (29.5)	
K-TIRADS 5	12 (6.0)	87 (55.8)	

re-FNAB: Repeat fine-needle aspiration biopsy; AUS-N: atypia of undetermined significance - nuclear atypia; ACR-TIRADS: American College of Radiology Thyroid Imaging Reporting and Data System; EU-TIRADS: European Thyroid Association Thyroid Imaging Reporting and Data System; K-TIRADS: Korean Thyroid Imaging Reporting and Data System.

Table 3. Binomial logistic regression model predicting malignancy

Predictor	β	SE	Z	p-value	OR (95% CI)
Shape (taller-than-wide)	2.89	1.09	2.65	0.008	17.95 (2.11–152.46)
Echogenic focus (peripheral calcification or microcalcifications)	2.27	0.41	5.53	<0.001	9.72 (4.34–21.75)
Margin (irregular/extrathyroidal)	2.13	0.42	5.08	<0.001	8.43 (3.70–19.20)
Nuclear atypia (present)	1.51	0.35	4.35	<0.001	4.52 (2.29–8.92)
Echogenicity (hypoechoic)	0.90	0.34	2.67	0.008	2.46 (1.27–4.75)
Composition (solid)	0.89	0.40	2.26	0.024	2.44 (1.12–5.31)
Intercept	–3.45	0.45	–7.73	<0.001	0.03 (0.01–0.08)

Model summary: Nagelkerke $R^2 = 0.615$; Overall model: $p < 0.001$. β: Beta coefficient; SE: standard error; OR: odds ratio; CI: confidence interval.

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Table 4. Derivation of point-based risk scoring system AUS-MRS from model coefficients

Predictor	β	Point ratio	Assigned points
Shape (taller-than-wide vs. wider-than-tall)	2.89	(2.89/0.89) = 3.25	6
Echogenic focus (high vs. low risk)	2.27	(2.27/0.89) = 2.55	5
Margin (irregular/extrathyroidal extension vs. regular)	2.13	(2.13/0.89) = 2.39	5
Nuclear atypia (present vs. absent)	1.51	(1.51/0.89) = 1.70	3
Echogenicity (hypoechoic vs. non-hypoechoic)	0.90	(0.90/0.89) = 1.01	2
Composition (solid vs. not solid)	0.89	(reference)	2

β: Beta coefficient.

Figure 1 presents the ROC curves comparing the diagnostic performance of AUS-MRS with three established ultrasound-based classification systems (ACR-TIRADS, EU-TIRADS, and K-TIRADS). The AUS-MRS yielded the highest discriminative power, with an AUC of 0.902 (95% CI: 0.870–0.934), followed by ACR-TIRADS (AUC = 0.871, 95% CI: 0.833–0.908), EU-TIRADS (AUC = 0.865, 95% CI: 0.824–0.906), and K-TIRADS (AUC = 0.845, 95% CI: 0.803–0.888). Pairwise comparison of AUCs via the DeLong test indicated that AUS-MRS had significantly higher diagnostic performance than ACR-TIRADS (p = 0.002), EU-TIRADS (p = 0.007), and K-TIRADS (p < 0.001). To minimize overfitting, internal validation using a split-sample approach was performed, with the AUS-MRS showing excellent discriminative performance in the validation cohort (AUC = 0.911, 95% CI: 0.854–0.968).

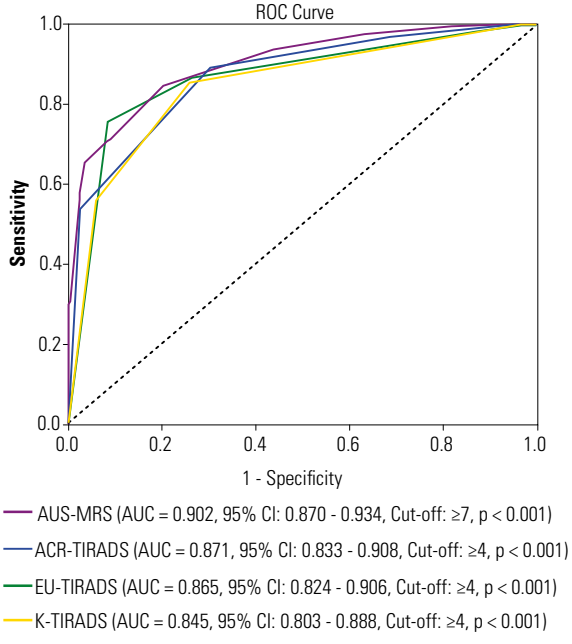


Figure 1. Receiver operating characteristic curve comparing the diagnostic performance of AUS-MRS and three established classification systems: ACR-TIRADS, EU-TIRADS, and K-TIRADS.

Optimal cut-off values for malignancy prediction, determined by the Youden index, were 7 for AUS-MRS and ≥4 for all TIRADS-based systems. All scoring systems demonstrated statistically significant discrimination between benign and malignant nodules (p < 0.001). AUS-MRS was further stratified into two risk categories: low-risk (<7 points) and high-risk (≥7 points). The malignancy rate increased markedly between these categories: 13.0% in the low-risk group and 76.3% in the high-risk group (**Table 5**).

Table 5. AUS Malignancy risk scoring system

Predictor	Score
Shape (taller-than-wide)	6
Echogenic focus (peripheral calcification or microcalcifications)	5
Margin (irregular or extrathyroidal extension)	5
Nuclear atypia (present)	3
Echogenicity (hypoechoic)	2
Composition (solid)	2
Total score range	0–23
Risk categories	**Malignancy rate (%)**
Low (<7 points)	13.0
High (≥7 points)	76.3

Comparison of the classification systems

To compare diagnostic performance and agreement, all classification systems were dichotomized using clinically relevant cut-off values: ≥4 for ACR-, EU-, and K-TIRADS, and ≥7 for AUS-MRS, as established by ROC curve analysis and the Youden index. Pairwise Cohen's kappa analyses demonstrated near-perfect agreement among the three conventional TIRADS systems, with the highest concordance between EU-TIRADS and K-TIRADS (κ = 0.98), followed by

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EU-TIRADS and ACR-TIRADS (κ = 0.92), and ACR-TIRADS and K-TIRADS (κ = 0.90). AUS-MRS showed slightly lower, though still high, levels of agreement with EU-TIRADS (κ = 0.86), K-TIRADS (κ = 0.84), and ACR-TIRADS (κ = 0.84) (**Figure 2**).

Diagnostic performance metrics for AUS-MRS and the three conventional systems are presented in **Table 6**. AUS-MRS yielded the highest diagnostic odds ratio (DOR = 21.8), with balanced sensitivity (84.6%) and specificity (79.6%). ACR-TIRADS achieved the highest sensitivity (89.1%) but lower specificity (69.7%). EU-TIRADS and K-TIRADS provided more balanced performance, with specificities of 73.6% and 74.1%, respectively. When AUS-N was incorporated into each TIRADS system, specificity increased notably in all models (to 85.1% for ACR-TIRADS, 86.6% for EU-TIRADS, and 86.1% for K-TIRADS), albeit with a concurrent reduction in sensitivity. The overall diagnostic odds ratios remained relatively unchanged.

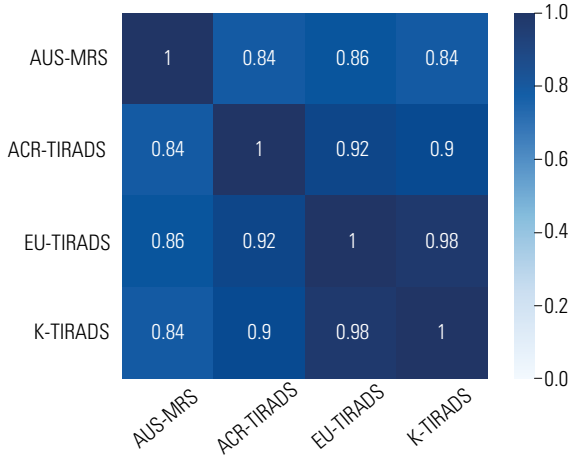


Figure 2. Heatmap illustrating the concordance of risk stratification among the classification systems. The values represent Cohen's kappa coefficients for inter-rater agreement, where a value of 1.00 indicates perfect agreement. The color intensity corresponds to the strength of agreement, with darker blue indicating a higher kappa coefficient.

DISCUSSION

The TBSRTC has provided a standardized framework for the cytological evaluation of thyroid nodules; however, the management of nodules classified as AUS remains a significant clinical challenge. The ambiguity surrounding the AUS category is further compounded by the inconsistency in malignancy rates reported across studies, reflecting the heterogeneous nature of this cytological classification. In clinical settings where molecular testing is not readily available, a repeat FNAB is often recommended for patients with an initial AUS diagnosis (21,22). Nevertheless, approximately 30–40% of these nodules remain within the AUS category even after repeat FNAB, thereby complicating clinical decision-making (23,24).

Recent studies have proposed alternative strategies to improve malignancy risk prediction, such as subclassification of AUS based on nuclear atypia and evaluation of suspicious ultrasonographic features (9–12,15–18,25). Building on these approaches, we hypothesized that integrating cytological subgroups with key sonographic parameters could yield a more accurate method for predicting malignancy in AUS-diagnosed nodules. In this retrospective study, a novel malignancy risk stratification model (i.e., the AUS-MRS) was developed and validated by combining cytological subclassification (AUS-N vs. AUS-O)

Table 6. Diagnostic performance of the classification systems in predicting thyroid nodule malignancy

Classification system	Cutt-off	Total case	Sensitivity (%)	Specificity (%)	LR+	LR-	DOR
AUS-MRS	≥7	173 (48.5)	84.6	79.6	4.15	0.19	21.8
ACR-TIRADS	≥4	200 (56.0)	89.1	69.7	2.94	0.16	18.4
EU-TIRADS	≥4	188 (52.7)	86.5	73.6	3.28	0.18	18.2
K-TIRADS	≥4	185 (51.8)	85.3	74.1	3.29	0.20	16.7
ACR-TIRADS with AUS-N	≥4	149 (41.7)	76.3	85.1	5.12	0.28	18.3
EU-TIRADS with AUS-N	≥4	142 (39.8)	73.7	86.6	5.5	0.30	18.3
K-TIRADS with AUS-N	≥4	141 (39.5)	72.4	86.1	5.2	0.32	16.3

LR+: Positive likelihood ratios; LR–: negative likelihood ratios; DOR: diagnostic odds ratios; AUS-MRS: atypia of undetermined significance malignancy risk scoring; ACR-TIRADS: American College of Radiology Thyroid Imaging Reporting and Data System; EU-TIRADS: European Thyroid Association Thyroid Imaging Reporting and Data System; K-TIRADS: Korean Thyroid Imaging Reporting and Data System; AUS-N: atypia of undetermined significance - nuclear atypia.

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with sonographic findings. The findings indicated that AUS-MRS was associated with higher diagnostic performance than three other conventional ultrasound-based risk classification systems (ACR-TIRADS, EU-TIRADS, and K-TIRADS) for malignancy prediction. This highlights the importance of integrating cytological atypia and ultrasonographic risk features for a more refined and clinically actionable malignancy risk assessment in AUS-diagnosed nodules.

Ultrasonographic features of thyroid nodules are among the most extensively studied predictors of malignancy, particularly in nodules classified as AUS. In this study, the predictive value of key US parameters for malignancy was comprehensively evaluated. Consistent with previous reports, the presence of microcalcifications, hypoechogenicity, and irregular or extrathyroidal margins were identified as independent predictors of malignancy (26–28). Notably, the "taller-than-wide" morphology emerged as one of the most powerful statistical predictors (OR = 17.95), reaffirming the diagnostic significance of this specific sonographic feature. Furthermore, in line with the third edition (2023) of the TBSRTC, the AUS category was subclassified into nuclear atypia (AUS-N) and other atypia (AUS-O), allowing for a more nuanced clinical assessment. The current analysis demonstrated that the AUS-N subgroup was an independent and statistically significant predictor of malignancy (OR = 4.52), corroborating the findings of Valderrabano and cols. (18), who emphasized the diagnostic significance of nuclear atypia in indeterminate thyroid cytology, and Glass and cols. (29), who advocated for AUS specimen subclassification based on the type of atypia.

In the present study, the AUS-MRS, a novel malignancy risk scoring system was developed using independent predictors identified via multivariable logistic regression based on sonographic parameters and cytological subclassification. Integration of nuclear atypia with high-risk ultrasound features allowed for a scoring system that stratified patients into two categories: low risk (<7 points) and high risk (≥7 points). The AUS-MRS demonstrated superior diagnostic accuracy compared to current ultrasound-based classification systems. A distinctive strength of AUS-MRS is the quantitative integration of nuclear atypia as an independent predictor, to which 3 points were assigned. In contrast, current ultrasound-based systems (i.e., ACR-TIRADS, EU-TIRADS, and K-TIRADS) do not include nuclear atypia in their algorithms.

Yoo and cols. (16) reported that categorizing thyroid nodules with AUS cytology using updated US risk stratification systems, particularly when combined with cytological subtyping, may facilitate the determination of the most appropriate management strategy for patients. To address this gap, we conducted additional analyses by incorporating nuclear atypia as a binary variable (present/absent) into each conventional TIRADS system. Although this modification led to a notable increase in specificity (>85%), it resulted in decreased sensitivity, and overall diagnostic accuracy did not significantly improve. These findings suggest that binary incorporation of nuclear atypia offers limited added value, whereas weighted integration (as in the AUS-MRS) provides a more balanced and effective risk prediction when combined with sonographic features. Accordingly, cytological subclassification should not be considered merely as a supplementary factor, but rather a structural component in malignancy risk models designed for AUS-diagnosed nodules.

Clinically, the AUS-MRS should not be viewed as a stand-alone determinant for managing AUS-diagnosed thyroid nodules, but rather as a complementary risk stratification tool to support existing clinical approaches. In particular, in clinical settings where high-quality US evaluation and experienced cytopathological assessment, AUS-MRS may enhance clinical decision-making. By integrating cytological subclassification (especially nuclear atypia) with sonographic risk features, the AUS-MRS can help contextualize malignancy risk within the heterogeneous AUS category. In this framework, a cut-off value of ≥7 identifies a subgroup of AUS nodules with substantially increased malignancy risk, in whom closer follow-up, repeat biopsy, molecular testing, or earlier surgical intervention may be warranted based on clinical context and patient-specific factors. Conversely, lower AUS-MRS scores may favor more conservative management, including surveillance or deferred intervention, particularly in the absence of other high-risk clinical features.

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This study has several limitations. First, the study population consisted exclusively of patients who underwent thyroidectomy following AUS cytology diagnosis, representing a preselected higher-risk cohort. This design introduces verification (work-up) bias, as analyses were limited to surgically treated nodules. As a result, the observed malignancy rate was higher than that reported in unselected or conservatively managed AUS populations, despite noninvasive follicular thyroid neoplasm with papillary-like nuclear features being classified as benign, and diagnostic performance metrics may have consequently been overestimated. Therefore, our findings should be interpreted in the context of surgically treated AUS nodules and may not be directly generalizable to all AUS cases. Second, the single-center and retrospective design of the study introduced potential selection and observation bias. Additionally, the AUS-MRS was not directly compared with molecular diagnostic methods, thus precluding a direct performance comparison.

Despite all US examinations being carried out by experienced endocrinologists, it is inherently operator-dependent and subjective. Consequently, the absence of interobserver agreement analysis for sonographic features constitutes a further significant limitation. Moreover, some predictors demonstrated wide confidence intervals, likely reflecting the low prevalence of certain high-risk sonographic features rather than model instability, and should be interpreted with caution. The diagnosis of AUS itself remains a subjective and interpretative category, with known interobserver variability among cytopathologists. Cytological specimens in this study were originally diagnosed by different pathologists and subsequently re-evaluated by an experienced cytopathologist who was blinded to the histopathological outcomes using the updated 2023 TBSRTC criteria. While this second assessment improved diagnostic consistency and confirmation of AUS categorization, it could not entirely eliminate pathologist-dependent variability. Consequently, the lack of full standardization—an inherent feature of AUS-based and ultrasound-driven studies—should be considered when applying and interpreting the AUS-MRS in routine clinical practice. More consistent application may be achieved in clinical settings with high-quality ultrasonography and experienced cytopathology, albeit reproducibility across operators and centers should not be overlooked. Although internal validation was performed, the absence of external validation is an additional limitation of this study.

The generalizability, reproducibility, and robustness of AUS-MRS across different populations and clinical settings remain to be established. Nevertheless, this study introduces the first scoring model integrating both cytological subclassification (particularly nuclear atypia) and essential sonographic features for malignancy risk stratification in AUS-diagnosed thyroid nodules. In this respect, the AUS-MRS is an innovative and comprehensive tool with the potential to enhance clinical decision-making. To validate and generalize our findings, prospective multicenter studies involving more diverse populations are warranted.

In conclusion, the AUS-MRS system was shown to improve malignancy prediction by integrating nuclear atypia with high-risk ultrasonographic features and was associated with higher diagnostic performance than existing TIRADS classification systems. Hence, this scoring model has the potential to serve as a valuable instrument to facilitate clinical decision-making in patients diagnosed with AUS. Nevertheless, external validation in larger, prospective, multicenter cohorts is necessary to confirm its generalizability and clinical utility.

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original

Evolution of thyroid fine-needle aspiration biopsy performance over 15 years: implications for risk stratification in clinical practice

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ABSTRACT

Objective: We aimed to evaluate temporal changes in the diagnostic performance of thyroid fine-needle aspiration biopsy (FNAB) over a 15-year period in a single-center, surgically confirmed cohort. **Subjects and methods:** We retrospectively analyzed thyroid FNABs performed between 2009 and 2023, dividing the data into two intervals (Group 1: 2009–2014; Group 2: 2015–2023). All specimens were interpreted by the same two experienced cytopathologists. Sensitivity, specificity, and Youden's J were calculated at four diagnostic thresholds (≥AUS/FLUS, ≥FN/SFN, ≥SM, and malignant only), and level-specific likelihood ratios (LRs) were compared between cohorts. Overall diagnostic discrimination was assessed with receiver operating characteristic (ROC) curves and compared by DeLong's test. **Results:** The study included 1,241 nodules; after excluding non-diagnostic cytology, 601 nodules in Group 1 and 553 in Group 2 were available for diagnostic performance analysis. At the ≥AUS/FLUS cutoff, sensitivity increased from 85.6% to 94.8%, while specificity declined from 58.4% to 33.0% (both $p<0.01$). Specificity at higher thresholds remained similar or increased in Group 2. The area under the ROC curve (AUC) was 0.799 for Group 1 and 0.831 for Group 2 ($p = 0.24$). Of the five Bethesda LRs, only that for AUS/FLUS changed significantly (1.24 vs. 0.37; $p<0.01$). **Conclusion:** The overall discriminative ability of the Bethesda System remained stable over 15 years. The decrease in the AUS/FLUS LR reflects a spectrum effect driven by more selective surgical referral patterns and should be interpreted as a change in cohort composition rather than diminished test performance.

Keywords: Biopsy; fine-needle; thyroid nodule; Bethesda system; diagnostic accuracy; cytopathology

INTRODUCTION

Thyroid nodules are among the most frequently encountered endocrine pathologies, and their prevalence is rising with the increased use of imaging modalities (1,2). Fine-needle aspiration biopsy (FNAB) remains the cornerstone of initial risk stratification and management in patients with sonographically suspicious thyroid nodules (1,3). The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) provides a standardized framework for interpreting FNAB results and estimating the risk of malignancy within specific diagnostic categories (4,5)

However, discrepancies between cytological findings and final histopathological diagnoses persist, mainly due to sampling errors and interpretive challenges (1,6). These issues underscore the importance of institutional audits to assess the diagnostic accuracy and clinical utility of FNAB in real-world settings. Such audits are particularly valuable for evaluating temporal trends and the applicability of cytological classifications to local populations (7).

Previously, we conducted a study at our institution evaluating the concordance between FNAB cytology and final histopathology in patients who underwent thyroid surgery from 2009 to 2014, with additional analysis of the impact of nodule size on

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diagnostic accuracy (6). Since then, advances in biopsy techniques, ultrasonographic evaluation, and cytopathological reporting have been introduced (8,9). Therefore, in the present study, we aimed to evaluate the diagnostic performance of FNAB in patients who underwent thyroid surgery from 2015 to 2023, compare these results with our previously published data (2009–2014), and assess diagnostic performance using multiple cut-offs, receiver operating characteristic (ROC)-based discrimination, and prevalence-independent likelihood ratios (LRs).

SUBJECTS AND METHODS

This retrospective study was conducted in the Department of Endocrinology and Metabolism. The study included all patients who underwent thyroid FNAB between January 1, 2015, and March 31, 2023, and subsequently underwent thyroid surgery with a final histopathological diagnosis available. The primary aim was to evaluate the diagnostic performance of FNAB and to compare the results with those from our previously published dataset covering the years 2009–2014, thereby assessing whether diagnostic performance changed over time with increasing institutional experience (6). All FNAB and surgical pathology specimens were consistently examined and reported by the same two pathologists throughout the study period. Patients who underwent FNAB and thyroid surgery between 2009 and 2014 were designated as Group 1, while those between 2015 and 2023 were assigned to Group 2. A comparative analysis of the data from both groups was subsequently performed to assess potential changes in diagnostic performance.

Patients were eligible for inclusion if they had undergone thyroid FNAB during the specified time period with available postoperative histopathological data. Patients who had incidental thyroid malignancy detected in non-biopsied nodules were excluded. Additionally, cases with non-diagnostic FNAB results were excluded from sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy analyses. Cases with missing data for specific variables were excluded from the corresponding analyses (variable-specific complete-case analysis).

For each patient, the following data were recorded: age, sex, thyroid function test results, thyroid autoantibody status, ultrasound-measured maximum nodule diameter, FNAB results, surgical indications, and final postoperative histopathological findings. FNAB results were classified according to the TBSRTC into six categories: nondiagnostic, benign, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), follicular neoplasm/suspicious for follicular neoplasm (FN/SFN), suspicious for malignancy (SM), and malignant. It should be noted that the AUS/FLUS terminology reflects the 2017 Bethesda System, which was in use during the study period; the 2023 revision, which eliminates the FLUS description, was introduced after completion of data collection (4,5). No molecular or immunocytochemical ancillary tests were performed in either time period, as such methods were not incorporated into routine practice at our institution during the study period.

In cases where multiple nodules were biopsied, each nodule was evaluated separately. If multiple FNABs were performed at different times for the same nodule, the highest-risk cytological category was used for classification. Postoperative specimens were classified as benign or malignant. For malignant cases, histological subtypes were recorded. Patients with incidental thyroid cancer in a nodule other than the one biopsied were excluded from the analysis.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 23 (IBM Corp., USA), with additional calculations carried out in Python (version 3.11). Categorical variables are presented as frequencies and percentages, while continuous variables are presented as means with standard deviations. Group comparisons of categorical variables were performed using the chi-square or Fisher's exact test, as appropriate. Continuous variables were compared using the independent-samples t-test.

To evaluate the diagnostic performance of FNAB, sensitivity, specificity, PPV, NPV, and overall accuracy were calculated using the histopathological reference standard. In the primary analysis, an FNAB result was considered positive if the cytological diagnosis was

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indeterminate (AUS/FLUS, FN/SFN, or SM) or malignant, and negative if benign; non-diagnostic specimens were excluded from the calculation of diagnostic indices. True positive (TP) cases were those with a positive FNAB and malignant final pathology; true negative (TN) cases were benign on both FNAB and final pathology; false positive (FP) cases had a positive FNAB but benign final pathology; and false negative (FN) cases had a benign FNAB but malignant final pathology. Sensitivity was calculated as TP / (TP + FN), specificity as TN / (TN + FP), PPV as TP / (TP + FP), NPV as TN / (TN + FN), and accuracy as (TP + TN) / (TP + TN + FP + FN). Wilson score 95% confidence intervals (CIs) were used for proportions (sensitivity, specificity, PPV, NPV, and accuracy). For LR, 95% CIs were derived from the normal approximation on the logarithmic scale, using the variance formula of Simel and cols. (10), a method recently adopted for standardizing biomarker interpretation in clinical cohorts (11). Diagnostic performance metrics for the 2015–2023 cohort (Group 2) were compared with those from the 2009–2014 cohort (Group 1) using two-proportion z-tests.

The analysis utilized four distinct cut-off values, as the choice of threshold for defining a positive FNAB result substantially influences the diagnostic performance of the test. The thresholds were AUS/FLUS or higher (Cut-off 1 (CO1), the conventional cut-off used in our primary analysis), FN/SFN or higher (CO2), suspicious for malignancy or higher (CO3), and malignant only (CO4). For each threshold and within each cohort, sensitivity, specificity, and Youden's J index (calculated as sensitivity + specificity − 1) were derived, again with Wilson score 95% CIs, and the two cohorts were compared using two-proportion z-tests.

To assess the overall discriminative ability of the Bethesda System independent of any single cut-off, receiver operating characteristic (ROC) curve analysis was performed for each cohort, with the Bethesda category treated as an ordinal test variable and the final histopathological diagnosis as the reference standard. Areas under the curve (AUCs) and their nonparametric standard errors were obtained in SPSS (12). The two AUCs were compared using the method described by DeLong and cols., implemented in Python; the resulting test statistic and *p*-value were verified against a two-sample z-test using the SPSS-derived nonparametric variance estimates (13).

Because the prevalence of malignancy differed substantially between the two cohorts, predictive values were not directly comparable across periods; therefore, prevalence-independent measures were necessary to assess changes in test characteristics. We calculated level-specific LRs for each Bethesda category in both cohorts as LR = (a/M)/(b/N), where 'a' is the number of malignant cases in the category, 'M' is the total number of malignant cases, 'b' is the number of benign cases in the category, and 'N' is the total number of benign cases. The variance of the natural logarithm of LR was estimated using the formula proposed by Simel and cols. (10): Var(ln LR) = 1/a − 1/M + 1/b − 1/N . Between-cohort differences in category-specific LRs were tested using a z-test on the difference of ln(LR), with the standard error calculated as the square root of the sum of the two variances. Although the categories within a single cohort are not statistically independent, between-cohort comparisons of level-specific LRs are conventionally conducted using this approach. A two-sided *p*-value of less than 0.05 was considered statistically significant for all analyses.

In the size-stratified analysis, only sensitivity and specificity were compared between cohorts, as positive and negative predictive values are influenced by disease prevalence, which differed substantially between subgroups, and because subgroup sample sizes were insufficient to support stable, level-specific likelihood ratio estimation.

This study was prepared in accordance with the STROBE guidelines, and the completed checklist is provided in the Supplementary Material (14,15).

RESULTS

The study included 1,241 nodules from patients who underwent thyroidectomy following FNAB, with 648 nodules in Group 1 and 593 in Group 2. The overall mean age was 49.7 ± 13.1 years, with a significant difference between Group 1 and Group 2 (48.2 ± 12.5 vs. 51.3 ± 13.6, $p < 0.01$). There was a female predominance overall (76.1%), with a modest but significant change in sex distribution between groups over time

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(p = 0.01). The proportion of nodules < 4 cm was significantly higher in Group 2 than in Group 1 (89.8% vs. 67.0%; p < 0.01). Similarly, the proportion of patients with positive thyroid autoantibodies was higher in Group 2 than in Group 1 (27.7% vs. 16.0%; p < 0.01). Normal thyroid-stimulating hormone (TSH) levels were more frequently observed in Group 2 (84.1% vs. 62.5%, p < 0.01) (**Table 1**).

The most common overall indication for thyroidectomy was cytology SM (25.6%), followed by pressure symptoms (17.0%), AUS/FLUS (15.6%), and malignant FNAB (12.0%). These indications varied markedly between the two time periods. Notably, AUS/FLUS was a more frequent indication in Group 2 (29.2%) compared to Group 1 (3.2%, p < 0.01). Conversely, follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) was a more common indication in Group 1 (17.3% vs. 2.5%, p < 0.01). Hyperthyroidism as an indication for thyroidectomy was also more prevalent in Group 1 than in Group 2 (20.4% vs. 1.9%, p < 0.01). Surgery for cosmetic reasons or during concomitant parathyroid procedures was rare (**Figure 1**).

Histopathological examination revealed benign lesions in 802 nodules (64.6%) and malignant lesions in 439 (35.4%). Papillary thyroid carcinoma was the predominant malignancy, accounting for 91.3% of malignant diagnoses. Other subtypes included medullary carcinoma (3.7%), follicular carcinoma (3.0%), anaplastic carcinoma (0.7%), and metastatic lesions (0.7%). The proportion of malignancy differed significantly between groups, rising from 22.1% in Group 1 to 49.9% in Group 2 (p < 0.01) (**Table 2**).

FNAB results were classified according to the Bethesda System. Malignancy rates by cytological category differed significantly between time periods. In the benign category, malignancy was observed less frequently in Group 1 than in Group 2 (6.5% vs. 14.7%, p = 0.01). For the FN/SFN category, the malignancy rate was lower in Group 1 than in Group 2 (15.8% vs. 42.9%, p = 0.03). The SM category showed a significantly lower malignancy rate in Group 1 than in Group 2 (44.9% vs. 71.2%, p < 0.01). No significant differences were observed in the AUS/FLUS, nondiagnostic, or malignant categories (**Table 3**).

Level-specific LRs were calculated for each Bethesda category. The LR for the AUS/FLUS category was 1.24 (95% CI, 0.54–2.88) in Group 1 and 0.37 (95% CI, 0.28–0.50) in Group 2 (Z = 2.65, p < 0.01). For other categories, LRs were 0.25 in Group 1 and 0.16 in Group 2 for benign (p = 0.19), 0.67 and 0.69 for FN/SFN (p = 0.96), 2.90 and 2.25 for SM (p = 0.22), and 30.79 and 19.00 for malignant (p = 0.52) (**Table 4**).

Table 1. Baseline patient characteristics of the study cohort

Variable	Total (n = 1241)	Group 1 (n = 648)	Group 2 (n = 593)	p
Age (year) mean (±SD)	49.7 (±13.1)	48.2±12.5	51.3 (±13.6)	< 0.01
Sex, n (%)				
Female	944 (76.1)	511 (78.9)	433 (73.0)	0.01
Male	297 (23.9)	137 (21.1)	160 (27.0)	
Nodule diameter, n (%)				
<4 cm	964 (77.9)	434 (67.0)	530 (89.8)	< 0.01
≥ 4 cm	274 (22.1)	214 (33.0)	60 (10.2)	
Thyroid antibody, n (%)				
Negative	973 (78.4)	544 (84.0)	429 (72.3)	< 0.01
Positive	268 (21.6)	104 (16.0)	164 (27.7)	
Thyroid function, n (%)				
Normal TSH	904 (72.8)	405 (62.5)	499 (84.1)	< 0.01
Low TSH	215 (17.3)	164 (25.3)	51 (8.6)	
High TSH	122 (9.8)	79 (12.2)	43 (7.3)	

p-Values show the comparison between Groups 1 and 2. The largest nodule diameter data was missing for three nodules, all of which were in Group 2.
TSH: thyroid stimulating hormone.

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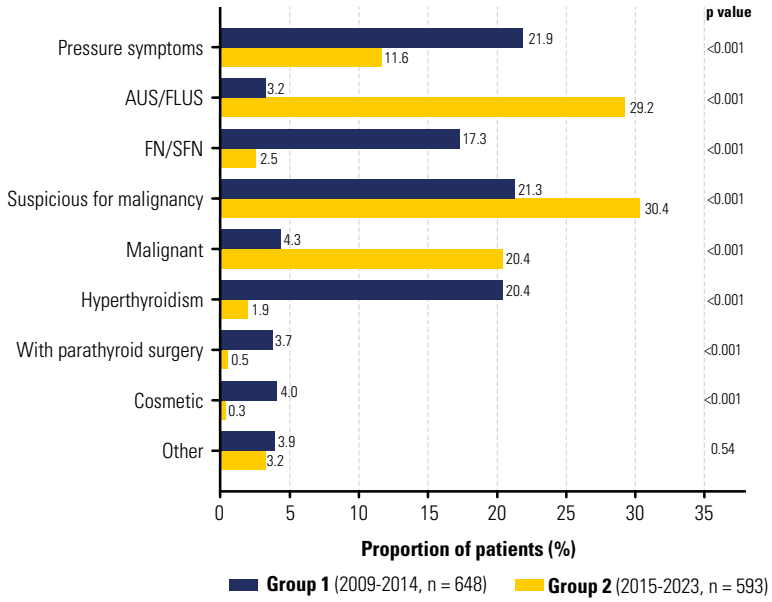


Figure 1. Indications for thyroidectomy in the study population by period. Bars represent the percentage of patients in each cohort for whom the listed indication was the primary reason for surgery. Group 1 (2009–2014) is shown in white with diagonal hatching, and Group 2 (2015–2023) in solid black. *p*-values represent two-proportion z-tests comparing the two periods. AUS/FLUS: Atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN: follicular neoplasm/suspicious for follicular neoplasm.

Table 2. Final histopathological diagnoses and distribution of malignant subtypes

Pathology	Total (n = 1241)	Group 1 (n = 648)	Group 2 (n = 593)	*p*
Benign, n (%)	802 (64.6)	505 (77.9)	297 (50.1)	< 0.01
Malignant, n (%)	439 (35.4)	143 (22.1)	296 (49.9)	< 0.01
PTC	401 (91.3)	126 (88.1)	275 (92.9)	0.01*
FTC	13 (3)	8 (5.6)	5(1.7)	
MTC	16 (3.6)	5 (3.5)	11 (3.7)	
Anaplastic	3 (0.7)	1 (0.7)	2 (0.7)	
Metastasis	3 (0.7)	3 (2.1)	0 (0.0)	
Unknown	3 (0.7)	-	3 (1.0)	

p-Values indicate the comparison between Groups 1 and 2. Histopathological subtype information for malignant nodules was unavailable for three patients in Group 2. Benign/malignant comparison: Pearson χ^2 test.

PTC: papillary thyroid carcinoma; FTC: follicular thyroid carcinoma; MTC: medullary thyroid carcinoma. *Fisher-Freeman-Halton exact test for subtype distribution (χ^2 assumptions not met; 5/10 cells with expected count <5).

Table 3. Bethesda category-specific malignancy rates across two time period

Variable	Total (n = 1241)		Group 1 (n = 648)		Group 2 (n = 593)		*p*
	FNAB result	MR	FNAB result	MR	FNAB result	MR	
Non-diagnostic, n (%)	87 (7.0)	18 (20.7)	47 (7.3)	11 (23.4)	40 (6.7)	7 (17.5)	0.68
Benign, n (%)	395 (31.8)	34 (8.6)	293 (45.2)	19 (6.5)	102 (17.2)	15 (14.7)	0.01
AUS/FLUS, n (%)	192 (15.5)	55 (28.6)	27 (4.2)	7 (25.9)	165 (27.8)	48 (29.1)	0.91
FN/SFN, n (%)	128 (10.3)	24 (18.8)	114 (17.6)	18 (15.8)	14 (2.4)	6 (42.9)	0.03
SM, n (%)	301 (24.3)	178 (59.1)	138 (21.3)	62 (44.9)	163 (27.5)	116 (71.2)	<0.01
Malign, n (%)	138 (11.1)	130 (94.2)	29 (4.5)	26 (89.7)	109 (18.4)	104 (95.4)	0.46

p-Values show the comparison of malignancy rates between Groups 1 and 2.

AUS: Atypia of undetermined significance; FLUS: follicular lesion of undetermined significance; MR: malignancy rate; FN: follicular neoplasm; SFN: suspicious for follicular neoplasm; SM: suspicious for malignancy.

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Table 4. Level-specific likelihood ratios for each Bethesda category, by cohort

Bethesda category	Group 1 (n = 601)		Group 2 (n = 553)		Z	*p*
	LR	95% CI	LR	95% CI		
Benign	0.25	0.16–0.38	0.16	0.09–0.27	+1.31	0.192
AUS/FLUS	1.24	0.54–2.88	0.37	0.28–0.50	+2.65	<0.01
FN/SFN	0.67	0.42–1.06	0.69	0.24–1.95	-0.05	0.96
SM	2.90	2.20–3.81	2.25	1.68–3.03	+1.22	0.22
Malignant	30.79	9.47–100.15	19.00	7.87–45.89	+0.64	0.52

Likelihood ratios were calculated from the category-specific cancer and non-cancer counts in **Table 3**, using the formula LR = (a/M)/(b/N), where a is the number of malignant cases in the category, M is the total number of malignant cases, b is the number of benign cases in the category, and N is the total number of benign cases. The variance of ln(LR) was estimated as 1/a – 1/M + 1/b – 1/N. Between-cohort comparisons were conducted using a z-test on the difference of ln(LR).

LR: Likelihood ratio; CI: confidence interval; AUS/FLUS: atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN: follicular neoplasm/suspicious for follicular neoplasm; SM: suspicious for malignancy.

When FNAB diagnostic performance was assessed using the conventional threshold of AUS/FLUS or higher as a positive result (CO1), sensitivity increased significantly from Group 1 to Group 2 (85.6% vs. 94.8%; $p < 0.01$), whereas specificity declined (58.4% vs. 33.0%; $p < 0.01$). At CO2, sensitivity was 80.3% in Group 1 and 78.2% in Group 2 ($p = 0.62$), and specificity was 62.7% and 77.3%, respectively ($p < 0.01$). At CO3, sensitivity was 66.7% in Group 1 and 76.1% in Group 2 ($p = 0.04$), while specificity was 83.2% and 80.3% ($p = 0.33$). At CO4, sensitivity was 19.7% in Group 1 and 36.0% in Group 2 ($p < 0.01$), with specificity of 99.4% and 98.1% ($p = 0.12$). Youden's J index was 0.440 in Group 1 and 0.278 in Group 2 at CO1; 0.430 and 0.555 at CO2; 0.498 and 0.564 at CO3; and 0.191 and 0.341 at CO4 (**Table 5**).

Table 5. Multi-cutoff diagnostic performance of thyroid FNAB across two periods

CO	Group	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Youden's J	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, %
CO1	1	85.6 (78.6–90.6)	58.4 (53.9–62.8)	0.440	36.7 (31.5–42.2)	93.5 (90.1–95.8)	64.4
	2	94.8 (91.6–96.8)	33.0 (27.6–38.8)	0.278	60.8 (56.2–65.2)	85.3 (77.1–90.9)	65.3
	p-value	<0.01	<0.01	—	<0.01	0.01	0.75
CO2	1	80.3 (72.7–86.2)	62.7 (58.2–66.9)	0.430	37.7 (32.3–43.5)	91.9 (88.4–94.4)	66.6
	2	78.2 (73.1–82.6)	77.3 (71.8–81.9)	0.555	79.0 (73.9–83.3)	76.4 (71.0–81.1)	77.8
	p-value	0.62	<0.01	—	<0.01	<0.01	<0.01
CO3	1	66.7 (58.3–74.1)	83.2 (79.5–86.3)	0.498	52.7 (45.1–60.1)	89.9 (86.7–92.4)	79.5
	2	76.1 (70.9–80.7)	80.3 (75.1–84.7)	0.564	80.9 (75.8–85.1)	75.4 (70.1–80.1)	78.1
	p-value	0.04	0.33	—	<0.01	<0.01	0.55
CO4	1	19.7 (13.8–27.3)	99.4 (98.1–99.8)	0.191	89.7 (73.6–96.4)	81.5 (78.1–84.4)	81.9
	2	36.0 (30.7–41.7)	98.1 (95.6–99.2)	0.341	95.4 (89.7–98.0)	58.3 (53.7–62.8)	65.6
	p-value	<0.01	0.12	—	0.23	<0.01	<0.01

Diagnostic performance metrics were calculated after excluding nodules with non-diagnostic cytology. Cut-off definitions: CO1, Bethesda category III and above classified as positive; CO2, Bethesda category IV and above classified as positive; CO3, Bethesda category V and above classified as positive; CO4, Bethesda category VI classified as positive. Ninety-five percent confidence intervals were computed using the Wilson score method. *p*-values represent two-proportion z-tests comparing Group 1 with Group 2.

CO: Cut-off; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval; AUS: atypia of undetermined significance; FLUS: follicular lesion of undetermined significance; FN: follicular neoplasm; SFN: suspicious for follicular neoplasm; SM: suspicious for malignancy.

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ROC curve analysis was performed for each cohort using the Bethesda category as an ordinal test variable. The AUC was 0.799 (95% CI, 0.755–0.842) in Group 1 and 0.831 (95% CI, 0.799–0.863) in Group 2. The difference of 0.032 was not statistically significant (DeLong test, Z = −1.18, p = 0.24) (**Figure 2**).

Stratification by nodule size was performed using CO1, with comparisons limited to sensitivity and specificity. For nodules ≥4 cm, sensitivity was 75.9% in Group 1 and 81.8% in Group 2 (p = 0.60), while specificity was 75.2% and 56.2% (p = 0.02). For nodules <4 cm, sensitivity was 88.3% in Group 1 and 95.9% in Group 2 (p < 0.01), while specificity was 49.3% and 30.1% (p < 0.01). Youden's J index was 0.510 in Group 1 and 0.381 in Group 2 for nodules ≥4 cm, and 0.377 and 0.260 for nodules <4 cm (**Table 6**).

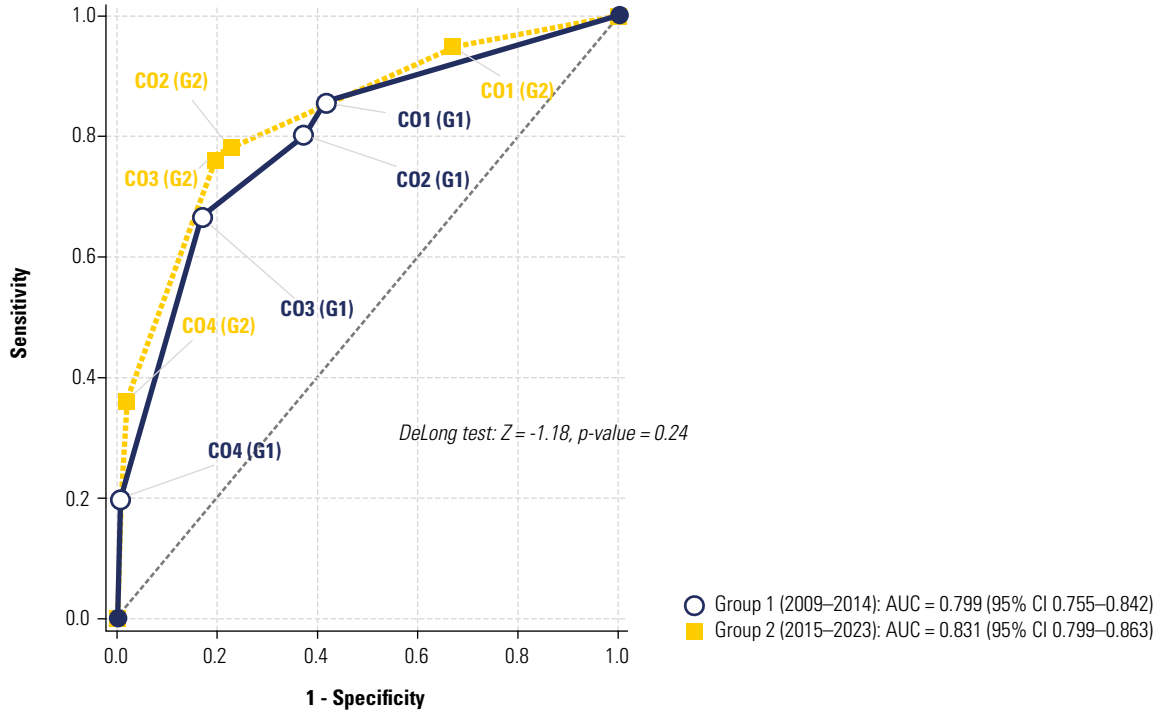


Figure 2. Receiver operating characteristic curves comparing the performance of thyroid fine-needle aspiration biopsy (FNAB) in Group 1 (2009–2014) and Group 2 (2015–2023). The Bethesda category was used as an ordinal test variable, with final histopathology serving as the reference standard. Each vertex along the curves corresponds to one of four cut-off thresholds for defining a positive FNAB result: CO1, ≥AUS/FLUS; CO2, ≥FN/SFN; CO3, ≥SM; CO4, malignant only. Areas under the curve (AUC) and 95% confidence intervals are provided in the legend. The diagonal dotted line represents an uninformative test. A comparison of the two AUCs using the DeLong test for two independent samples revealed no statistically significant difference (Z = −1.18, p = 0.238), indicating that the overall discriminative performance of the Bethesda System remained stable between the two periods.

Table 6. Temporal comparison of thyroid FNAB diagnostic performance by nodule size

Variable	Nodule size ≥4 cm			Nodule size <4 cm		
	Group 1 (n = 194)	Group 2 (n = 54)	p	Group 1 (n = 407)	Group 2 (n = 496)	p
Sensitivity* (95% CI)	75.9 (57.9–87.8)	81.8 (61.5–92.7)	0.60	88.3 (80.7–93.2)	95.9 (92.8–97.7)	<0.01
Specificity* (95% CI)	75.2 (68.0–81.1)	56.2 (39.3–71.8)	0.02	49.3 (43.8–54.9)	30.1 (24.6–36.4)	<0.01
Youden's J	0.510	0.381	-	0.377	0.260	-

Diagnostic indices in this stratified analysis were calculated using the conventional ≥AUS/FLUS cut-off only, after excluding nodules with non-diagnostic cytology. Nodule diameter was missing for three patients in Group 2, who were therefore excluded from this stratified analysis. 95% Confidence intervals were computed using the Wilson score method. p-values represent two-proportion z-tests comparing Group 1 with Group 2 for sensitivity and specificity. Youden's J index is provided as a descriptive summary of the sensitivity–specificity balance (calculated as sensitivity + specificity - 1) and is not subjected to formal statistical comparison between cohorts. Positive predictive value, negative predictive value, and accuracy were excluded from this stratified analysis because they are influenced by disease prevalence, which differed substantially between subgroups. Subgroup sample sizes, particularly the 54 cases of nodules ≥4 cm in Group 2, were insufficient to support stable level-specific likelihood ratio estimation or multi-cutoff ROC-based analysis. CI: confidence interval.

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DISCUSSION

In this large, 15-year retrospective cohort study, the diagnostic performance of thyroid FNAB exhibited a temporal shift in the sensitivity-specificity balance, rather than a uniform improvement in overall accuracy. At the conventional CO1 threshold, sensitivity increased from 85.6% to 94.8%, while specificity decreased from 58.4% to 33.0%. However, multi-cutoff analysis revealed that this loss of specificity was limited to the CO1 threshold; at higher cutoffs (CO2, CO3, or CO4), specificity in the recent cohort was comparable to, or higher than, that in the earlier period. This shift was also evident in the level-specific LR for the AUS/FLUS category, which declined from 1.24 to 0.37 ($p < 0.01$). Because likelihood ratios are mathematically independent of disease prevalence, this finding cannot be attributed to the increase in the overall malignancy rate from 22.1% to 49.9%. A spectrum effect is a more plausible explanation. After the 2015 American Thyroid Association (ATA) guidelines (16), FNAB and surgical referral practices shifted from a size-based approach to one based on sonographic risk stratification. Consequently, the case mix of benign and malignant nodules reaching surgery shifted toward higher-suspicion lesions. As a result, the category-specific conditional probabilities that define the LR changed, even though cytopathological interpretations continued to be performed by the same two pathologists throughout the study period.

The LR estimate for Group 1 was also statistically unstable; its 95% CI (0.54–2.88) crossed unity, indicating that the point estimate of 1.24 was not well established. The Group 2 CI (0.28–0.50) was considerably narrower, situating AUS/FLUS within the low-risk category for this surgical cohort. Clinically, in a cohort with an initial probability of malignancy of approximately 50%, an AUS/FLUS LR produces a post-test probability of 29.1%, below the cohort average. This demonstrates that the interpretation of an indeterminate cytological category depends on the pre-test probability of the population in which it is applied. The LRs reported in this study should, therefore, be considered as characteristics of this surgically confirmed cohort rather than as population-level estimates.

Moreover, the area under the ROC curve remained stable (0.799 vs. 0.831, $p = 0.24$), indicating that the overall discriminative ability of the Bethesda System did not change significantly. These findings suggest that the divergence in metrics observed at the conventional CO1 threshold reflects a shift in the operating characteristics for clinical decision-making rather than a global change in diagnostic power. The substantial increase in PPV from 36.7% to 60.8% should be interpreted with caution, as it was driven by a more than twofold increase in malignancy prevalence within this surgically confirmed cohort. This finding aligns with recent meta-analyses in high-resource settings (17) and underscores the importance of evaluating FNAB performance across multiple thresholds and in various local prevalence contexts. Importantly, the LRs reported here should be regarded as characterizing the present surgically confirmed population, not as updated population-level estimates for the Bethesda System as a whole.

The shift in cohort composition that underlies these findings was driven by significant changes in surgical indications over time. There was a decrease in surgeries for hyperthyroidism and benign nodular disease, coupled with an increase in procedures prompted by cytological suspicion, such as AUS/FLUS, SM, and malignant cytology (Figure 1). This likely reflects increasing reliance on FNAB-based decision-making and more selective surgical referral, has led to a more "malignancy-enriched" surgical cohort. This may account for the concurrent rise in the proportion of malignant nodules among resected specimens observed in this study and other recent reports (18–20).

Malignancy rates across Bethesda categories showed distinct temporal shifts. The most pronounced change was seen in the FN/SFN category, where the malignancy rate nearly tripled from 15.8% to 42.9%, exceeding the traditionally expected range (21–24). A similar trend was observed in the SM category, with an increase from 44.9% to 71.2%, consistent with existing literature (1,5,22). These increases are likely attributable to more selective surgical referral, rather than to fundamental changes in cytopathological interpretation. Supporting this, level-specific LRs for the benign, FN/SFN, SM, and malignant categories

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did not differ significantly between periods (Table 4), suggesting that the inherent discriminative value of these categories remained stable despite apparent shifts in malignancy rates. The AUS/FLUS malignancy rate remained stable between periods, though it exceeded the classically cited range in both cohorts (1,5). Notably, the 2016 reclassification of non-invasive encapsulated follicular variant of papillary thyroid carcinoma as noninvasive follicular thyroid neoplasm with papillary-like nuclear features may have affected historical malignancy estimates, particularly in the earlier cohort, where such lesions would have been classified as malignant (25).

The elevated malignancy rate in Bethesda II is also noteworthy. Both cohorts exceeded the 0–3% range reported in the 2017 TBSRTC (5); however, only surgically treated nodules were included in this study, altering the interpretation. In routine practice, Bethesda II nodules rarely undergo surgery; when they do, it is usually because of suspicious sonographic features, compressive symptoms, cosmetic concerns, or a coexisting nodule requiring surgery. Thus, the observed malignancy rate reflects a selection effect; only Bethesda II nodules with additional clinical or sonographic concerns are surgically excised. Two further factors may contribute to this finding. First, exact nodule-level matching in multinodular glands is challenging, so a small proportion of cases may represent incidental carcinoma in adjacent nodules. Second, tertiary referral centers see a higher pre-test probability of malignancy than community settings (26), thereby increasing malignancy rates in all cytological categories, including benign.

Several factors may underlie the differences observed between the two cohorts. Cytopathology was consistently performed by the same two pathologists throughout the study, limiting potential interobserver variability. Nevertheless, clinical practice evolved, with higher-resolution ultrasound becoming available in the later period. FNABs were performed, throughout, by endocrinology fellows rotating during their three-year training, so operator experience varied within—though not systematically between—the two cohorts. The decline in specificity was confined to CO1; at CO2, CO3, and CO4 thresholds, specificity was higher or unchanged. Together with the absence of a significant difference in AUC, these results suggest that the overall discriminatory power of the Bethesda System to distinguish malignant from benign nodules was preserved. Similarly, level-specific LRs were unchanged across four of the five Bethesda categories (Table 4). The exception, AUS/FLUS, is better explained by the shift in cohort composition than by a change in test characteristics.

Stratification by nodule size revealed a consistent pattern. In both subgroups, specificity declined and Youden's J index decreased at the CO1 threshold, consistent with the unstratified analysis (Table 6), though the small number of nodules ≥4 cm in Group 2 (n = 54) limits the strength of any conclusions drawn from this subgroup.

Our study has several strengths. Conducted over 15 years at a single institution, all FNABs and surgical specimens were interpreted by the same two experienced cytopathologists; this design is uncommon in the longitudinal FNAB literature. Previous studies comparing pre- and post-Bethesda periods typically involved multiple observers or shorter follow-up intervals (27–29), complicating temporal comparison. The present study also combined three analytic approaches that are rarely used together in this context: multi-cutoff evaluation across four thresholds, ROC-based comparison of discriminative performance between periods, and prevalence-independent level-specific LRs. This comprehensive analysis offers a more nuanced perspective on changes in FNAB performance over time than single-threshold sensitivity–specificity analyses.

This study has several limitations. First, as a single-center retrospective analysis at a tertiary referral hospital, findings may not be generalizable to broader clinical practice. Second, only surgically treated nodules were included, resulting in higher malignancy rates than would be observed in an unselected biopsy population. Third, the 2016 reclassification of non-invasive follicular variant papillary thyroid carcinoma as noninvasive follicular thyroid neoplasm with papillary-like nuclear features could not be retrospectively applied to the earlier cohort, potentially inflating historical malignancy rates. Fourth, the sample sizes for

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size-stratified analysis and the FN/SFN category in Group 2 were small (n = 54 and n = 14, respectively), warranting cautious interpretation of those subgroups. Finally, no molecular or immunocytochemical ancillary testing was performed during the study period, limiting comparability with centers where such diagnostic modalities are available for indeterminate nodules.

In conclusion, this 15-year study demonstrates that the Bethesda System's ability to distinguish malignant from benign thyroid nodules remained stable over time, as shown by multi-cutoff analyses, ROC curves, and level-specific LRs. The only exception, the decline in the AUS/FLUS LR, is most likely explained by a spectrum effect rather than a change in cytopathologic interpretation. This likely reflects a shift toward a more selective, sonographically risk-stratified surgical population following the introduction of the 2015 ATA guidelines. As malignancy rates in surgical series are shaped by local referral patterns, these LRs should be interpreted within that specific context and not generalized to unselected biopsy populations.

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Association of physical activity with arterial stiffness: the mediating role of the visceral fat metabolism score

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ABSTRACT

Objective: We investigated the association between physical activity (PA) and arterial stiffness (AS) in Chinese middle-aged and older adults, systematically evaluating the potential mediating role of the Metabolic Score For Visceral Fat. **Subjects and methods:** Data were drawn from the 2015 China Health and Retirement Longitudinal Study. PA was assessed using the International Physical Activity Questionnaire, and AS was determined by estimated pulse wave velocity. Multivariable logistic regression, restricted cubic spline models, and Bootstrap mediation analysis were applied for statistical analyses. **Results:** In the fully adjusted model, each standard deviation increase in PA intensity was associated with a 22% reduction in AS risk (odds ratio [OR] = 0.78, 95% confidence interval [CI]: 0.72–0.84, $p < 0.001$). Compared to low-intensity PA, both moderate-intensity (OR = 0.75, 95% CI: 0.61–0.92, $p = 0.005$) and high-intensity PA (OR = 0.57, 95% CI: 0.48–0.68, $p < 0.001$) were significantly associated with a lower risk of AS. Restricted cubic spline analysis revealed a nonlinear association, with AS risk decreasing as PA intensity increased (p-nonlinear = 0.034). Mediation analysis indicated that Metabolic Score For Visceral Fat significantly mediated the negative correlation between PA and AS, accounting for 11.4% of the total effect. **Conclusion:** Among middle-aged and older Chinese adults, higher levels of PA are associated with a lower risk of AS, with this association partially mediated by improvements in visceral fat metabolism.

Keywords: Physical activity; arterial stiffness; metabolic score for visceral fat; estimated pulse wave velocity

INTRODUCTION

Globally, cardiovascular disease (CVD) accounts for a substantial proportion of deaths and disabilities. Effective primary prevention of CVD depends on the early detection and management of vascular abnormalities during the subclinical phase (1,2). Among the various early markers of vascular function, arterial stiffness (AS), a core manifestation of vascular aging, is a well-established independent predictor of incident CVD and all-cause mortality (3–5). Therefore, developing accurate and convenient tools to assess AS is key for risk stratification in large populations. Currently, carotid-femoral pulse wave velocity is considered the "gold standard" for evaluating AS. However, the complex method involved hinders its widespread use in population-based research (6). To address this challenge, estimated pulse wave velocity (ePWV), calculated from routine parameters such as age and blood pressure, has been proposed. It is a proven independent predictor of mortality from all causes and specifically from CVD in the general population, making it an ideal alternative for assessing vascular health in large cohorts (7).

Routine physical activity (PA) is established as an effective non-pharmacological intervention for improving and slowing arterial aging as part of a vascular-healthy lifestyle (8). This effect has been validated across diverse populations; for instance, sustained aerobic exercise has been reported to significantly reduce the degree of AS in middle-aged and older women (9). Among patients with metabolic syndrome, moderate-to-vigorous PA has been associated with marked improvements in AS parameters (10). Notably, visceral fat (VF) is likely central to the beneficial effects of PA on arterial AS (11). Evidence suggests that

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higher PA levels are strongly associated with lower VF content (12), and accumulation of VF is closely linked with an increased risk of AS (13). Therefore, reductions in VF likely mediate the improvement in AS observed with increased PA.

Despite growing evidence for multiple pathways, the specific pathways by which PA modulates AS warrant further investigation (14). As a critical nexus between metabolic dysregulation and vascular aging, the role of VF in the relationship between PA and AS has not been systematically validated in large populations (15). Utilizing data from the China Health and Retirement Longitudinal Study (CHARLS), this study aimed to investigate the association between PA and AS in middle-aged and older Chinese adults, by analyzing the interconnected roles of PA, the Metabolic Score for Visceral Fat (METS-VF), and ePWV. Specifically, we sought to determine whether VF mediates the association between PA and AS, thereby providing novel epidemiological evidence elucidating the relationship between PA and vascular health.

SUBJECTS AND METHODS

Study design

This study used data from the 2015 CHARLS survey, a nationally representative longitudinal survey conducted in China. CHARLS systematically collects multidimensional information on health status, socioeconomic characteristics, and other factors among Chinese individuals aged ≥45 years. The survey adopted a multistage, stratified probability sampling method, covering 28 provinces (including autonomous regions and municipalities) across China. Its rigorous method and high-quality data collection are well established (16). Notably, all data collection protocols for the CHARLS database were reviewed and approved by the Biomedical Ethics Committee of Peking University. As written informed consent was secured from all participants at enrollment, a separate ethical review for this analysis was not required.

The initial sample comprised 21,095 participants. An iterative filtering process was applied based on predefined criteria: (1) exclusion of individuals who did not participate in the 2015 physical examination (n = 4,704); (2) exclusion of those aged <45 years (n = 894); (3) exclusion of those with missing data on PA-related variables (n = 8,328); (4) exclusion of those with missing data for AS assessment indicators (n = 70); and (5) exclusion of those with incomplete covariate data (n = 743). Ultimately, 6,356 participants were included in the final analysis. The participant selection flowchart is presented in **Figure 1**.

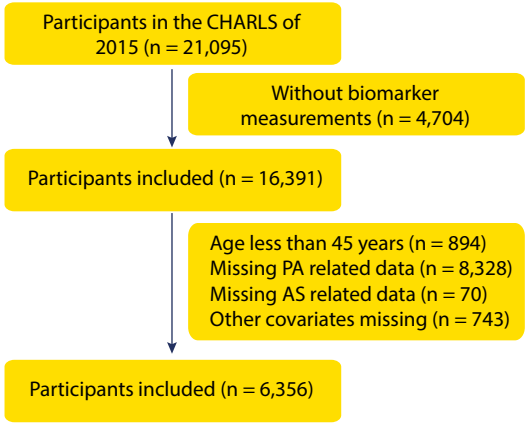


Figure 1. Inclusion and exclusion criteria.

Definition and measurement of variables

Exposure variable

Physical activity level was defined as the exposure variable, assessed using the internationally recognized metabolic equivalent of task (MET) system and strictly following the standardized protocol of the International Physical Activity Questionnaire (17). Based on questionnaire responses regarding activity duration, daily PA time was categorized into five levels (0 min, 10–29 min, 30–119 min, 120–239 min, and ≥240 min), with midpoint values used for quantitative analysis. Activity types were classified into three distinct intensity grades: vigorous-intensity PA (assigned a MET value of 8.0), including strenuous activities such as climbing, running, and heavy farming; moderate-intensity PA (MET value of 4.0), including brisk walking and Tai Chi; and low-intensity PA (MET value of 3.3), including light activities such as leisurely walking. Total weekly PA was calculated as MET value × number of activity days per week × daily activity duration (minutes). Following International Physical Activity Questionnaire criteria, intensity was categorized as low (<600 MET-min/week), moderate (600–3000 MET-min/week), or high (>3000 MET-min/week).

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To ensure data quality, outliers were identified using the interquartile range (IQR) method. Values falling below Q1 − 1.5 × IQR or above Q3 + 1.5 × IQR were treated as missing to maintain the reliability of the study findings.

Mediating variable

The METS-VF, a composite indicator of visceral adiposity derived from metabolic profiles, was used as the mediating variable (18,19). This scoring system comprises two core components: the metabolic score for insulin resistance (METS-IR) and the waist-to-height ratio (WHtR). METS-IR was calculated as [ln(2 × fasting glucose + triglycerides) × body mass index (BMI)] / ln(high-density lipoprotein cholesterol), effectively assessing the metabolic activity of adipose tissue using blood glucose, lipid, and body weight. The formula for WHtR is waist circumference/height, a morphological indicator that accurately characterizes the fat distribution features of central obesity. METS-VF was then computed using a validated multiple regression equation: $4.466 + 0.011 \times (\ln(\text{METS-IR}))^3 + 3.239 \times (\ln(\text{WHtR}))^3 + 0.319 \times \text{sex} + 0.594 \times (\ln(\text{age}))$. All laboratory indicators were measured with standardized methods: fasting glucose and triglycerides in mg/dL; high-density lipoprotein cholesterol in mg/dL; BMI in kg/m^2; waist circumference and height in cm; and sex coded as a binary variable (male = 1, female = 0). By integrating metabolic, morphological, and demographic information, this scoring system provides a more robust assessment of the pathological burden imposed by VF.

Outcome variable

Arterial stiffness was the outcome variable, measured using the widely validated, noninvasive ePWV calculated from age and blood pressure. ePWV was derived using a published regression formula: $9.587 - 0.402 \times \text{age} + 4.560 \times 10^{-3} \times \text{age}^2 - 2.621 \times 10^{-5} \times \text{age}^2 \times \text{mean blood pressure} + 3.176 \times 10^{-3} \times \text{age} \times \text{mean blood pressure} - 1.832 \times 10^{-2} \times \text{mean blood pressure}$, where mean blood pressure was defined as diastolic blood pressure + 0.4 × (systolic blood pressure - diastolic blood pressure). Consistent with current consensus in cardiovascular research, AS was defined as ePWV ≥10 m/s (20,21). The cutoff value was based on the following evidence: (1) validation studies have demonstrated that ePWV ≥10 m/s exhibits good consistency and diagnostic accuracy compared with the gold standard carotid-femoral artery pulse wave velocity (≥10 m/s) (22); (2) large prospective cohort studies have verified that ePWV ≥10 m/s is significantly associated with increased risk of cardiovascular and cerebrovascular events, such as myocardial infarction and stroke (23); and (3) several recent studies utilizing representative databases, including NHANES and CHARLS (24–26), have adopted ePWV ≥10 m/s as the operational definition of AS to enhance the comparability of findings across populations.

Covariates

Based on previous evidence, we accounted for potential confounders that could affect the PA-AS association across multiple domains. Demographic characteristics included age, sex (male/female), level of education (illiterate/primary school or below/secondary school or above), marital status (with or without partner), and residential area (rural/urban) (27). Lifestyle factors included current smoking status (yes/no) and current alcohol drinking (yes/no). Health status-related variables included BMI [categorized as normal (<24 kg/m^2), overweight (24–28 kg/m^2), and obese (≥28 kg/m^2)], and three common chronic diseases: diabetes, dyslipidemia, and CVD (28). Diagnostic criteria were as follows: Diabetes was defined based on any of the following: (1) fasting plasma glucose ≥126 mg/dL; (2) glycosylated hemoglobin ≥6.5%; or (3) self-reported physician diagnosis. Dyslipidemia was defined by any of the following: (1) total triglycerides ≥150 mg/dL; (2) total cholesterol ≥240 mg/dL; (3) high-density lipoprotein cholesterol <40 mg/dL; (4) low-density lipoprotein cholesterol ≥160 mg/dL; or (5) self-reported physician diagnosis. These cutoff values were referenced from several previous studies using the CHARLS database (29–31) and have been widely applied in large epidemiological cohorts to identify individuals with clinically significant dyslipidemia, including within CHARLS. Cardiovascular disease was assessed via standardized questions: participants were classified as having CVD if they reported

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a physician diagnosis of heart attack, coronary heart disease, angina, congestive heart failure, or stroke.

Statistical analysis

All statistical analyses were performed using R software (v. 4.4.2). Baseline characteristics were summarized by AS status. Categorical variables are presented as n (%), and continuous variables as mean. Comparisons between groups were conducted using analysis of variance for continuous variables and the chi-square test for categorical variables, with the *tableone* package used to generate the baseline table. PA was stratified into three levels based on intensity: low, moderate, and high. A multivariable logistic regression model was employed to assess the association between different PA levels and AS. Model 1 was unadjusted. Model 2 adjusted for age, sex, level of education, marital status, and residential area. Model 3 further adjusted for smoking, alcohol consumption, BMI, diabetes, dyslipidemia, and CVD in addition to variables in Model 2. All logistic regression analyses used the *stats* package, with the low-intensity group serving as the reference. To explore the heterogeneity in the PA-AS association across population subgroups, we performed subgroup analyses stratified by age and sex, and tested for interaction effects. All subgroup and interaction analyses were conducted using the *jstable* package. Restricted cubic spline (RCS) models were used to examine potential nonlinear relationships between PA and AS, with RCS curves plotted using the *rms* package.

Potential mediating effects of VF-related indicators on the association between PA and AS were examined using the bootstrap method with 500 resamplings. We evaluated whether METS-VF and its core components (i.e., METS-IR and WHtR) mediated the association between PA and AS, and estimated the direct, indirect, and total effects separately. To mitigate multicollinearity, the mediating effects of METS-VF, METS-IR, and WHtR were each assessed in separate models, with only one mediator included per model. All models were fully adjusted for covariates including age, sex, level of education, marital status, residential area, smoking, alcohol consumption, diabetes, dyslipidemia, and CVD. The BMI was retained in all models to control for its confounding influence, enabling a more precise estimation of the independent mediating effect of METS-VF after accounting for general adiposity. Given the cross-sectional design, the reported mediation effects should be interpreted as statistical associations and considered exploratory evidence for potential mechanistic pathways. All mediation analyses were performed using the mediation package. Furthermore, considering that the applicability of defining AS as ePWV ≥10 m/s may vary by population, we conducted supplementary sensitivity analyses by defining AS as ePWV above the 75th percentile to confirm the robustness of our primary findings. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

This study included 6,356 participants with a mean age of 61.0 ± 9.4 years, among whom 2,504 (39.4%) were diagnosed with AS. Compared with their non-AS counterparts, participants with AS were older and had a higher prevalence of male sex, single marital status, and current smoking, along with lower educational attainment ($p < 0.001$). In terms of health status, the AS group also had a markedly higher prevalence of diabetes, dyslipidemia, and CVD ($p < 0.001$). Additionally, both systolic and diastolic blood pressure levels were significantly elevated in the AS group. Regarding PA, the proportion of participants engaging in moderate- and high-intensity activities was significantly lower in the AS group ($p < 0.001$), suggesting a substantial association between PA levels and AS status (**Table 1**).

Association between PA and the risk of AS

Multivariable logistic regression analysis was conducted to assess the relationship between PA levels and AS risk, adjusting for potential confounders. Detailed results are shown in **Table 2**. The analysis revealed a significant negative correlation between PA and the risk of AS. In the fully adjusted Model 3, each standard deviation increase in PA intensity was associated with a 22% lower risk of AS (OR = 0.78, 95% CI: 0.72–0.84). Analysis by PA intensity

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level demonstrated a clear dose-response relationship. Compared with the low PA group, both moderate- and high-intensity PA were associated with a lower risk of AS. In the fully adjusted Model 3, moderate-intensity activity was linked to a 25% reduction in AS risk (OR = 0.75, 95% CI: 0.61–0.92), while high-intensity activity corresponded to a 43% decreased risk (OR = 0.57, 95% CI: 0.48–0.68). Sensitivity analyses yielded findings consistent with the primary analyses (**Supplementary Table S1**).

Table 1. Baseline characteristics of the study participants

Variable	Total, n (%)	Arterial stiffness, mean (SD)		p-value
		Absent	Present	
n	6356	3852	2504	
Age (years)	61.0 (9.4)	55.8 (6.1)	69.0 (7.6)	<0.001
Sex (%)				
Female	3376 (53.1)	2179 (56.6)	1197 (47.8)	<0.001
Male	2980 (46.9)	1673 (43.4)	1307 (52.2)	
Level of education (%)				
Illiterate	1636 (25.7)	824 (21.4)	812 (32.4)	<0.001
Elementary school or below	2674 (42.1)	1532 (39.8)	1142 (45.6)	
Middle school or above	2046 (32.2)	1496 (38.8)	550 (22.0)	
Marital status (%)				
Single	778 (12.2)	258 (6.7)	520 (20.8)	<0.001
Married	5578 (87.8)	3594 (93.3)	1984 (79.2)	
Residential area (%)				
Rural	3972 (62.5)	2404 (62.4)	1568 (62.6)	0.886
Urban	2384 (37.5)	1448 (37.6)	936 (37.4)	
Smoking status (%)				
No	3586 (56.4)	2302 (59.8)	1284 (51.3)	<0.001
Yes	2770 (43.6)	1550 (40.2)	1220 (48.7)	
Alcohol consumption (%)				
No	4164 (65.5)	2490 (64.6)	1674 (66.9)	0.074
Yes	2192 (34.5)	1362 (35.4)	830 (33.1)	
Body mass index (kg/m^2)	23.9 (3.8)	23.9 (3.6)	24.0 (4.1)	0.735
Diabetes (%)				
No	5325 (83.8)	3307 (85.9)	2018 (80.6)	<0.001
Yes	1031 (16.2)	545 (14.1)	486 (19.4)	
Dyslipidemia (%)				
No	3676 (57.8)	2313 (60.0)	1363 (54.4)	<0.001
Yes	2680 (42.2)	1539 (40.0)	1141 (45.6)	
Cardiovascular disease (%)				
No	5235 (82.4)	3328 (86.4)	1907 (76.2)	<0.001
Yes	1121 (17.6)	524 (13.6)	597 (23.8)	
Systolic blood pressure (mm/Hg)	127.4 (20.0)	118.3 (14.2)	141.5 (19.7)	<0.001
Diastolic blood pressure (mm/Hg)	75.0 (11.7)	71.9 (10.0)	79.7 (12.6)	<0.001
Physical activity				
Light	1074 (16.9)	516 (13.4)	558 (22.3)	<0.001
Moderate	1430 (22.5)	808 (21.0)	622 (24.8)	
Vigorous	3852 (60.6)	2528 (65.6)	1324 (52.9)	

SD: standard deviation.

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Table 2. Association between physical activity and arterial stiffness

Variable	Model 1		Model 2		Model 3	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)	p-value
Per-SD	0.74 (0.70–0.78)	<0.001	0.75 (0.70–0.80)	<0.001	0.78 (0.72–0.84)	<0.001
Categorical						
Light	1 (Reference)		1 (Reference)		1 (Reference)	
Moderate	0.71 (0.61–0.83)	<0.001	0.76 (0.62–0.93)	0.009	0.75 (0.61–0.92)	0.005
Vigorous	0.48 (0.42–0.56)	<0.001	0.55 (0.47–0.66)	<0.001	0.57 (0.48–0.68)	<0.001
p (trend)		<0.001		<0.001		<0.001

Model 1 was unadjusted. **Model 2** was adjusted for age, sex, educational level, marital status, and residential area. **Model 3** was further adjusted for age, sex, educational level, marital status, residential area, smoking, alcohol consumption, body mass index, diabetes, dyslipidemia, and cardiovascular disease. OR: Odds ratio; CI: confidence interval.

Dose-response association between PA and AS

We used RCS to investigate the potential nonlinear dose-response relationship between PA and AS risk. As shown in **Figure 2**, a nonlinear dose-response relationship was observed between PA and the risk of AS (p for overall association < 0.001, p for nonlinearity = 0.034).

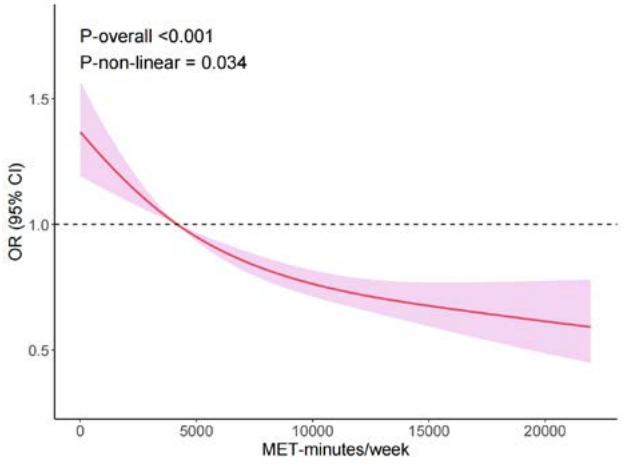


Figure 2. Dose-response association between physical activity and arterial stiffness.

Subgroup analysis and interaction tests

To assess the consistency of the association between PA and AS across various populations, subgroup analyses were conducted, stratified by age, sex, and chronic disease status (**Table 3**). A markedly lower AS risk with high-intensity PA was observed across all subgroups, although no statistically significant interactions were detected (p > 0.05). These results suggest that the protective effect of PA against AS is robust and generally consistent across individuals with different ages, sexes, and chronic disease statuses.

Mediation analysis

To further investigate the biological pathways through which PA affects AS, we systematically evaluated the mediating role of METS-VF and its core components (i.e., METS-IR and WHtR) in the association between PA and AS using the bootstrap method. The mediation analysis (**Figure 3**) revealed that the relationship between PA and AS was significantly mediated by METS-VF, with a mediated proportion of 11.4% (**Figure 3A**). METS-IR and WHtR also acted as notable mediators in the PA-AS relationship, with mediated proportions of 5.3% and 7.4%, respectively (**Figure 3B-C**).

DISCUSSION

Based on the CHARLS database, this study systematically explored the association between PA and AS risk among middle-aged and older Chinese adults, as well as the potential mediating role of METS-VF within such relationship. A significant negative correlation was identified between PA and AS risk, with more pronounced protective effects observed for moderate-to-high-intensity PA and an overall nonlinear dose-response relationship. No significant interaction effects were observed. Mediation analyses further indicated that METS-VF partially mediated the association between PA and AS, with a mediated proportion of approximately 11.4%. These findings provide novel epidemiological evidence for the biological pathways linking PA to vascular health. Moreover, the results suggested that future vascular health interventions should not only promote regular PA but also consider METS-VF as an auxiliary indicator for risk screening and dynamic monitoring, thereby facilitating early identification of individuals at high risk for AS and providing an objective evaluation of intervention efficacy.

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Table 3. Subgroup analysis

Variable	n	Level	OR (95%CI)	*p*-value	$p_{(interaction)}$
Age (years)					
		Light	Reference		
<60	2911	Moderate	0.65(0.42–1.01)	0.055	
		Vigorous	0.68(0.47–0.97)	0.035	0.128
		Light	Reference		
≥60	3445	Moderate	0.77(0.61–0.98)	0.031	
		Vigorous	0.54(0.45–0.67)	<0.001	
Sex					
		Light	Reference		
Female	3376	Moderate	0.69(0.52–0.92)	0.011	
		Vigorous	0.49(0.39–0.63)	<0.001	0.203
		Light	Reference		
Male	2980	Moderate	0.84(0.62–1.12)	0.230	
		Vigorous	0.67(0.52–0.86)	0.002	
Hypertension					
		Light	Reference		
No	3677	Moderate	0.66(0.48–0.92)	0.012	
		Vigorous	0.51(0.39–0.67)	<0.001	0.146
		Light	Reference		
Yes	2679	Moderate	0.96(0.70–1.31)	0.804	
		Vigorous	0.78(0.59–1.01)	0.054	
Diabetes					
		Light	Reference		
No	5325	Moderate	0.77(0.61–0.96)	0.022	
		Vigorous	0.58(0.48–0.7)	<0.001	0.850
		Light	Reference		
Yes	1031	Moderate	0.67(0.42–1.06)	0.086	
		Vigorous	0.54(0.36–0.80)	0.003	
Dyslipidemia					
		Light	Reference		
No	3676	Moderate	0.72(0.54–0.95)	0.022	
		Vigorous	0.59(0.47–0.75)	<0.001	0.772
		Light	Reference		
Yes	2680	Moderate	0.76(0.57–1.02)	0.072	
		Vigorous	0.54(0.42–0.7)	<0.001	
Cardiovascular disease					0.384
		Light	Reference		
No	5235	Moderate	0.76(0.61–0.96)	0.021	
		Vigorous	0.60(0.50–0.73)	<0.001	
		Light	Reference		
Yes	1121	Moderate	0.67(0.43–1.05)	0.084	
		Vigorous	0.47(0.31–0.70)	<0.001	

The model was fully adjusted for covariates. OR: Odds ratio; CI: confidence interval.

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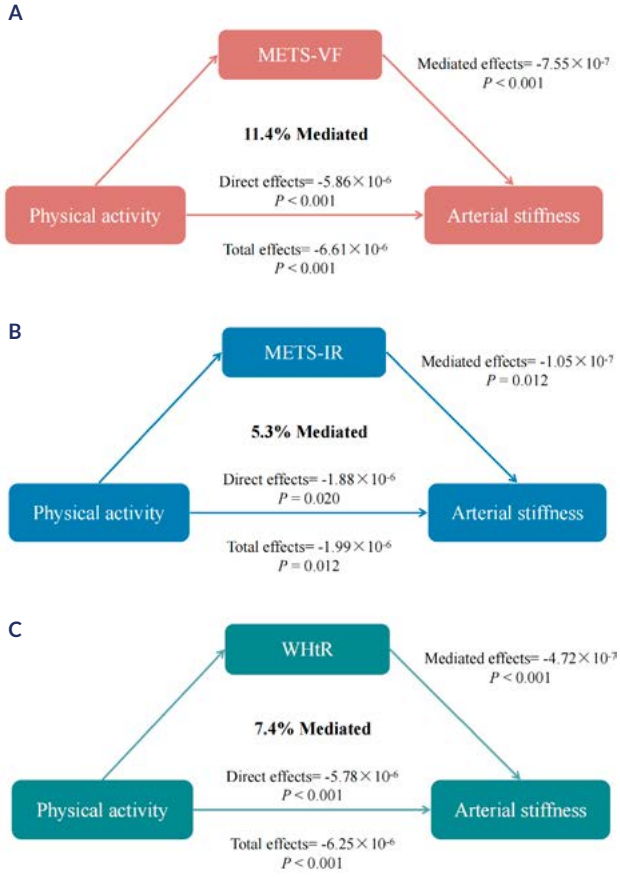


Figure 3. The mediating role of visceral fat metabolism-related indicators in the association between physical activity and arterial stiffness.

In this study, moderate-to-high-intensity PA was significantly correlated with a lower risk of AS. This result is consistent with findings from the Jackson Heart Study among Black African descent populations, which reported that higher overall PA was independently associated with reduced AS (32). The consistent epidemiological evidence across various populations and ethnicities strongly supports the intrinsic link between PA and vascular health. Notably, ePWV used in this study is an estimated indicator calculated based on age, blood pressure, and other parameters, rather than a direct measurement of AS. Previous evidence has established that PA substantially affects blood pressure, a core component of ePWV calculation. Accordingly, the observed association in this study may, in part, reflect PA-induced alterations in blood pressure rather than structural alterations in the arterial wall itself. Such distinction should be fully considered when interpreting ePWV as a surrogate marker of vascular health.

Furthermore, previous studies have reported a similar ceiling effect in the inverse relationship between PA and CVD risk reduction, indicating that further decreases in CVD risk plateau when weekly PA reaches around 3000 MET-min (33). Additionally, long-term, high-intensity endurance training has been associated with aortic medial fibrosis, potentially detrimental vascular remodeling, and transient adverse effects on endothelial function (34–36). In accordance with these prior reports, our RCS analysis revealed a reverse J-shaped curve: protective effects increased rapidly from low to moderate PA levels, then gradually plateaued with further elevations in PA intensity. This dose-response pattern suggests that moderate increases in PA are sufficient to achieve major vascular health benefits, whereas excessive exercise yields limited marginal gain. Nevertheless, the aforementioned vascular risks are primarily derived from observations among elite athletes or those with extreme exercise exposure, which may not be generalizable to routine high-intensity PA among the general population.

This study did not detect any harmful association between high-intensity PA and elevated AS risk, nor did it identify extreme exercise exposure among participants. Therefore, exercise interventions targeting individuals at high risk for AS should follow individualized and progressive principles, prioritizing the establishment of sustainable daily activity habits rather than pursuing excessive high-intensity exercise.

Subgroup analyses showed that high-intensity PA was consistently associated with reduced AS risk across all predefined subgroups, without significant interactions detected. These findings highlight the robustness of the observed association across diverse demographic and baseline health characteristics, providing population-based support among Chinese adults for the concept of "Exercise is Medicine" (37). In age-stratified analyses, the inverse correlation between high-intensity PA and AS risk was stronger among individuals aged ≥60 years and older than in those younger than 60. This may be attributed to age-related structural deterioration of blood vessels in older adults, leaving greater room for functional

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interventions to generate measurable health benefits (38–40). In analyses stratified by sex, high-intensity PA was linked to a greater reduction in AS risk among females. Existing literature indicates that estrogen may modulate vascular endothelial function, and the female vascular system exhibits higher sensitivity to hemodynamic adjustments after exercise-induced workload changes (41–43). Moreover, the inverse association between high-intensity PA and AS risk was more pronounced in participants with diabetes, dyslipidemia, or a history of CVD. Such patients typically present with severe endothelial dysfunction and chronic inflammation, both of which are strongly related to increased AS susceptibility (44–47). Regular PA may ameliorate these pathologies by improving endothelial function and modulating inflammatory responses (48,49), thereby conferring greater protection in these vulnerable groups.

As a comprehensive indicator integrating metabolic and morphological characteristics, METS-VF may better reflect the pathophysiological activity of VF than single indicators such as body fat percentage or waist circumference (50). Our study found that METS-VF mediated 11.4% of the association between PA and AS prevalence. Visceral adipose tissue, as a metabolically active endocrine organ, produces free fatty acids and pro-inflammatory cytokines; excess accumulation leads to systemic chronic inflammation and insulin resistance (51,52). Such metabolic disturbances are closely implicated in endothelial impairment, vascular smooth muscle cell proliferation, collagen deposition, and ultimately, the development and progression of AS (53). Additionally, PA may regulate VF deposition and metabolic homeostasis through multiple biological pathways; notably, PA reduces VF accumulation by elevating energy expenditure (54).

Exercise training also induces adaptive adipose tissue remodeling, including enhanced mitochondrial biogenesis and improved mitochondrial function (55,56), which improve insulin sensitivity and glucose uptake (57,58). Enhanced mitochondrial oxidative capacity supports lipolytic metabolism and maintains overall metabolic homeostasis (59,60). Furthermore, PA modulates adipose tissue immune microenvironments and alleviates VF-related chronic inflammation (61,62). These adaptive changes attenuate the detrimental effects of visceral fat on the vascular system, suggesting that VF burden and metabolic status reflected by METS-VF may serve as a potential intermediate pathway linking PA to AS risk. Nevertheless, despite adjusting statistical models for multiple confounding factors (including BMI), interpreting the observed mediating effects remains complex. Therefore, further validation in prospective cohort or interventional studies is required to clarify the underlying molecular mechanisms.

Despite being based on a nationally representative sample and rigorous mediation analysis, several limitations should be acknowledged. First, the cross-sectional design inherently limits the ability to establish causality. Although mediation analysis suggests plausible physiological pathways, definitive causality among variables require validation through more robust study designs. Second, key variables including PA, AS, and VF were assessed using self-reported data or indirect estimations rather than gold-standard measurements, potentially introducing non-differential misclassification bias and attenuating effect estimates. Third, as the study was based on the CHARLS database, only participants aged ≥45 years were included, which aligns with the core design purpose of this survey. Nevertheless, such age restriction may have limited generalizability of our findings to younger populations. Lastly, diagnostic criteria for dyslipidemia used apply specifically to middle-aged and older Chinese adults aged ≥45 years. Given the discrepancies in lipid reference ranges, ethnic genetic backgrounds, lifestyle patterns, and disease spectrums across regions and countries, caution should be exercised when extrapolating our findings to non-Chinese populations or other age subgroups. Building on our results, future randomized controlled trials in various populations should aim to confirm and elucidate the mechanisms by which exercise may ameliorate AS through VF reduction.

In conclusion, based on the CHARLS database, this study demonstrated that increased PA, particularly at moderate-to-vigorous intensity, is an effective strategy to prevent AS among middle-aged and older Chinese adults. Moreover, the association between

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PA and AS was found to be partially mediated by VF levels. These findings advance understanding of the mechanisms linking PA to vascular health. From a public health perspective, the results suggest that, alongside promoting regular PA, incorporating VF assessment indicators (e.g., the METS-VF) into health screening systems for middle-aged and elderly populations can provide a reference for identifying individuals at high risk of AS and developing potential exercise-related health intervention strategies.

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SUPPLEMENTARY MATERIAL

Table S1. Association between PA and the redefined AS

Variable	Model 1		Model 2		Model 3	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)	p-value
Per-SD	0.66 (0.62–0.71)	<0.001	0.69(0.64–0.74)	<0.001	0.71(0.66–0.76)	<0.001
Categorical						
Light	1 (Reference)		1 (Reference)		1 (Reference)	
Moderate	0.75(0.63–0.89)	<0.001	0.85(0.69–1.03)	0.102	0.83(0.68–1.02)	0.075
Vigorous	0.42(0.36–0.49)	<0.001	0.49(0.41–0.58)	<0.001	0.50(0.42–0.60)	<0.001
p (trend)		<0.001		<0.001		<0.001

Model 1 was unadjusted. **Model 2** was adjusted for age, sex, educational level, marital status, and residential area. **Model 3** was further adjusted for age, sex, educational level, marital status, residential area, smoking, alcohol consumption, body mass index, diabetes, dyslipidemia, and cardiovascular disease. OR: Odds ratio; CI: confidence interval.

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original

Comparison of clinical characteristics and predictive performance of severity scoring systems in hypertriglyceridemic versus non-hypertriglyceridemic acute pancreatitis: a retrospective cohort study

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ABSTRACT

Objective: To compare early clinical and biochemical characteristics of hypertriglyceridemic acute pancreatitis (HTG-AP) and non-hypertriglyceridemic acute pancreatitis (NHTG-AP), and to evaluate the predictive performance of commonly used severity scoring systems for adverse in-hospital outcomes. **Subjects and methods:** This single-center retrospective cohort study included 268 consecutive adults with acute pancreatitis admitted from January to December 2023. Patients were classified as HTG-AP (n = 128) or NHTG-AP (n = 140) according to predefined etiological criteria. Demographic data, laboratory results within 24 hours, and six scoring systems, including BISAP, MCTSI, modified Marshall score, Ranson criteria, qSOFA, and HAPS, were analyzed. The primary endpoint was a composite adverse outcome comprising persistent organ failure, pancreatic or peripancreatic necrosis requiring intervention, intensive care unit stay longer than 7 days, or in-hospital mortality. Predictors were identified using LASSO-selected multivariable logistic regression and assessed by temporal validation, receiver operating characteristic analysis, calibration, and bootstrap resampling. **Results:** Compared with NHTG-AP, HTG-AP patients were younger, had higher body mass index and recurrence rates, showed greater systemic inflammation and atherogenic dyslipidemia, and had lower free thyroxine levels. Composite adverse outcomes were more frequent in HTG-AP than in NHTG-AP. BISAP and MCTSI were independent predictors of adverse outcomes. The combined BISAP-MCTSI model showed good discrimination in derivation and validation cohorts and outperformed Ranson, qSOFA, and HAPS after Bonferroni correction. **Conclusion:** HTG-AP represents a distinct clinical phenotype with worse in-hospital outcomes. A combined BISAP-MCTSI model may improve early risk stratification and warrants prospective validation.

Keywords: hypertriglyceridemic acute pancreatitis; clinical characteristics; BISAP; Modified CT Severity Index; severity scoring systems; retrospective cohort study

INTRODUCTION

Acute pancreatitis (AP), a common and potentially life-threatening gastrointestinal disorder, involves inflammation of the pancreatic tissue. Globally, the incidence of AP has ranged from 13 to 45 cases per 100,000 population annually and has continued to rise, imposing substantial clinical and economic burdens on healthcare systems (1). Acute pancreatitis etiologies are diverse; the most common causes have included gallstone disease, excessive alcohol consumption, hypertriglyceridemia, and drug-induced injury (2). Among these, hypertriglyceridemic acute pancreatitis (HTG-AP) has garnered increasing attention as a distinct clinical subtype, particularly given its rising prevalence among younger individuals and patients with metabolic syndrome (3). A serum triglyceride (TG) level exceeding 11.3 mmol/L (1000 mg/dL) typically defines HTG-AP, and its incidence

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has paralleled the global surge in obesity, diabetes, and dyslipidemia (4). Unlike biliary or alcoholic pancreatitis, HTG-AP exhibits unique pathophysiological mechanisms, including elevated circulating chylomicrons, free fatty acid-induced cytotoxicity, and pancreatic microvascular ischemia (5). These metabolic derangements amplify systemic inflammatory responses and may predispose patients to more severe disease trajectories. Elucidating the distinct clinical and biochemical profiles of HTG-AP compared to non-hypertriglyceridemic acute pancreatitis (NHTG-AP) is therefore essential to refine diagnostic precision and optimize early therapeutic interventions.

Early and accurate AP severity assessment is paramount to guide clinical decision-making, including monitoring intensity, fluid resuscitation strategies, and escalation to critical care (6). Investigators have developed and validated multiple scoring systems to predict AP severity and prognosis. These include the bedside index for severity in acute pancreatitis (BISAP), the modified computed tomography severity index (MCTSI), the modified Marshall score, Ranson's criteria, the quick sequential organ failure assessment (qSOFA), and the harmless acute pancreatitis score (HAPS) (7–9). Each tool stratifies patients by risk using distinct clinical, laboratory, or radiological parameters. However, the applicability and predictive accuracy of these scoring systems across different AP etiological subtypes, particularly HTG-AP, have remained insufficiently characterized. Previous studies developed and validated most existing scoring models predominantly in cohorts with biliary or alcoholic pancreatitis; this potentially limits their generalizability to HTG-AP, which exhibits unique metabolic and inflammatory phenotypes. Moreover, small sample sizes, heterogeneous outcome definitions, and a lack of standardized temporal assessment protocols have constrained previous comparative studies. These limitations have generated diagnostic uncertainty, potentially leading to suboptimal risk stratification or resource allocation for patients with HTG-AP (10). Establishing more robust risk stratification strategies for HTG-AP thus requires a systematic comparative evaluation of these scoring systems within distinct etiological subgroups using standardized methodology and composite clinical endpoints.

Despite growing recognition of HTG-AP as a distinct clinical entity, several critical knowledge gaps persist. First, although prior studies have documented differences in lipid profiles between HTG-AP and other etiologies, the comprehensive characterization of a broader spectrum of metabolic derangements, (e.g., alterations in glucose homeostasis, thyroid function, and inflammatory cascade activation) remains incomplete. Second, investigators have not rigorously evaluated the comparative predictive performance of commonly utilized severity scoring systems specifically within populations with HTG-AP while adequately controlling for temporal bias and confounding related to etiological heterogeneity. Third, existing prognostic models may succumb to circular reasoning when employing organ dysfunction scores to predict outcomes defined by organ failure itself; avoiding this requires adopting composite clinical endpoints that encompass the full range of adverse outcomes. Lastly, the notably high recurrence rate observed in HTG-AP and its relationship to the baseline metabolic phenotype warrant further investigation to inform evidence-based secondary prevention strategies.

Given the above, we sought to (1) to systematically compare early clinical, metabolic, and biochemical characteristics between patients with HTG-AP and those with NHTG-AP who were admitted within 24 hours of symptom onset, and (2) evaluate and compare the predictive accuracy of six commonly used severity scoring systems (i.e., BISAP, MCTSI, modified Marshall score, Ranson criteria, qSOFA, and HAPS) for a predefined composite adverse in-hospital outcome, determining whether a combined scoring approach offered superior risk stratification across etiological subtypes.

SUBJECTS AND METHODS

Study design and setting

This single-center retrospective cohort study was conducted at the First Affiliated Hospital of Soochow University (China). Consecutive adult patients admitted with AP between January 1 and December 31, 2023, were screened. The primary analysis included

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patients admitted within 24 hours of symptom onset; a sensitivity analysis included admissions within 72 hours. For temporal validation, patients admitted from January to October 2023 formed the derivation cohort (n = 214, ~80%), and those admitted from November to December 2023 formed the temporal validation cohort (n = 54, ~20%). The institutional review board approved the study, and informed consent was waived due to the retrospective design.

Patient selection and eligibility criteria

Acute pancreatitis was diagnosed according to the 2012 Revised Atlanta Classification (11), requiring at least two of the following: (1) characteristic abdominal pain; (2) serum amylase or lipase ≥3× the upper limit of normal; (3) imaging findings consistent with AP. Inclusion criteria comprised age ≥18 years; admission within the predefined time window; TG measured within 24 hours of admission prior to lipid-lowering therapy; and sufficient data to calculate severity scores and ascertain outcomes. Exclusion criteria included chronic pancreatitis; a history of pancreatic surgery; malignancy-associated pancreatitis; pregnancy; transfer from another institution with delayed presentation; and incomplete records precluding score calculation or outcome assessment. For recurrent admissions, only the index admission was analyzed.

Etiological classification

HTG-AP was defined as a TG level ≥11.3 mmol/L (1000 mg/dL) measured within 24 hours of admission in the absence of definitive alternative etiologies, or a TG level of 5.65–11.3 mmol/L (500–1000 mg/dL) with lipemic serum and the exclusion of other causes. NHTG-AP etiologies included biliary, alcohol-related, post-endoscopic retrograde cholangiopancreatography, drug-induced, and idiopathic pancreatitis, diagnosed based on standard criteria. In patients with multiple potential etiological factors, the predominant cause was determined using a hierarchical adjudication protocol. First, a biliary etiology was considered definitive if gallstones or biliary sludge were confirmed on imaging (ultrasound, computed tomography [CT], or magnetic resonance cholangiopancreatography) with concordant elevation of hepatobiliary enzymes (ALT >3× the upper limit of normal within 24 hours of admission), regardless of concomitant TG levels. Second, alcohol-related pancreatitis was diagnosed given documented significant alcohol consumption (>50 g/day for >5 years or a binge episode within 48 hours prior to symptom onset) and no identified biliary cause, even if TG levels were mildly elevated. Third, HTG-AP was diagnosed only when (a) serum TG were ≥11.3 mmol/L (1000 mg/dL), or (b) TG were 5.65–11.3 mmol/L (500–1000 mg/dL) with lipemic serum, and no definitive biliary, alcohol-related, or other identifiable cause was present. Cases with competing etiologies that could not be resolved by these criteria (e.g., TG 5.65–11.3 mmol/L with concurrent moderate alcohol use but no clear biliary disease) were independently reviewed by two senior gastroenterologists blinded to clinical outcomes. Disagreements were resolved by consensus involving a third adjudicator. Inter-rater agreement was excellent (Cohen's κ = 0.89, 95% confidence interval [CI]: 0.82–0.96). A total of 32 cases (10.7% of the final cohort) required formal adjudication; of these, 18 were classified as HTG-AP and 14 as NHTG-AP.

Data collection

We extracted demographics, comorbidities, clinical presentation, and vital signs from electronic medical records using a standardized form. We recorded laboratory values obtained within the first 24 hours, including inflammatory markers, metabolic indices, lipid profiles, renal and liver function, and thyroid function tests. Patients underwent contrast-enhanced CT according to routine clinical practice.

Severity scoring systems

This study evaluated six commonly used severity scoring systems. **Supplementary Table S1** provides detailed definitions, individual components, score ranges, interpretation thresholds, and assessment time windows, which we summarize below.

1) Bedside index for severity in acute pancreatitis (BISAP): A 5-point clinical score assessed within the first 24 hours of admission, comprising five dichotomous variables: blood urea nitrogen (BUN) >25 mg/dL

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(>7.14 mmol/L), impaired mental status (Glasgow coma scale [GCS] <15), the presence of systemic inflammatory response syndrome (SIRS; defined as ≥2 of the following: temperature >38 or <36 ℃, heart rate >90 beats/min, respiratory rate >20 breaths/min or $PaCO_2$ <32 mmHg, white blood cell count >12,000 or <4,000/mm^3), age >60 years, and pleural effusion detected on imaging. A score ≥3 indicates a high risk for severe disease and in-hospital mortality (12).

2) Modified computed tomography severity index (MCTSI): A 10-point imaging-based score derived from contrast-enhanced computed tomography findings, assessed when the imaging procedure was performed according to routine clinical practice (typically within 72 hours of admission). The MCTSI comprises three domains: degree of pancreatic inflammation (0, 2, or 4 points), extent of pancreatic necrosis (0 = none, 2 = ≤30%, 4 = >30%), and the presence of extrapancreatic complications (2 points for any of the following: pleural effusion, ascites, vascular complications, or extrapancreatic parenchymal complications). A total score ≥4 indicates moderate-to-severe disease (13).

3) Modified Marshall scoring system: A physiological score evaluating organ dysfunction across three systems: respiratory (PaO_2/FiO_2 ratio), renal (serum creatinine), and cardiovascular (systolic blood pressure, with or without response to fluid resuscitation). The system scores each category from 0 to 4. A score ≥2 in any single system indicates organ failure. We initially calculated scores using the worst values recorded within the first 24 hours of admission and reassessed them at 48 hours to determine persistence. In accordance with the Revised Atlanta Classification, we defined persistent organ failure as organ failure lasting ≥48 hours (14).

4) Ranson's criteria: An 11-point score incorporating two distinct assessment time points. The score evaluates five criteria at admission: age >55 years, white blood cell count >16,000/mm^3, blood glucose >200 mg/dL (>11.1 mmol/L), lactate dehydrogenase >350 IU/L, and aspartate aminotransferase >250 IU/L. Six additional criteria apply at 48 hours: a hematocrit decrease >10 percentage points, a BUN increase >5 mg/dL (>1.79 mmol/L), serum calcium <8 mg/dL (<2.0 mmol/L), PaO_2 <60 mmHg, base deficit >4 mEq/L, and estimated fluid sequestration >6 L. A total score ≥3 predicts severe disease. We collected the 48-hour components when available, according to the original scoring definition (15).

5) Quick sequential organ failure assessment (qSOFA): A 3-point bedside screening score assessed within the first 24 hours of admission, comprising three dichotomous criteria: altered mental status (GCS <15), systolic blood pressure ≤100 mmHg, and respiratory rate ≥22 breaths/min. A score ≥2 suggests an elevated risk for a poor clinical outcome (16).

6) Harmless acute pancreatitis score (HAPS): A rule-out score assessed within the first 24 hours of admission, designed to identify patients likely to have a non-severe clinical course. The score comprises three criteria: the absence of rebound abdominal tenderness or guarding, a normal hematocrit (30–44%), and normal serum creatinine (≤2.0 mg/dL or ≤177 μmol/L). A score of 3 (all three criteria fulfilled) predicts a harmless course with a high negative predictive value (17).

For all scoring systems, we calculated scores using the worst clinical and laboratory values obtained within the specified assessment time windows. When multiple measurements were available within the assessment window, we selected the most abnormal value to ensure we captured peak disease severity.

Outcomes

The primary outcome was an in-hospital composite adverse endpoint, defined as any of the following: (1) persistent organ failure (modified Marshall score ≥2 for ≥48 hours); (2) pancreatic or peripancreatic necrosis requiring continuous intervention (percutaneous, endoscopic, or surgical); (3) intensive care unit (ICU) stay >7 days; or (4) in-hospital mortality. Secondary outcomes included individual components of the composite endpoint, pancreatic necrosis, ICU admission, and length of stay.

The composite adverse outcome was selected a priori based on the following rationale. First, individual severe outcomes in AP (mortality, persistent organ failure, necrotizing pancreatitis requiring intervention) are relatively infrequent in moderate-sized cohorts, which limits statistical power for single-endpoint analyses. Second, a composite endpoint encompassing the

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full spectrum of clinically meaningful adverse events captures the aggregate burden of severe disease and aligns with endpoints used in recent severity studies. Third, to prevent circular reasoning, the composite outcome was deliberately constructed so it does not simply recapitulate a single scoring system's definition (e.g., modified Marshall ≥2 alone); rather, it integrates multiple independent clinical domains. To facilitate clinical interpretation regarding their relative contributions, the individual components of the composite outcome are also reported separately as secondary endpoints (Table 1).

Table 1. Baseline demographic, clinical, and comorbidity characteristics and clinical outcomes of patients with acute pancreatitis stratified by etiology

Variable	Total (n=268)	HTG-AP (n=128)	NHTG-AP (n=140)	Test statistic	*p*-Value	Effect size
Demographics						
Age (years), mean ± SD	43.25 ± 12.38	39.84 ± 10.52	46.38 ± 13.15	t = –4.321	<0.001*	0.53[a]
Male sex, n (%)	189 (70.5)	102 (79.7)	87 (62.1)	χ^2 = 10.127	0.001*	0.19[b]
BMI (kg/m^2), mean ± SD	26.47 ± 4.32	28.15 ± 4.68	24.96 ± 3.51	t = 6.189	<0.001*	0.76[a]
Clinical presentation						
Time from onset to admission (hours), median (IQR)	18.5 (12.0–30.0)	16.0 (10.0–26.0)	21.0 (14.0–36.0)	U = 7342	0.009*	0.21[c]
Abdominal pain on admission, n (%)	265 (98.9)	127 (99.2)	138 (98.6)	Fisher's exact	1.000	0.03[b]
Nausea/vomiting, n (%)	218 (81.3)	108 (84.4)	110 (78.6)	χ^2 = 1.523	0.217	0.08[b]
Fever (>38 °C), n (%)	87 (32.5)	48 (37.5)	39 (27.9)	χ^2 = 2.936	0.087	0.10[b]
Primary Etiology (mutually exclusive)†				χ^2 = 162.34	<0.001*	0.78[b]
Hypertriglyceridemia, n (%)	128 (47.8)	128 (100.0)	0 (0.0)	—	—	—
Biliary, n (%)	74 (27.6)	0 (0.0)	74 (52.9)	—	—	—
Alcohol-related, n (%)	35 (13.1)	0 (0.0)	35 (25.0)	—	—	—
Post-ERCP/biliary surgery, n (%)	16 (6.0)	0 (0.0)	16 (11.4)	—	—	—
Drug-induced, n (%)	7 (2.6)	0 (0.0)	7 (5.0)	—	—	—
Idiopathic, n (%)	8 (3.0)	0 (0.0)	8 (5.7)	—	—	—
Precipitating/Contributing Factors (non-exclusive)‡						
Overeating/binge eating, n (%)	104 (38.8)	67 (52.3)	37 (26.4)	χ^2 = 18.72	<0.001*	0.26[b]
Concurrent alcohol use, n (%)	58 (21.6)	18 (14.1)	40 (28.6)	χ^2 = 8.45	0.004*	0.18[b]
Concurrent biliary disease, n (%)	14 (5.2)	8 (6.3)	6 (4.3)	χ^2 = 0.529	0.467	0.04[b]
Any coexisting etiological factor, n (%)	47 (17.5)	28 (21.9)	19 (13.6)	χ^2 = 3.195	0.074	0.11[b]
Comorbidities						
Recurrent pancreatitis, n (%)	78 (29.1)	51 (39.8)	27 (19.3)	χ^2 = 13.945	<0.001*	0.23[b]
Diabetes mellitus, n (%)	92 (34.3)	58 (45.3)	34 (24.3)	χ^2 = 13.483	<0.001*	0.22[b]
Hypertension, n (%)	98 (36.6)	52 (40.6)	46 (32.9)	χ^2 = 1.823	0.177	0.08[b]
Known dyslipidemia, n (%)	145 (54.1)	96 (75.0)	49 (35.0)	χ^2 = 42.687	<0.001*	0.40[b]
Coronary artery disease, n (%)	36 (13.4)	21 (16.4)	15 (10.7)	χ^2 = 1.928	0.165	0.09[b]
Chronic kidney disease, n (%)	18 (6.7)	10 (7.8)	8 (5.7)	χ^2 = 0.502	0.478	0.04[b]
Lifestyle Factors						
Current/former smoker, n (%)	127 (47.4)	67 (52.3)	60 (42.9)	χ^2 = 2.546	0.111	0.10[b]
Regular alcohol consumption, n (%)	129 (48.1)	64 (50.0)	65 (46.4)	χ^2 = 0.360	0.548	0.04[b]
Initial severity assessment						
SIRS criteria met on admission, n (%)	156 (58.2)	84 (65.6)	72 (51.4)	χ^2 = 5.734	0.017*	0.15[b]
Organ failure on admission, n (%)	42 (15.7)	26 (20.3)	16 (11.4)	χ^2 = 4.228	0.040*	0.13[b]
Respiratory failure, n (%)	28 (10.4)	18 (14.1)	10 (7.1)	χ^2 = 3.542	0.060	0.12[b]
Cardiovascular failure, n (%)	19 (7.1)	12 (9.4)	7 (5.0)	χ^2 = 2.013	0.156	0.09[b]
Renal failure, n (%)	15 (5.6)	10 (7.8)	5 (3.6)	χ^2 = 2.465	0.116	0.10[b]
ICU admission required, n (%)	68 (25.4)	39 (30.5)	29 (20.7)	χ^2 = 3.563	0.059	0.12[b]

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Variable	Total (n=268)	HTG-AP (n=128)	NHTG-AP (n=140)	Test statistic	*p*-Value	Effect size
Disease Severity (Revised Atlanta)[§]				χ^2 = 12.845	0.002*	0.22[b]
Mild AP, n (%)	168 (62.7)	72 (56.3)	96 (68.6)	—	—	—
Moderately severe AP, n (%)	58 (21.6)	28 (21.9)	30 (21.4)	—	—	—
Severe AP, n (%)	42 (15.7)	28 (21.9)	14 (10.0)	—	—	—
Clinical Outcomes						
Composite adverse outcome, n (%)[‖]	57 (21.3)	36 (28.1)	21 (15.0)	χ^2 = 6.980	0.008*	0.16[b]
Components (non-exclusive):						
Persistent organ failure (≥48 h), n (%)	36 (13.4)	24 (18.8)	12 (8.6)	χ^2 = 5.867	0.015*	0.15[b]
Necrosis requiring intervention, n (%)	31 (11.6)	19 (14.8)	12 (8.6)	χ^2 = 2.663	0.103	0.10[b]
ICU stay >7 days, n (%)	28 (10.4)	19 (14.8)	9 (6.4)	χ^2 = 5.172	0.023*	0.14[b]
In-hospital mortality, n (%)	6 (2.2)	4 (3.1)	2 (1.4)	Fisher's exact	0.426	0.06[b]
Other outcomes:						
Pancreatic necrosis (any), n (%)	38 (14.2)	24 (18.8)	14 (10.0)	χ^2 = 4.389	0.036*	0.13[b]
Infected necrosis, n (%)	15 (5.6)	10 (7.8)	5 (3.6)	χ^2 = 2.465	0.116	0.10[b]
Any intervention required, n (%)	31 (11.6)	19 (14.8)	12 (8.6)	χ^2 = 2.663	0.103	0.10[b]
Length of hospital stay (days), median (IQR)	9.0 (6.0–14.0)	10.0 (7.0–16.0)	8.0 (5.0–12.0)	U = 7256	0.007*	0.22[c]

HTG-AP: hypertriglyceridemic acute pancreatitis; NHTG-AP: non-hypertriglyceridemic acute pancreatitis; BMI: body mass index; IQR: interquartile range; ERCP: endoscopic retrograde cholangiopancreatography; SIRS: systemic inflammatory response syndrome; ICU: intensive care unit; AP: acute pancreatitis. *Statistically significant at $p < 0.05$ (two-sided).

Effect size measures: [a] Cohen's d for independent-samples t-tests; [b] Cramér's V (ϕc) for χ^2 and Fisher's exact tests; [c] rank-biserial correlation (r) for Mann-Whitney U tests.

[†] Primary etiology was assigned as a mutually exclusive category using the hierarchical adjudication protocol described in the Methods section. By definition, all patients in the HTG-AP group had hypertriglyceridemia as the primary etiology, and all patients in the NHTG-AP group had a non-hypertriglyceridemic primary etiology. The overall χ^2 test compares the distribution across all primary etiology categories.

[‡] Precipitating/contributing factors are non-mutually exclusive and reflect coexisting etiological risk factors not classified as the primary etiology. A single patient may have more than one contributing factor. The detailed distribution of overlapping etiologies is provided in **Supplementary Table S3**.

[§] Disease severity was classified according to the Revised Atlanta Classification (2012): mild (no organ failure, no local/systemic complications), moderately severe (transient organ failure <48 h and/or local complications), severe (persistent organ failure ≥48 h).

[‖] Composite adverse outcome was defined as any of the following during the index hospitalization: (1) persistent organ failure (Modified Marshall score ≥2 in any organ system for ≥48 hours); (2) pancreatic or peripancreatic necrosis requiring intervention (percutaneous drainage, endoscopic necrosectomy, or surgical necrosectomy); (3) ICU stay >7 days; or (4) in-hospital mortality. Components are non-mutually exclusive; among 57 patients meeting the composite endpoint, 32 (56.1%) satisfied two or more criteria.

Statistical analysis

We summarized continuous variables as means ± standard deviation (SD) or medians (interquartile ranges [IQRs]), depending on their distributions as assessed by the Shapiro-Wilk test, and compared them between groups using independent-samples t tests or Mann-Whitney U tests, as appropriate. We expressed categorical variables as frequencies (percentages) and compared them using χ^2 tests or Fisher's exact tests. For multiple laboratory comparisons (**Table 2**), we applied the Benjamini-Hochberg false discovery rate procedure to control for multiplicity.

To identify independent predictors of the composite adverse outcome from among the six candidate severity scores (BISAP, MCTSI, modified Marshall, Ranson, qSOFA, and HAPS), we employed a two-stage modeling strategy. In the first stage, we performed least absolute shrinkage and selection operator (LASSO) logistic regression to simultaneously address multicollinearity and variable selection. We standardized all continuous severity scores to zero mean and unit variance prior to penalization to ensure equal weighting during regularization. We selected the regularization parameter λ using 10-fold cross-validation, choosing the λ within one standard error of the minimum deviance (λ_{1se}) to favor a parsimonious model and reduce overfitting risk. At the optimal λ_{1se} = 0.042, the model retained BISAP and MCTSI with non-zero coefficients (LASSO-penalized β: BISAP = 1.86, MCTSI = 0.43) and shrank the remaining four scores to zero. In the second stage, we entered the LASSO-selected variables into an unpenalized multivariable logistic regression model for final coefficient estimation and statistical inference, as LASSO-penalized coefficients are inherently biased and do not yield valid standard errors for hypothesis testing.

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Table 2. Comparative laboratory and biochemical parameters on admission in patients with hypertriglyceridemic versus non-hypertriglyceridemic acute pancreatitis

Variable	Total (n=268)	HTG-AP (n=128)	NHTG-AP (n=140)	Mean difference (95% CI)	Test statistic	*p*-Value	Effect size
Pancreatic enzymes							
Serum amylase (U/L), median (IQR)	856 (412-1654)	792 (385-1521)	918 (456-1742)	-	U = 8234	0.156	0.11
Serum lipase (U/L), median (IQR)	1245 (587-2876)	1156 (542-2654)	1328 (618-3021)	-	U = 8456	0.234	0.09
Inflammatory Markers							
WBC count (×10^9/L), mean ± SD	12.85 ± 4.67	13.94 ± 5.12	11.86 ± 4.02	2.08 (0.89-3.27)	t = 3.456	0.001*	0.45
Neutrophil percentage (%), mean ± SD	78.5 ± 9.8	81.2 ± 8.9	76.1 ± 10.2	5.1 (2.4-7.8)	t = 3.287	0.001*	0.53
CRP (mg/L), median (IQR)	54.3 (18.6-126.5)	86.7 (32.4-168.2)	38.9 (12.8-94.3)	-	U = 6234	<0.001*	0.34
PCT (ng/mL), median (IQR)	0.42 (0.12-1.86)	0.58 (0.18-2.34)	0.31 (0.09-1.45)	-	U = 7123	0.018*	0.19
Lipid profile							
Triglycerides (mmol/L), median (IQR)	5.82 (2.14-18.45)	22.67 (14.32-36.84)	2.08 (1.32-4.56)	-	U = 1245	<0.001*	0.92
Total cholesterol (mmol/L), mean ± SD	6.24 ± 2.87	8.15 ± 3.24	4.52 ± 1.68	3.63 (2.95-4.31)	t = 10.234	<0.001*	1.41
HDL-C (mmol/L), mean ± SD	0.96 ± 0.34	0.78 ± 0.28	1.12 ± 0.32	-0.34 (-0.42-0.26)	t = -8.456	<0.001*	1.12
LDL-C (mmol/L), mean ± SD	3.45 ± 1.56	4.28 ± 1.82	2.71 ± 0.98	1.57 (1.19-1.95)	t = 8.123	<0.001*	1.04
TG/HDL-C ratio, median (IQR)	6.18 (2.34-19.87)	28.45 (18.34-46.72)	1.86 (1.18-4.05)	-	U = 876	<0.001*	0.95
LDL/HDL-C ratio, mean ± SD	3.72 ± 1.85	5.68 ± 2.14	2.43 ± 0.96	3.25 (2.81-3.69)	t = 11.567	<0.001*	1.96
Liver function tests							
Total bilirubin (μmol/L), median (IQR)	18.6 (10.2-34.8)	14.3 (8.7-24.5)	28.4 (15.6-52.3)	-	U = 5678	<0.001*	0.41
Direct bilirubin (μmol/L), median (IQR)	7.8 (3.2-18.6)	5.2 (2.4-12.4)	12.6 (5.8-28.7)	-	U = 5845	<0.001*	0.38
ALT (U/L), median (IQR)	98 (42-256)	68 (32-164)	142 (58-384)	-	U = 6234	<0.001*	0.35
AST (U/L), median (IQR)	86 (38-218)	58 (28-142)	126 (52-312)	-	U = 5987	<0.001*	0.37
ALP (U/L), median (IQR)	112 (78-168)	98 (72-148)	128 (86-192)	-	U = 7234	0.002*	0.25
GGT (U/L), median (IQR)	124 (56-248)	98 (48-196)	156 (72-298)	-	U = 6987	0.005*	0.22
Albumin (g/L), mean ± SD	36.8 ± 5.6	35.4 ± 5.8	38.1 ± 5.2	-2.7 (-4.0–-1.4)	t = -3.987	<0.001*	0.49
Renal function							
Serum creatinine (μmol/L), mean ± SD	78.5 ± 32.4	84.6 ± 38.7	72.9 ± 24.1	11.7 (3.2-20.2)	t = 2.734	0.007*	0.35
BUN (mmol/L), mean ± SD	6.84 ± 3.56	7.52 ± 4.12	6.23 ± 2.84	1.29 (0.34-2.24)	t = 2.678	0.008*	0.36
eGFR (mL/min/1.73 m^2), mean ± SD	94.6 ± 24.8	89.2 ± 27.6	99.4 ± 20.8	-10.2 (-16.1–-4.3)	t = -3.456	0.001*	0.41
Metabolic parameters							
Fasting blood glucose (mmol/L), mean ± SD	9.45 ± 4.28	11.34 ± 5.12	7.76 ± 2.68	3.58 (2.54-4.62)	t = 6.789	<0.001*	0.85
HbA1c (%)	6.8 ± 1.6	7.6 ± 1.8	6.1 ± 1.2	1.5 (1.1-1.9)	t = 7.234	<0.001*	0.98
Serum calcium (mmol/L), mean ± SD	2.08 ± 0.28	2.01 ± 0.31	2.14 ± 0.24	-0.13 (-0.20–-0.06)	t = -3.678	<0.001*	0.46
Corrected calcium (mmol/L)††	2.18 ± 0.26	2.12 ± 0.29	2.23 ± 0.22	-0.11 (-0.18–-0.04)	t = -3.234	0.001*	0.42
Coagulation profile							
D-dimer (mg/L), median (IQR)	1.24 (0.48-3.86)	1.56 (0.62-4.52)	0.98 (0.38-3.12)	-	U = 7456	0.042*	0.16
Fibrinogen (g/L), mean ± SD	4.52 ± 1.68	4.98 ± 1.84	4.12 ± 1.45	0.86 (0.38-1.34)	t = 3.456	0.001*	0.51
PT (seconds), mean ± SD	12.8 ± 1.6	13.2 ± 1.8	12.5 ± 1.4	0.7 (0.2-1.2)	t = 2.678	0.008*	0.43
APTT (seconds), mean ± SD	32.4 ± 5.8	33.6 ± 6.4	31.3 ± 5.1	2.3 (0.5-4.1)	t = 2.456	0.015*	0.39
Thyroid Function							
TSH (mIU/L), median (IQR)	1.86 (0.92-3.42)	1.68 (0.78-3.12)	2.04 (1.08-3.68)	-	U = 8123	0.124	0.12
Free T4 (pmol/L), mean ± SD	14.8 ± 3.6	12.6 ± 3.2	16.8 ± 3.4	-4.2 (-5.2–-3.2)	t = -7.892	<0.001*	1.26
Free T3 (pmol/L), mean ± SD	3.8 ± 0.9	3.2 ± 0.8	4.3 ± 0.8	-1.1 (-1.3–-0.9)	t = -8.456	<0.001*	1.38
Electrolytes							
Serum sodium (mmol/L), mean ± SD	138.6 ± 4.2	137.8 ± 4.6	139.3 ± 3.7	-1.5 (-2.6–-0.4)	t = -2.678	0.008*	0.36
Serum potassium (mmol/L), mean ± SD	3.92 ± 0.58	3.84 ± 0.62	3.99 ± 0.54	-0.15 (-0.31–0.01)	t = -1.867	0.063	0.26

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Variable	Total (n=268)	HTG-AP (n=128)	NHTG-AP (n=140)	Mean difference (95% CI)	Test statistic	*p*-Value	Effect size
Arterial blood gas[§§]							
pH, mean ± SD	7.38 ± 0.08	7.36 ± 0.09	7.40 ± 0.07	-0.04 (-0.06–-0.02)	t = -3.456	0.001*	0.49
PaO2/FiO2 ratio, mean ± SD	348 ± 86	326 ± 94	368 ± 75	-42 (-65–-19)	t = -3.678	<0.001*	0.49
Lactate (mmol/L), median (IQR)	1.8 (1.2-3.4)	2.2 (1.4-4.1)	1.5 (1.0-2.8)	-	U = 6789	0.003*	0.24

HTG-AP: hypertriglyceridemic acute pancreatitis; NHTG-AP: non-hypertriglyceridemic acute pancreatitis; IQR: interquartile range; WBC: white blood cell; CRP: C-reactive protein; PCT: procalcitonin; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglycerides; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; BUN: blood urea nitrogen; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; PT: prothrombin time; APTT: activated partial thromboplastin time; TSH: thyroid-stimulating hormone; T3: triiodothyronine; T4: thyroxine; PaO_2: partial pressure of oxygen; FiO_2: fraction of inspired oxygen.

We evaluated collinearity among predictors at two levels. For the final multivariable model, we calculated variance inflation factors and the condition number of the design matrix. Additionally, we constructed a pairwise Spearman correlation matrix of all six candidate severity scores to characterize the interrelationships among the scoring systems and provide context for the LASSO selection results (**Supplementary Table S2**).

We assessed model discrimination by calculating the area under the receiver operating characteristic curve (AUC) using DeLong's method. We evaluated calibration using calibration plots (observed vs. predicted probabilities across deciles of predicted risk), the Hosmer-Lemeshow goodness-of-fit test, and calibration slope and intercept metrics. We performed internal validation using bootstrap resampling (2000 iterations) to estimate the optimism-corrected C-statistic. We conducted temporal validation in a held-out cohort comprising patients admitted from November to December 2023 (n = 54, ~20% of the total sample).

We performed 15 pairwise AUC comparisons among the seven models (six individual scores plus the combined BISAP-MCTSI model) using DeLong's test. To balance the risk of type I error inflation against the potential for excessive conservatism, we applied both the Bonferroni correction (adjusted α = 0.05/15 = 0.0033) and the Benjamini-Hochberg false discovery rate procedure as a complementary sensitivity approach. We present the results under both correction methods in **Table 3,** allowing readers to interpret the findings under their preferred framework for multiple comparison adjustment.

As an additional sensitivity analysis, we performed propensity score matching to balance baseline characteristics between the HTG-AP and NHTG-AP groups. We applied a 1:1 nearest-neighbor matching algorithm without replacement, using a caliper width of 0.2 standard deviations of the logit of the propensity score. The covariates included in the propensity score model were age, sex, body mass index (BMI), diabetes mellitus, hypertension, and time from symptom onset to admission. We confirmed the adequacy of balance using standardized mean differences, with a target threshold of <0.10 for all matched covariates.

We conducted all statistical analyses using the software SPSS v. 26.0 (IBM Corp., USA) and R v. 4.3.1 (R Foundation for Statistical Computing, Austria). We used the R packages glmnet (LASSO regression), pROC (ROC analysis), and MatchIt (propensity score matching) for specialized analyses. All tests were two-sided, and $p < 0.05$ was considered statistically significant unless otherwise specified for multiple comparison adjustments.

RESULTS

Study population

During the study period, we screened 312 consecutive patients with AP. Of these, 268 met the eligibility criteria and comprised the final cohort (HTG-AP, n = 128; NHTG-AP, n = 140). **Figure 1** summarizes the exclusion details. Cases with competing etiologies were adjudicated with high inter-rater agreement (κ = 0.89). **Table 1** presents the baseline characteristics and clinical features.

Baseline characteristics

Patients with HTG-AP were younger (39.84 ± 10.52 vs. 46.38 ± 13.15 years; $p < 0.001$), more frequently male (79.7% vs. 62.1%; $p = 0.001$), and had a higher BMI (28.15 ± 4.68 vs. 24.96 ± 3.51 kg/m^2; $p < 0.001$) than those with NHTG-AP. Metabolic comorbidities

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Table 3. Comparison of clinical severity scoring systems between hypertriglyceridemic and non-hypertriglyceridemic acute pancreatitis cohorts

Scoring system	Total (n=268)	HTG-AP (n=128)	NHTG-AP (n=140)	Mean difference (95% CI)	Test statistic	*p*-Value	Effect size	AUC for composite adverse outcome (95% CI)
BISAP Score								
Total score, mean ± SD	1.24 ± 0.96	1.52 ± 1.05	0.99 ± 0.81	0.53 (0.28-0.78)	t=4.287	<0.001*	0.56	0.842 (0.768-0.916)
Score ≥3, n (%)	34 (12.7)	24 (18.8)	10 (7.1)	-	χ^2=8.234	0.004*	0.18	-
Individual BISAP components, n (%)								
BUN >7.14 mmol/L	78 (29.1)	46 (35.9)	32 (22.9)	-	χ^2=5.432	0.020*	0.14	-
Impaired mental status	18 (6.7)	12 (9.4)	6 (4.3)	-	Fisher's exact	0.126	0.11	-
SIRS present	156 (58.2)	84 (65.6)	72 (51.4)	-	χ^2=5.734	0.017*	0.15	-
Age >60 years	42 (15.7)	12 (9.4)	30 (21.4)	-	χ^2=7.654	0.006*	0.17	-
Pleural effusion	68 (25.4)	38 (29.7)	30 (21.4)	-	χ^2=2.456	0.117	0.10	-
Modified CT severity index (MCTSI)								
Total score, mean ± SD	2.86 ± 1.48	3.24 ± 1.62	2.52 ± 1.26	0.72 (0.34-1.10)	t=3.789	<0.001*	0.49	0.768 (0.682-0.854)
Score ≥4, n (%)	98 (36.6)	58 (45.3)	40 (28.6)	-	χ^2=8.234	0.004*	0.18	-
MCTSI components								
Pancreatic inflammation score, median (IQR)	2 (2-4)	2 (2-4)	2 (2-4)	-	U=8234	0.234	0.09	-
Pancreatic necrosis, n (%)	38 (14.2)	24 (18.8)	14 (10.0)	-	χ^2=4.389	0.036*	0.13	-
Extrapancreatic complications, n (%)	82 (30.6)	48 (37.5)	34 (24.3)	-	χ^2=5.432	0.020*	0.14	-
Modified Marshall Score								
Total score, mean ± SD	1.34 ± 1.86	1.84 ± 2.24	0.89 ± 1.32	0.95 (0.48-1.42)	t=3.987	<0.001*	0.50	0.831 (0.755-0.907)
Score ≥2 (organ failure), n (%)	42 (15.7)	26 (20.3)	16 (11.4)	-	χ^2=4.228	0.040*	0.13	-
Modified Marshall Components, mean ± SD								
Respiratory score	0.54 ± 0.92	0.72 ± 1.08	0.38 ± 0.74	0.34 (0.10-0.58)	t=2.789	0.006*	0.36	-
Cardiovascular score	0.42 ± 0.78	0.58 ± 0.94	0.28 ± 0.58	0.30 (0.09-0.51)	t=2.678	0.008*	0.37	-
Renal score	0.38 ± 0.82	0.54 ± 0.96	0.23 ± 0.64	0.31 (0.10-0.52)	t=2.789	0.006*	0.37	-
Ranson Score								
Total score, mean ± SD	2.48 ± 1.68	2.64 ± 1.78	2.34 ± 1.58	0.30 (-0.14–0.74)	t=1.345	0.180	0.18	0.698 (0.604-0.792)
Score ≥3, n (%)	98 (36.6)	52 (40.6)	46 (32.9)	-	χ^2=1.823	0.177	0.08	-
qSOFA Score								
Total score, median (IQR)	1 (0-1)	1 (0-2)	0 (0-1)	-	U=6789	0.002*	0.25	0.724 (0.632-0.816)
Score ≥2, n (%)	58 (21.6)	38 (29.7)	20 (14.3)	-	χ^2=9.234	0.002*	0.19	-
qSOFA Components, n (%)								
Altered mental status (GCS <15)	18 (6.7)	12 (9.4)	6 (4.3)	-	Fisher's exact	0.126	0.11	-
Systolic BP ≤100 mmHg	68 (25.4)	42 (32.8)	26 (18.6)	-	χ^2=7.234	0.007*	0.17	-
Respiratory rate ≥22/min	124 (46.3)	72 (56.3)	52 (37.1)	-	χ^2=10.123	0.001*	0.19	-
HAPS Score								
Total score, mean ± SD	2.18 ± 0.56	2.08 ± 0.58	2.27 ± 0.54	-0.19 (-0.33–-0.05)	t=-2.678	0.008*	0.34	0.356‡‡ (0.264-0.448)
Score=3 (harmless), n (%)	96 (35.8)	38 (29.7)	58 (41.4)	-	χ^2=4.123	0.042*	0.12	-
HAPS Components (absence indicates harmless), n (%)								
No rebound tenderness/guarding	192 (71.6)	86 (67.2)	106 (75.7)	-	χ^2=2.543	0.111	0.10	-
Normal hematocrit	168 (62.7)	72 (56.3)	96 (68.6)	-	χ^2=4.432	0.035*	0.13	-
Normal serum creatinine	224 (83.6)	102 (79.7)	122 (87.1)	-	χ^2=2.876	0.090	0.10	-
Composite risk stratification								
Low risk (BISAP 0–1 and MCTSI 0–2)	120 (44.8%)	46 (35.9%)	74 (52.9%)	-	χ^2=7.823	0.005*	0.17	-
Intermediate risk (BISAP ≥2 OR MCTSI 3–4, not both high)	96 (35.8%)	46 (35.9%)	50 (35.7%)	-	-	-	-	-
High risk (BISAP ≥2 AND MCTSI ≥4)	52 (19.4%)	36 (28.1%)	16 (11.4%)	-	χ^2=12.456	<0.001*	0.22	-

HTG-AP: hypertriglyceridemic acute pancreatitis; NHTG-AP: non-hypertriglyceridemic acute pancreatitis; BISAP: Bedside Index for Severity in Acute Pancreatitis; MCTSI: Modified CT Severity Index; qSOFA: quick Sequential Organ Failure Assessment; HAPS: Harmless Acute Pancreatitis Score; BUN: blood urea nitrogen; SIRS: systemic inflammatory response syndrome; GCS: Glasgow Coma Scale; BP: blood pressure; IQR: interquartile range; AUC: area under the receiver operating characteristic curve; SAP: severe acute pancreatitis; CI: confidence interval.

* Statistically significant at $P < 0.05$ (two-sided).

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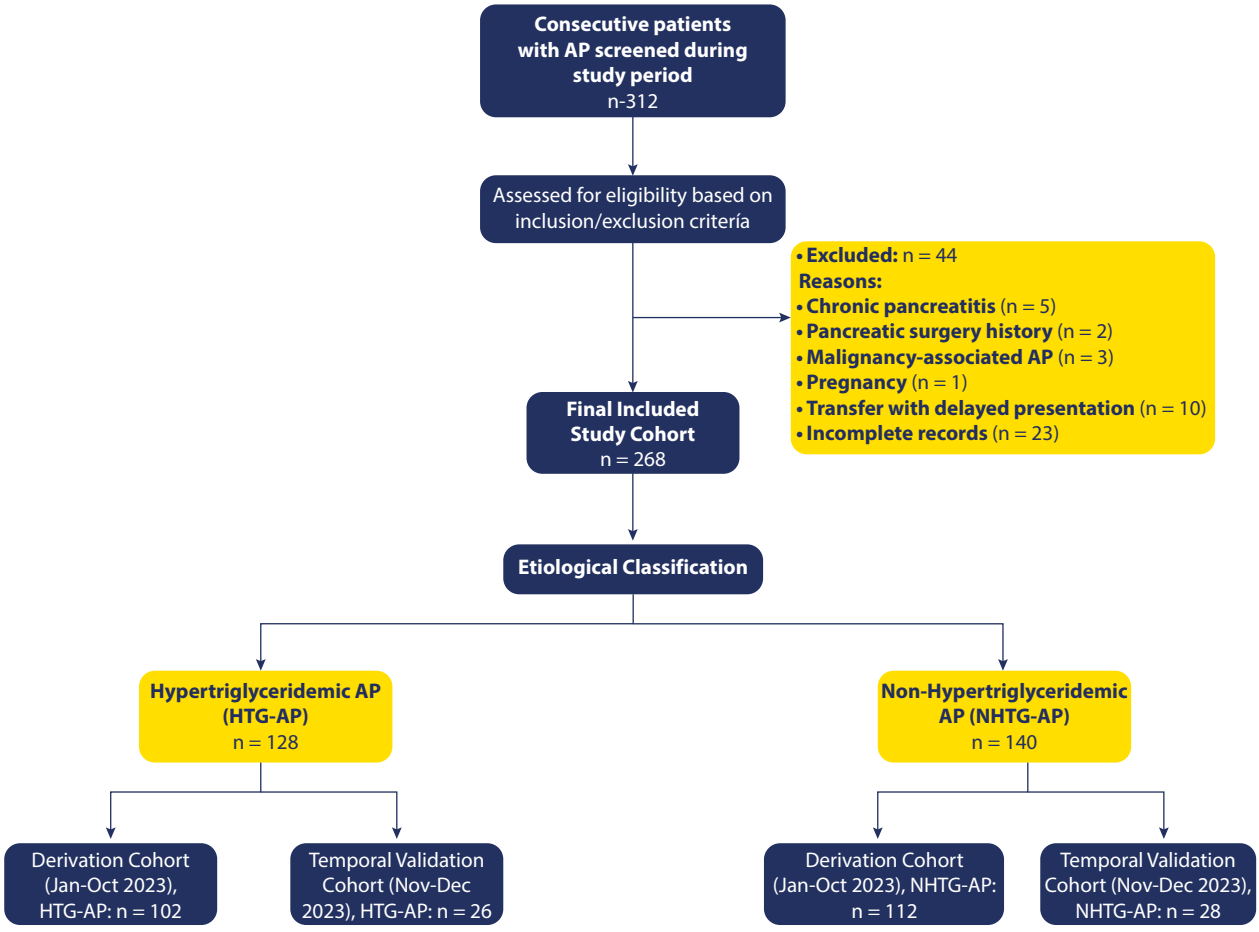


Figure 1. Study flow diagram and patient selection.

were more prevalent in the HTG-AP group, including diabetes mellitus (45.3% vs. 24.3%; $p < 0.001$) and known dyslipidemia (75.0% vs. 35.0%; $p < 0.001$). Recurrent pancreatitis was also more common in this group (39.8% vs. 19.3%; $p < 0.001$). On admission, patients with HTG-AP more frequently met the SIRS criteria (65.6% vs. 51.4%; $p = 0.017$) and exhibited organ failure (20.3% vs. 11.4%; $p = 0.040$) (**Table 1**).

We observed etiological overlap in a subset of patients. Across the entire cohort, 47 patients (17.5%) had more than one identifiable etiological risk factor. In the HTG-AP group, 28 patients (21.9%) had at least one coexisting etiological factor (18 with concomitant alcohol use, 8 with biliary disease, and 2 with both), compared with 19 patients (13.6%) in the NHTG-AP group ($p = 0.074$). A hierarchical adjudication protocol determined the predominant etiology in these cases (see the Methods section). **Supplementary Table S3** details the distribution of coexisting etiological risk factors.

Laboratory findings

Table 2 presents the comparisons of laboratory parameters obtained on admission. Patients with HTG-AP exhibited a greater inflammatory burden, indicated by a higher WBC count (13.94 ± 5.12 vs. 11.86 ± 4.02×10^9/L; $p = 0.001$) and higher CRP levels (median 86.7 vs. 38.9 mg/L; $p < 0.001$). As expected, TG were markedly elevated in the HTG-AP group (median 22.67 vs. 2.08 mmol/L; $p < 0.001$). These patients also showed lower HDL-C and higher LDL-C levels (both $p < 0.001$), yielding substantially higher TG/HDL-C and LDL/HDL-C ratios (both $p < 0.001$). Furthermore, the HTG-AP group displayed worse glycemic indices (fasting glucose and HbA1c; both $p < 0.001$) and lower thyroid hormone levels (FT4 and FT3; both $p < 0.001$), although TSH levels did not differ between groups. Hepatobiliary markers (bilirubin and liver enzymes) were lower in patients with HTG-AP, consistent with the different etiological distributions (**Table 2**).

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Severity scores and clinical outcomes

Table 3 summarizes the between-group comparisons of severity scoring systems. Patients with HTG-AP had higher BISAP scores (1.52 ± 1.05 vs. 0.99 ± 0.81; $p < 0.001$) and MCTSI scores (3.24 ± 1.62 vs. 2.52 ± 1.26; $p < 0.001$). The qSOFA scores were also higher in this group ($p = 0.002$), whereas Ranson scores did not differ significantly between groups ($p = 0.180$) (Table 3). The composite adverse outcome occurred more frequently in patients with HTG-AP than in those with NHTG-AP (28.1% vs. 15.0%; $p = 0.008$). Among the components of this outcome, persistent organ failure (≥48 h) was more common in the HTG-AP group (18.8% vs. 8.6%; $p = 0.015$), as was prolonged ICU stay >7 days (14.8% vs. 6.4%; $p = 0.023$). The rate of necrosis requiring intervention did not differ significantly between groups (14.8% vs. 8.6%; $p = 0.103$). HTG-AP was also associated with a longer hospital length of stay (median 10 vs. 8 days; $p = 0.007$) and a higher incidence of pancreatic necrosis (18.8% vs. 10.0%; $p = 0.036$). In-hospital mortality remained low in both groups (3.1% vs. 1.4%; $p = 0.426$) (Table 1).

Predictive model development and validation

Table 4 presents the multivariable modeling results. We retained BISAP and MCTSI in the final model using LASSO-regularized selection followed by multivariable logistic regression. Both BISAP (adjusted odds ratio [OR] = 8.42, 95% CI 2.89–24.51; $p < 0.001$) and MCTSI (adjusted OR = 1.68, 95% CI 1.21–2.33; $p = 0.002$) were independently associated with the composite adverse outcome (Table 4).

Table 3 and Figure 2 summarize the discriminative performance of each score and the combined model. BISAP achieved the highest AUC among the individual scores, and the combined BISAP-MCTSI model demonstrated improved discrimination. Following Bonferroni correction for pairwise comparisons, the combined model outperformed the Ranson, qSOFA, and HAPS scores, although its difference compared with the Modified Marshall score was not statistically significant (Figure 2, Table 3).

We assessed the calibration of the BISAP-MCTSI model using calibration plots and the Hosmer-Lemeshow goodness-of-fit test. In the derivation cohort, the Hosmer-Lemeshow test was non-significant ($\chi^2 = 6.82$, df = 8, $p = 0.556$), indicating no evidence of poor fit. The calibration plot showed close agreement between the predicted and observed probabilities across the deciles of predicted risk (calibration slope = 0.94, intercept = −0.03). In the temporal validation cohort, calibration remained adequate (Hosmer-Lemeshow $p = 0.412$; calibration slope = 0.89, intercept = −0.06), albeit with an expected slight attenuation. Figure 3 provides the calibration plots for both cohorts.

To facilitate clinical application, we derived the optimal cutoff for the combined BISAP-MCTSI predicted probability using Youden's index in the derivation cohort and subsequently evaluated it in the temporal validation cohort. The optimal probability threshold was 0.22 (corresponding approximately to a BISAP ≥ 2 combined with an MCTSI ≥ 4). In the temporal validation cohort, this threshold yielded a sensitivity of 82.1% (95% CI: 70.2–90.8%), a specificity of 78.4% (95% CI: 72.1–83.9%), a positive predictive value of 41.8% (95% CI: 32.6–51.5%), a negative predictive value of 95.8% (95% CI: 91.9–98.2%), a positive likelihood ratio of 3.80, and a negative likelihood ratio of 0.23.

For clinical practicability, we propose a simplified three-tier risk stratification scheme based on the two component scores:

1. Low risk (BISAP 0–1 AND MCTSI 0–2): Predicted adverse outcome probability <10%; ward-level monitoring with standard reassessment may be appropriate.
2. Intermediate risk (BISAP ≥ 2 OR MCTSI 3–4): Predicted probability 10–30%; close monitoring with clinical and biochemical reassessment at 24–48 hours is recommended.
3. High risk (BISAP ≥2 AND MCTSI ≥4): Predicted probability >30%; early ICU admission or step-up care should be considered.

These cutoffs are intended as clinical guidance pending external validation; they should be interpreted within the overall clinical context, including patient comorbidities, clinical trajectory, and institutional resources.

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Table 4. Multivariable logistic regression analysis identifying independent predictors of the composite adverse outcome

Variable	β coefficient	Standard error	Wald χ^2	p-value	OR	95% CI	VIF†
Final model (LASSO-selected multivariable logistic regression)							
BISAP score	2.131	0.546	15.24	<0.001	8.42	2.89–24.51	1.62
MCTSI score	0.519	0.168	9.55	0.002	1.68	1.21–2.33	1.62
Constant	-4.121	0.623	43.70	<0.001	0.016	0.005–0.053	—

Model performance: AUC (temporal validation) = 0.843 (95% CI: 0.762–0.924); optimism-corrected C-statistic = 0.831 (bootstrap 2000 iterations). BISAP: Bedside Index for Severity in Acute Pancreatitis; MCTSI: Modified CT Severity Index; OR: Odds Ratio; VIF: Variance Inflation Factor; CI: confidence interval.

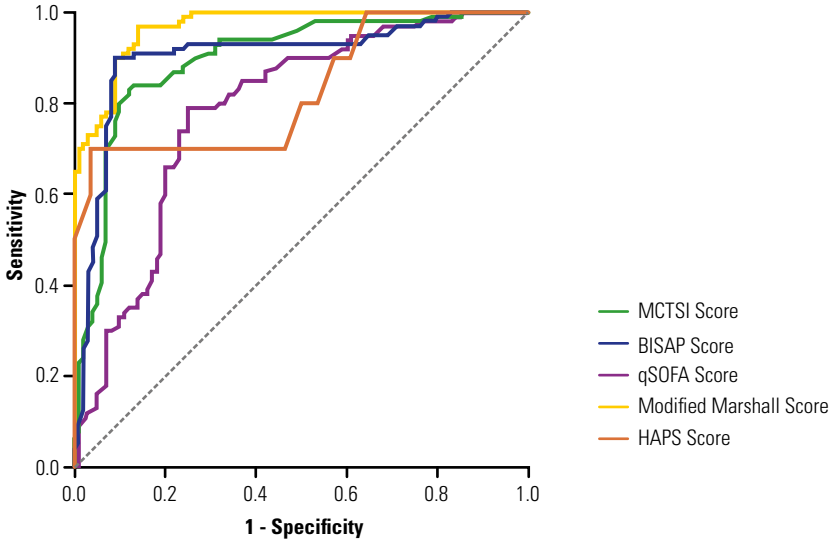


Figure 2. Receiver operating characteristic curves comparing the predictive performance of severity scoring systems for composite adverse outcomes.

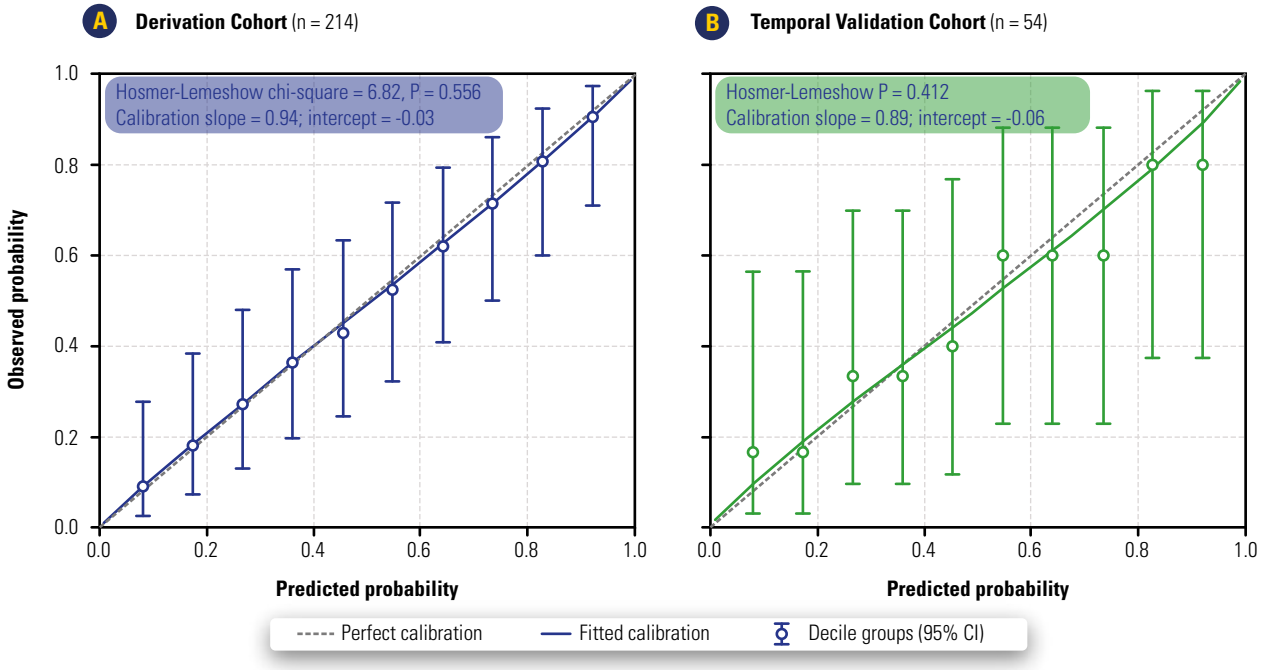


Figure 3. Calibration plots of the combined BISAP-MCTSI predictive model for the composite adverse outcome. (**A**) Derivation cohort (n = 214). Hosmer-Lemeshow test: $\chi^2 = 6.82$, $p = 0.556$; calibration slope = 0.94, intercept = −0.03. (**B**) Temporal validation cohort (n = 54). Hosmer-Lemeshow test: $p = 0.412$; calibration slope = 0.89, intercept = −0.06. The diagonal line represents perfect calibration. Data points represent decile groups of predicted risk with 95% confidence intervals.

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Subgroup and sensitivity analyses

Table 5 provides the subgroup and sensitivity analyses of the BISAP-MCTSI model. Model discrimination remained high in both the HTG-AP and NHTG-AP subgroups (AUC, 0.902 vs. 0.864; interaction p = 0.186). Performance also remained consistent across the diabetes and obesity strata, as well as across the admission time subgroups. Sensitivity analyses, including a complete-case analysis, the inclusion of equivocal-etiology cases, and an expanded admission window, yielded similar AUCs, supporting the robustness of the model (Table 5).

To evaluate the potential impact of etiological misclassification, we performed a sensitivity analysis excluding all 47 patients with overlapping etiologies (i.e., more than one identifiable risk factor). In this restricted cohort (n = 221; HTG-AP, n = 100; NHTG-AP, n = 121), the composite adverse outcome rate remained significantly higher in the HTG-AP group than in the NHTG-AP group (28.9% vs. 13.2%; p = 0.004). The BISAP-MCTSI model maintained robust discrimination (AUC = 0.879; 95% CI: 0.812–0.946), consistent with the primary analysis (AUC = 0.887). Furthermore, among the 47 patients with mixed

Table 5. Subgroup and sensitivity analyses of the BISAP-MCTSI predictive model performance

Analysis type	Subgroup/condition	n	AUC (95% CI)	Sensitivity (%)	Specificity (%)	*p*-value for interaction /comparison
Primary subgroup analyses						
Etiology	HTG-AP	128	0.902 (0.842–0.962)	85.3	82.6	0.186
	NHTG-AP	140	0.864 (0.789–0.939)	80.9	79.8	
Diabetes status	Diabetic patients	92	0.893 (0.824–0.962)	83.7	81.2	0.542
	Non-diabetic patients	176	0.881 (0.815–0.947)	82.4	80.5	
Obesity status	Obese (BMI ≥30)	115	0.879 (0.808–0.950)	81.8	79.4	0.634
	Non-obese (BMI <30)	153	0.892 (0.829–0.955)	83.9	81.7	
Time to admission	0–12 hours	98	0.895 (0.828–0.962)	84.2	82.1	0.287
	12–24 hours	116	0.878 (0.810–0.946)	81.6	79.8	
Sex	Male	189	0.882 (0.821–0.943)	82.5	80.3	0.724
	Female	79	0.896 (0.826–0.966)	84.1	82.4	
Disease severity	Mild AP	168	0.854 (0.778–0.930)	79.8	78.2	0.312
	Moderately severe/severe AP	100	0.908 (0.852–0.964)	86.4	83.5	
Etiological overlap analyses						
Mixed etiology subgroup	Patients with ≥2 risk factors	47	0.856 (0.756–0.956)	79.2	77.1	—
Excluding mixed etiologies	Single etiology only	221	0.879 (0.812–0.946)	82.8	80.6	0.623[§]
Sensitivity analyses						
Missing data handling	Complete-case analysis (no imputation)	248	0.884 (0.825–0.943)	82.6	80.4	0.756[§]
	Original analysis (with imputation)	268	0.887 (0.828–0.946)	83.1	80.9	
Etiology classification	Including equivocal-etiology patients	280	0.881 (0.823–0.939)	82.2	79.8	0.623[§]
	Excluding equivocal-etiology patients	268	0.887 (0.828–0.946)	83.1	80.9	
Admission time window	0–72 hours admission	312	0.869 (0.812–0.926)	80.5	78.6	0.184[§]
	0–24 hours admission (primary)	268	0.887 (0.828–0.946)	83.1	80.9	
Outcome definition	Necrosis >30% threshold	268	0.892 (0.835–0.949)	83.8	81.4	0.714[§]
Propensity score matching	Matched HTG-AP vs NHTG-AP	78 pairs (n=156)	0.875 (0.802–0.948)	82.0	79.5	0.012[††]

HTG-AP: hypertriglyceridemic acute pancreatitis; NHTG-AP: non-hypertriglyceridemic acute pancreatitis; AUC: area under the receiver operating characteristic curve; CI: confidence interval; BMI: body mass index; AP: acute pancreatitis; OR: odds ratio; BISAP: Bedside Index for Severity in Acute Pancreatitis; MCTSI: Modified CT Severity Index.
Sensitivity and specificity are reported at the optimal Youden's index cutoff for each subgroup.
P-values for interaction were derived from logistic regression models including a subgroup × BISAP–MCTSI predicted probability interaction term.
[§] P-value for comparison of AUC between sensitivity analysis and primary analysis using DeLong's test.
[††] P-value for composite outcome rate comparison between propensity score–matched HTG-AP and NHTG-AP groups using McNemar's test.
Propensity score matching was performed using 1:1 nearest-neighbor matching without replacement (caliper = 0.2 SD of the logit of the propensity score), adjusting for age, sex, BMI, diabetes mellitus, hypertension, and time from onset to admission. Standardized mean differences for all matched covariates were <0.10 after matching.

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etiologies, the composite adverse outcome rate was 25.5% (12/47), and the BISAP-MCTSI model demonstrated adequate discrimination within this subgroup (AUC = 0.856; 95% CI: 0.756–0.956). These findings indicated that etiological misclassification in overlap cases did not substantially drive the primary results (**Table 5**).

DISCUSSION

This retrospective cohort study of 268 patients with AP yielded three principal findings. First, HTG-AP constituted a distinct clinical phenotype characterized by younger age, male predominance, higher BMI, greater recurrence rates, and worse in-hospital outcomes. Second, HTG-AP exhibited a characteristic early biochemical signature featuring heightened systemic inflammation, profound atherogenic dyslipidemia, disturbed glucose homeostasis, and lower circulating thyroid hormones. Third, the combined BISAP-MCTSI model provided superior prediction of the composite adverse outcome compared with several commonly used individual scoring tools. These findings supported etiology-aware, integrated risk stratification in AP.

Comparison with existing literature

Our findings aligned with previous studies that have identified HTG-AP as an increasingly common subtype, particularly among younger patients (18). Previous studies have reported a rising incidence of HTG-AP in populations with a high prevalence of obesity, type 2 diabetes, and metabolic syndrome. These comorbid conditions contribute to elevated serum TG levels and remain well-established risk factors for HTG-AP (19,20).

Compared with biliary-induced pancreatitis, which commonly affects older adults, previous research has frequently associated HTG-AP with more severe systemic inflammation and an increased risk of early organ dysfunction (21). This aligned with our observation of elevated inflammatory biomarkers and metabolic ratios (TG/HDL, LDL/HDL) in the HTG-AP group, which indicated a more aggressive clinical course even in the early phase of the disease.

Previous studies have validated the BISAP score as a practical tool for the early identification of high-risk patients with AP because of its simplicity and reliance on readily available clinical parameters (22,23). Our findings reinforced this conclusion, demonstrating that the BISAP score, alongside the Modified Marshall score, remained a robust and independent predictor of severe disease within the specific context of HTG-AP. These results supported the broader applicability of these tools across etiological subtypes and highlighted their clinical utility in diverse patient populations.

Although both BISAP and MCTSI are well-established severity assessment tools for AP, and combining bedside clinical scores with imaging-based assessments is not unprecedented, the specific contribution of the present study merits clarification. To our knowledge, previous literature has not formally evaluated the combination of BISAP and MCTSI as an integrated predictive model for a composite adverse outcome in a cohort enriched for HTG-AP. Furthermore, no prior research has systematically compared its performance against four other commonly used scoring systems within this specific etiological context using LASSO-based variable selection, temporal validation, and rigorous multiple comparison correction. The principal contribution of this study lies not in proposing a novel scoring instrument per se, but rather in providing empirical evidence that this specific combination offers complementary prognostic information. Specifically, BISAP captured early systemic physiological derangement, and MCTSI captured local pancreatic and peripancreatic disease burden. Furthermore, our findings demonstrated that this combination maintained robust discriminative performance across both the HTG-AP and NHTG-AP subgroups (AUC, 0.902 vs. 0.864; interaction $p = 0.186$), an observation not previously reported.

Clinical implications

These findings carry important clinical implications for the management of AP, particularly in patients with hypertriglyceridemia-induced disease. Given the younger age and higher recurrence rate observed in HTG-AP, early identification and close monitoring are essential, especially in patients presenting with recurrent episodes or known metabolic disorders. Our results support a two-tier approach to early risk

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stratification. For immediate bedside assessment, the BISAP score demonstrated the strongest individual predictive performance and can be calculated within 24 hours of admission using readily available clinical and laboratory data. When contrast-enhanced CT is performed—as is routine in many centers within the first 72 hours—incorporating the MCTSI into a combined BISAP-MCTSI model further improves prognostic accuracy. This combined approach outperformed Ranson, qSOFA, and HAPS after correction for multiple comparisons, and performed comparably to the Modified Marshall score, which has the inherent limitation of partial circularity when used to predict organ failure-defined outcomes.

Furthermore, lipid-related markers (e.g., TG/HDL-C and LDL/HDL-C ratios) may serve as additional risk stratification indicators in hypertriglyceridemic patients. Although not currently integrated into standard scoring systems, these ratios reflect the extent of metabolic disturbance and inflammatory burden and warrant further investigation as potential adjuncts in clinical decision-making. Rapid and accurate severity assessment is critical to guide early fluid resuscitation, ICU admission, and escalation of care when appropriate (22).

Building on the quantitative risk stratification results, the proposed three-tier classification (low/intermediate/high risk based on BISAP and MCTSI thresholds) provides a pragmatic framework for clinical decision-making. Notably, the high negative predictive value (95.8%) of the low-risk category may be particularly useful for identifying patients who can be safely managed in general ward settings, thereby optimizing resource allocation. Conversely, the high-risk category (BISAP ≥2 AND MCTSI ≥4), although associated with a moderate positive predictive value (41.8%), identifies a subgroup with more than a 30% probability of adverse outcomes, warranting proactive escalation of care. We emphasize that these thresholds require prospective external validation before formal incorporation into clinical protocols.

Pathophysiological mechanisms underlying enhanced inflammation in HTG-AP

The heightened inflammatory response observed in HTG-AP is likely multifactorial. Markedly elevated TG increase circulating chylomicrons, and intrapancreatic hydrolysis of TG by pancreatic lipase generates excess free fatty acids (24). When albumin-binding capacity is exceeded, unbound free fatty acids exert direct cytotoxic effects on acinar cells and vascular endothelium, aggravating local pancreatic injury and increasing capillary permeability. Free fatty acids can also promote mitochondrial dysfunction and oxidative stress, amplifying inflammatory signaling (e.g., NF-κB activation) and downstream cytokine release (e.g., TNF-α, IL-1β, and IL-6) (24,25). In addition, hypertriglyceridemia increases blood viscosity and impairs pancreatic microcirculation, predisposing to microvascular ischemia and tissue hypoxia, which further augments systemic inflammation and may contribute to early organ dysfunction (26).

The lower FT4 and FT3 levels observed in HTG-AP are consistent with non-thyroidal illness syndrome, a common response to acute critical illness. This hormonal pattern may reflect inflammatory severity and altered peripheral thyroid hormone metabolism rather than primary thyroid disease; however, whether thyroid hormone changes are merely markers of severity or contribute mechanistically to disease progression requires prospective study.

Limitations and future directions

Several limitations of this study merit consideration. First, the single-center, retrospective design conducted at a tertiary referral hospital introduces potential biases at multiple levels. Referral bias may have resulted in an overrepresentation of HTG-AP cases and disproportionately severe disease compared with the general AP population. Although standardized data abstraction forms with dual verification were employed, the information bias inherent to retrospective data extraction cannot be fully excluded. Furthermore, residual confounding by unmeasured variables, such as dietary habits, medication adherence, genetic predisposition to hypertriglyceridemia, and socioeconomic factors, may have influenced our results.

While multiple analytical strategies were used to mitigate confounding, including LASSO regularization, propensity score matching, and comprehensive subgroup and sensitivity analyses, these approaches

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cannot fully substitute for prospective randomization. Accordingly, external validation in multicenter, geographically diverse cohorts encompassing both tertiary and community hospital settings is essential before the BISAP-MCTSI model can be recommended for widespread clinical adoption. Although temporal split validation and bootstrap resampling provide some protection against overfitting, these internal validation approaches are not a substitute for true external validation in geographically and demographically distinct populations. To address this, we initiated a multicenter collaboration with three additional institutions spanning different geographic regions and healthcare settings. This effort aims to prospectively validate the BISAP-MCTSI model in an independent cohort of approximately 500 patients with AP, with results anticipated within 18 months. Until external validation is completed, the current findings should be considered hypothesis-generating, and the proposed risk stratification scheme should be applied with clinical judgment rather than treated as a definitive decision rule.

Second, the temporal validation cohort comprised only 54 patients with an estimated 11 to 12 composite outcome events, which limits the precision of the validation AUC estimate (95% CI: 0.762–0.924) and the statistical power of the calibration assessment. Consequently, the non-significant Hosmer-Lemeshow test (p = 0.412) in this small sample should be interpreted cautiously, as this test has limited power to detect miscalibration when event counts are low. Larger prospective validation studies with adequate event rates are needed to confirm the model's calibration and discrimination.

Third, the MCTSI relies on contrast-enhanced CT and is therefore inherently influenced by the timing of image acquisition and interobserver variability in radiological interpretation. In clinical practice, contrast-enhanced CT is not uniformly performed within a standardized time window, which may introduce heterogeneity into MCTSI scoring. Future multicenter studies incorporating standardized imaging protocols, predefined scan timing, and reproducibility assessments (e.g., automated or semi-automated imaging metrics) would strengthen the generalizability and reliability of this scoring component.

Fourth, we focused exclusively on in-hospital outcomes and did not assess longer-term endpoints, such as recurrent pancreatitis, the development of endocrine or exocrine pancreatic insufficiency, post-discharge mortality, or quality of life. Given the notably high recurrence rate observed in the HTG-AP group (39.8%), future prospective cohorts should incorporate longitudinal follow-up to evaluate whether early triglyceride-lowering strategies (e.g., fibrate therapy, insulin infusion, or plasmapheresis) and comprehensive metabolic risk modification can reduce disease recurrence and improve long-term outcomes.

Fifth, although a standardized hierarchical adjudication protocol with independent review was employed, and sensitivity analyses excluding patients with overlapping etiologies yielded consistent results, etiological classification in AP remains inherently imperfect when multiple risk factors coexist. In the present study, 47 patients (17.5%) had more than one identifiable etiological factor. While inter-rater agreement was excellent (κ = 0.89), misclassification cannot be entirely excluded. Future multicenter studies employing prospective, protocolized etiological assessments, preferably incorporating genetic testing for familial hypertriglyceridemia, standardized quantification of alcohol consumption using validated instruments, and systematic biliary evaluation, would further enhance classification accuracy and strengthen causal inference.

Sixth, although we adopted a composite endpoint specifically designed to mitigate circularity concerns, the potential overlap between predictor and outcome components warrants acknowledgment. The BISAP score incorporates SIRS criteria, which partially overlap with the clinical parameters used to define organ failure. Notably, the LASSO variable selection procedure did not retain the modified Marshall score, whose components directly define one element of the composite outcome (persistent organ failure), thereby providing some reassurance against circular prediction. Nevertheless, this inherent limitation should be considered when interpreting the predictive performance of the BISAP score. Future studies may benefit from employing strictly outcome-independent predictors or alternative endpoint definitions to further address this concern.

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Lastly, missing data affected 20 patients (7.5%) in the primary analysis. Although imputation was performed and a complete-case sensitivity analysis (n = 248) yielded concordant results (AUC = 0.884 vs. 0.887), the impact of missing data on model performance cannot be entirely excluded. Future prospective studies utilizing protocolized data collection will help minimize this concern.

In conclusion, HTG-AP is a metabolically distinct subtype of acute pancreatitis characterized by younger age, higher BMI, greater disease recurrence, and a stronger inflammatory response; furthermore, it is associated with a higher risk of adverse in-hospital outcomes. For early risk stratification, a combined BISAP-MCTSI approach demonstrated better predictive performance than several commonly used tools, supporting integrated bedside and imaging-based assessments when CT is available. Additionally, lipid-related indices may provide further prognostic information and warrant continued validation.

Authors' contributions: Zhen Chen contributed to the conceptualization, methodology, data collection, and writing of the original draft. Min-xing Liu was responsible for data analysis, investigation, and writing — review and editing. Zhen-yu Chu performed data curation and validation, and contributed to writing — review and editing. Li-min Jia, as the corresponding author, was in charge of supervision, project administration, and writing — review and editing. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Definitions, components, score ranges, and assessment time windows of severity scoring systems

Scoring System	Components	Score Range	Assessment Time Window	High-Risk Threshold	Reference
BISAP	BUN >25 mg/dL; impaired mental status (GCS <15); SIRS (≥2 criteria); age >60 years; pleural effusion	0-5	Within 24 h of admission	≥3	Wu et al., 2008
MCTSI	Pancreatic inflammation (0/2/4); pancreatic necrosis (0/2/4); extrapancreatic complications (0/2)	0-10	At time of CECT (typically within 72 h)	≥4	Mortele et al., 2004
Modified Marshall	Respiratory (PaO2/FiO2); renal (creatinine); cardiovascular (SBP) - each scored 0-4	0-4 per system	Within 24 h; reassessed at 48 h for persistence	≥2 in any system	Banks et al., 2013
Ranson's Criteria	5 criteria at admission + 6 criteria at 48 h (see Methods for details)	0-11	Admission + 48 h	≥3	Ranson et al., 1974
qSOFA	Altered mental status (GCS <15); SBP <=100 mmHg; RR ≥22/min	0-3	Within 24 h of admission	≥2	Singer et al., 2016
HAPS	No rebound tenderness/guarding; normal hematocrit; normal creatinine	0-3	Within 24 h of admission	=3 (harmless)	Lankisch et al., 2009

BISAP: Bedside Index for Severity in Acute Pancreatitis; BUN: blood urea nitrogen; GCS: Glasgow Coma Scale; SIRS: systemic inflammatory response syndrome; MCTSI: Modified CT Severity Index; CECT: contrast-enhanced computed tomography; SBP: systolic blood pressure; PaO2: partial pressure of arterial oxygen; FiO2: fraction of inspired oxygen; qSOFA: quick Sequential Organ Failure Assessment; RR: respiratory rate; HAPS: Harmless Acute Pancreatitis Score.

Supplementary Table S2. Spearman correlation matrix of six severity scoring systems (n = 268)

	BISAP	MCTSI	Modified Marshall	Ranson	qSOFA	HAPS
BISAP	1.00	0.52	0.68	0.46	0.58	-0.44
MCTSI	0.52	1.00	0.49	0.42	0.38	-0.36
Modified Marshall	0.68	0.49	1.00	0.44	0.62	-0.48
Ranson	0.46	0.42	0.44	1.00	0.35	-0.32
qSOFA	0.58	0.38	0.62	0.35	1.00	-0.41
HAPS	-0.44	-0.36	-0.48	-0.32	-0.41	1.00

Values represent Spearman rank correlation coefficients (rho).

All correlations were statistically significant at $P < 0.01$ (two-sided) except where noted.

HAPS scores are inversely correlated with the other scoring systems because higher HAPS scores indicate a predicted "harmless" (non-severe) course, whereas higher scores on the other systems indicate greater severity.

BISAP: Bedside Index for Severity in Acute Pancreatitis; MCTSI: Modified CT Severity Index; qSOFA: quick Sequential Organ Failure Assessment; HAPS: Harmless Acute Pancreatitis Score.

Supplementary Table S3. Distribution of coexisting etiological risk factors by study group

Coexisting Etiological Factor	Total (n=268)	HTG-AP (n=128)	NHTG-AP (n=140)	Chi-square/Fisher's	P-value
Any coexisting etiological factor, n (%)	47 (17.5)	28 (21.9)	19 (13.6)	chi-square = 3.195	0.074
TG ≥5.65 mmol/L + alcohol use, n (%)	30 (11.2)	18 (14.1)	12 (8.6)	chi-square = 2.053	0.152
TG ≥5.65 mmol/L + biliary disease, n (%)	14 (5.2)	8 (6.3)	6 (4.3)	chi-square = 0.529	0.467
TG ≥5.65 mmol/L + alcohol + biliary, n (%)	3 (1.1)	2 (1.6)	1 (0.7)	Fisher's exact	0.607
Single identifiable etiology only, n (%)	221 (82.5)	100 (78.1)	121 (86.4)	chi-square = 3.195	0.074

Coexisting etiological factors refer to the presence of more than one identifiable risk factor for acute pancreatitis in the same patient (e.g., hypertriglyceridemia plus significant alcohol consumption, or hypertriglyceridemia plus biliary disease).

The predominant etiology for each patient was assigned using the hierarchical adjudication protocol described in the Methods section.

TG: triglycerides; HTG-AP: hypertriglyceridemic acute pancreatitis; NHTG-AP: non-hypertriglyceridemic acute pancreatitis.

P-values were calculated using chi-square test or Fisher's exact test as appropriate.

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original

Glycemic responses to low-intensity, short-duration aerobic exercise sessions in hospitalized women with gestational diabetes: a pilot study

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ABSTRACT

Objective: This study aimed to investigate the effects of low-intensity aerobic exercise performed once (30 min; A30) or twice a day (15+15 min; A15+15) on pre- and postprandial capillary blood glucose (CBG) levels on the day of the exercise session (D1) and the following day (D2) in hospitalized women with gestational diabetes mellitus (GDM). **Subjects and methods:** In this crossover study, nine participants adhered to a standard hospital diet and completed 2-day trials under three conditions: A30 (30 min in the afternoon), A15+15 (15 min in the morning and afternoon), and resting (REST). Participants completed the REST condition first. Preprandial (immediately before meals) and postprandial (60 min after main meals) CBG levels were measured. Exercise sessions comprised cycling at 50%–65% of maximal heart rate using a friction-based pedal. Data were analyzed using generalized estimating equations with Bonferroni post hoc analysis. **Results:** Pooled data from D1 and D2 (six measurements each day) showed lower CBG levels in the A15+15 condition (5.7 ± 0.3 mmol/L) compared to REST (6.3 ± 0.6 mmol/L) (~-9.5%) (p = 0.001); however, this finding carried limited clinical significance. There were no differences among the three experimental conditions when considering the same pre- and postprandial time points. **Conclusion:** Short-duration, low-intensity aerobic exercise sessions (A15+15) attenuated overall daily (pre- and postprandial) glycemia in hospitalized women with GDM. This attenuation was only observed in the pooled data, as glucose levels did not differ at specific postprandial time points.

Keywords: Gestational diabetes; blood glucose; postprandial period; aerobic exercise; hospitalization

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized by carbohydrate intolerance diagnosed during pregnancy. It represents a significant health concern that increases the risk of unfavorable perinatal outcomes (1). The rising prevalence of GDM parallels the obesity epidemic among women of childbearing age (2). Maintaining maternal glycemic control is associated with lower rates of miscarriage, congenital malformations, fetal growth restriction, fetal macrosomia, and neonatal hypoglycemia.

GDM affects approximately 17% of pregnancies globally (3,4). Affected women face an increased risk of subsequently developing obesity, type 2 diabetes mellitus (T2DM), recurrent GDM, and cardiovascular morbidity (5). Data from the Brazilian Pelotas cohort study demonstrated that only about one-sixth of pregnant women engage in leisure-time physical activity (6). Initiating an exercise routine during pregnancy remains safe for most women, with few contraindications (7,8). Because patients typically interact more frequently with the healthcare system during pregnancy, this period provides an opportune window for establishing lasting lifestyle modifications.

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Diet and physical exercise represent the first-line treatments for GDM, supplemented by pharmacological therapy when necessary (9). Unlike insulin or oral antidiabetic agents, exercise incurs minimal costs and avoids medication-related side effects. Some health guidelines suggest dividing daily exercise into smaller blocks to make daily goal more achievable (7,10). In this regard, recent evidence has shown that pregnant women with GDM who engaged in 20 minutes (min) of interval walking after each main meal (breakfast, lunch, and dinner) over a 4-day period presented reduced glucose excursions at specific time points (3). Although lifestyle modifications can mitigate perinatal risks, some sedentary women may still require hospitalization to correct their glycemic values.

Most women with GDM fail to meet recommended physical activity levels due to time constraints, tiredness, household work, raising children, safety concerns, and/or cultural beliefs that pregnancy requires absolute resting (10–12). Consequently, relatively short-duration (≤ 20–30 min), low-intensity sessions offer a practical strategy to increase exercise adherence among patients experiencing fatigue and limited time. Intermittent walking training has also been shown to improve cardiometabolic risk factors, including blood lipids, blood pressure, and body composition, in women with obesity (13). Exercise is an adjuvant tool for improving the metabolic health of women and their fetus, in addition to potentially reducing healthcare costs by shortening hospital stays. Because current guidelines recommend 60–150 min of aerobic exercise per week for women with GDM, clinicians must evaluate different continuous aerobic exercise (CAE) regimens of equivalent daily volume (e.g., 30 min) to increase exercise compliance in these individuals' daily routines. Therefore, we aimed to investigate the effects of low-intensity, short-duration aerobic exercise sessions performed once daily (30 min) or divided into two 15-min bouts (15+15 min) on pre- and postprandial glucose levels during the day of exercise and the following day in hospitalized women with GDM.

SUBJECTS AND METHODS

Participants

We recruited participants from June 2022 to May 2023 at a tertiary public assistance and delivery hospital in southern Brazil. After screening medical and nursing records at the hospital's Division of Obstetrics and Gynecology, we invited potentially eligible hospitalized patients to participate. Interested individuals were fully informed about the experimental procedures and provided informed consent. The hospital operates under an open-door policy, focusing on the care of women with high-risk pregnancies, including preterm labor, endocrine disorders, infectious or hemodynamic complications, and other disturbances. All participants received outpatient GDM management at specialized ambulatory care (endocrinology and metabolism, obstetrics, and nursing services) before and after this hospital admission. This study was approved by the local Ethics Committee (registration no. 31570720.5.0000.5317) and registered in the Brazilian Registry of Clinical Trials (ID: RBR-3xsnyd).

The inclusion criteria comprised (a) presence of GDM (fasting glycemia 5.1–6.9 mmol/L and/or 2-h glycemia 8.5–11.0 mmol/L following a 75-g oral glucose tolerance test) (14) in the current pregnancy; (b) gestational age between 26 and 37 weeks (third trimester); (c) age between 18 and 40 years; (d) body mass index between 20 and 45 kg/m^2; (e) a protein/creatinine ratio < 30 mg/mmol; and (f) physical inactivity (no planned and structured exercise in the previous three months). We excluded patients with type 1 diabetes mellitus or T2DM, infectious diseases, or significant impairment of renal or hepatic function. We also excluded participants with relative contraindications to exercise (severe anemia, uncontrolled hypertension or preeclampsia, orthopedic limitations, and heavy smoking) or absolute contraindications (hemodynamically significant heart disease, incompetent cervix/cerclage, persistent second- or third-trimester bleeding, placenta previa, premature labor during the current pregnancy, or ruptured embryonic membranes) (7).

Study design

This crossover study was designed to minimize alterations to the hospital's routine care. Participants acted as their own controls in this one-group repeated-measures counterbalanced design, enabling comparisons between a resting condition and two CAE protocols. Participants were instructed to only

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consume the diet provided by the Nutrition Service during hospitalization, consisting of three main meals and three snacks. Medical staff prescribed pharmacological treatments for GDM (i.e., insulin and metformin) during hospitalization according to established recommendations (15).

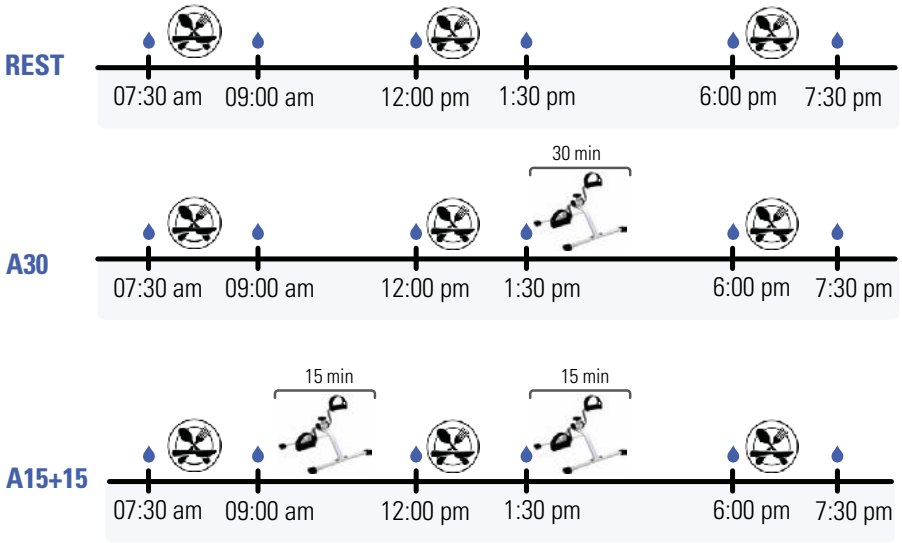
Participants completed 2-day trials under three conditions separated by at least 48 hours: 30 min of aerobic exercise in the afternoon (A30), 15 min of aerobic exercise in the morning and 15 min in the afternoon (A15+15), or a resting condition (REST). Day 1 (D1) was considered the exercise day, and Day 2 (D2) was the subsequent non-exercise day. Despite a systematic bias, REST was the first condition accomplished, since exams (e.g., cervical dilation, screening for preeclampsia, ultrasonography imaging, hepatic, thyroid, and renal biochemical parameters) were done or asked by medical staff in the first hours/days of hospitalization for complete screening of each case. After medical staff establishing the clinical management plan (including glycemic control), we randomized the exercise protocols (A30 and A15+15) using a simple coin toss (1:1 ratio). Only participants who completed at least two experimental conditions (REST plus A30 or REST plus A15+15) before discharge were considered in the statistical analysis.

In A30 condition, exercise sessions were conducted at 1:30 pm; in the A15+15 condition, sessions began at 9:00 am and 1:30 pm. Participants performed the A30 and A15+15 protocols approximately 60 min after finishing breakfast or lunch, matching the typical time of peak glycemia (4). Throughout their hospital stay, we measured patients' capillary blood glucose (CBG) levels at the following time points: 7:30 am (fasting), 9:00 am (~60 min after finishing breakfast), 12:00 pm (immediately before lunch), 1:30 pm (~60 min after finishing lunch), 6:00 pm (immediately before dinner), and 7:30 pm (~60 min after finishing dinner). Hospitalized patients also received snacks between breakfast and lunch, between lunch and dinner, and between dinner and bedtime. CBG levels were also measured immediately before and after each exercise session. **Figure 1** presents the study design for the exercise day (D1).

Experimental conditions

The following experimental conditions were employed:

- **A30:** Aerobic exercise for 30 min in the afternoon.
- **A15+15:** Aerobic exercise for 15 min in the morning and 15 min in the afternoon.
- **REST:** Resting, in which participants did not exercise.



REST: resting condition; A15+15: 15 min of aerobic exercise in the morning and 15 min in the afternoon; A30: 30 min of aerobic exercise in the afternoon.

Figure 1. Study design. : Capillary blood glucose measurement; : meal; : cycling.

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Participants performed all exercise sessions at 50%–65% of their maximal heart rate (HR_{max}) (7,16) using a friction-based pedal device (seated cycling) in their hospital rooms. Exercise physiologists supervised the sessions to continuously adjust pedal rate or resistance to maintain target heart rate (HR), monitor intensity, and provide constant motivation. Participants wore a finger pulse oximeter during exercise to measure oxygen saturation and HR every 3 min. Water was provided *ad libitum* during the exercise sessions.

Glucose measurements and diet control

CBG levels were measured using a handheld glucose meter (On Call® Plus II, ACON, USA) at the time points detailed above. Participants would ingest a 20-g carbohydrate gel if their CBG levels were ≤ 4 mmol/L before starting any exercise protocol. Participants were strongly encouraged to only consume the hospital's diet to minimize nutritional bias. Total energy and carbohydrate intake provided by the Nutrition Service were compared each day of the experimental period.

Statistical analysis

Glycemic response (primary outcome) and secondary outcome variables were analyzed using generalized estimating equations (GEE). We based model selection for the best overall fit on the lowest Quasi information criterion, employing an unstructured working correlation matrix (robust estimator), a linear or gamma distribution (according to lower Quasi information criterion value), and an identity link function. The main effects were determined by Wald's χ^2 statistic and a sequential Bonferroni post hoc test was conducted for pairwise comparisons.

GEE analyses were performed to examine the main effects of the three conditions (A30, A15+15, REST) and six time points (7:30 am, 12:00 pm, and 6:00 pm on D1 and D2; or 9:00 am, 1:30 pm, and 7:30 pm on both D1 and D2), as well as their respective interaction effects. Separate GEE analyses were conducted for preprandial and postprandial data. For dietary variables, insulin and metformin dosages, and mean CBG levels across days, GEE analyses assessed the main effects of the three conditions (A30, A15+15, REST) and two time points (D1 and D2), as well as their respective interaction effects. An additional GEE analysis compared CBG levels before and after each exercise session (A30, A15+15 in the morning, and A15+15 in the afternoon). Comparisons among the exercise conditions (A30, A15+15, and REST) for HR and CBG delta changes (after minus before CBG values) were analyzed using a general linear model. This model considered a normal distribution, and the main effects were assessed using pairwise comparisons with sequential Bonferroni post hoc tests. All statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS v. 20.0, USA) with an alpha level of 5%. Results are presented as the mean ± standard deviation.

RESULTS

Thirteen patients with GDM who met the eligibility criteria were invited to participate in the study (convenience sample). Two women declined to participate due to the perceived demands of the protocol, and two women were discharged from the hospital before completing at least one entire exercise protocol (A30 or A15+15). Descriptive data for the remaining participants (n = 9) and their childbirths are shown in **Table 1**.

Participants were ~32 years old, had 3.0 ± 1.2 total (prior and current) pregnancies, and delivered at a mean gestational age of ~37.6 weeks. The average length of

Table 1. Descriptive characteristics of the sample (n = 9) in their current pregnancy

Variable	Mean ± SD or absolute frequency
Gestational age at study entry (weeks)	32.2 ± 3
Age (years)	32.1 ± 3.3
Body mass index (kg/m²)	31.9 ± 6
Body weight (kg)	84.2 ± 13.2
Glycated hemoglobin (%)	5.4 ± 0.6
Systolic blood pressure (mmHg)	122 ± 7.8
Diastolic blood pressure (mmHg)	68.2 ± 6.6
Use of neutral protamine Hagedorn insulin (n)	4
Use of regular insulin (n)	2
Use of extended-release metformin (n)	7
Gestational age at delivery (weeks)	37.6 ± 0.6
Cesarean/vaginal birth (n)	8/1

SD: standard deviation.

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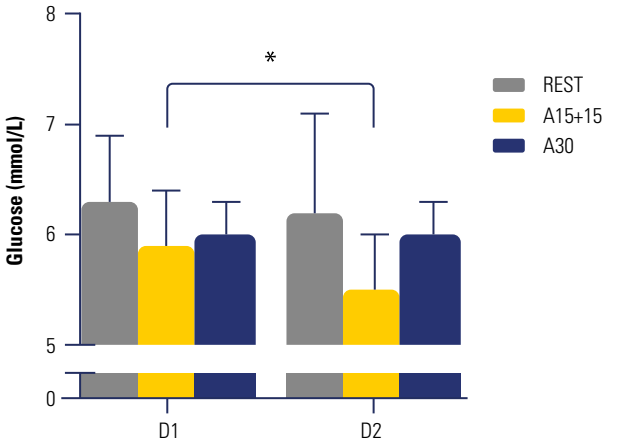
hospital stay was 8.4 ± 3.0 days. Eight participants did not give birth during their stay at the hospital (i.e., they were discharged and readmitted later for delivery). There were no multifetal gestations, and no infants were born weighing > 4 kg (macrosomia). Nine participants (100%) completed the REST condition, while the A30 and A15+15 conditions were each completed before hospital discharge by seven participants (77.8%).

Total daily energy intake provided by the Nutrition Service during hospitalization and experimental conditions on D1 and D2, respectively, was: 1628.0 ± 154.2 and 1628.0 ± 154.2 kcal (REST); 1605.7 ± 82.9 and 1669.3 ± 121.1 kcal (A15+15); and 1606.3 ± 145.7 and 1666.8 ± 103.6 kcal (A30). In this line, provided carbohydrate intake on D1 and D2, respectively, was: 176.6 ± 19.2 and 176.6 ± 19.2 g (REST); 184.9 ± 14.6 and 175.7 ± 13.0 g (A15+15); and 176.4 ± 16.2 and 182.6 ± 12.2 g (A30). Regarding a possible nutritional bias, there were no significant effects for total energy ($p = 0.963$ for condition, $p = 0.174$ for time/day, and $p = 0.397$ for interaction effects) or carbohydrate ($p = 0.778$ for condition, $p = 0.753$ for time/day, and $p = 0.120$ for interaction effects) intake across days and experimental conditions.

During the hospital stay, seven women used extended-release metformin, four received neutral protamine Hagedorn insulin, and two received regular insulin. Mean daily insulin dosages on D1 and D2, respectively, were: 1.1 ± 2.2 and 4.9 ± 5.4 IU (REST); 7.3 ± 7.9 and 8.0 ± 8.8 IU (A15+15); and 3.6 ± 5.0 and 5.8 ± 6.3 IU (A30). No differences were observed in condition ($p = 0.264$) or interaction ($p = 0.379$); however, a significant time/day effect was found ($\chi^2 = 5.696$, $p = 0.017$). These results indicate higher mean insulin dosages (pooled data from A30, A15+15, and REST) in D2 (6.2 ± 5.6 UI) than in D1 (4.0 ± 4.3 UI). Mean extended-release metformin dosages on D1 and D2, respectively, were: 1.0 ± 0.8 and 1.1 ± 0.5 g (REST); 0.6 ± 0.5 and 0.6 ± 0.5 g (A15+15); and 0.8 ± 0.8 and 0.6 ± 0.8 g (A30). No significant main effects of condition ($p = 0.057$) or time/day ($p = 0.651$), nor interaction effects ($p = 0.698$), were found.

CBG values measured immediately before and after each exercise session, respectively, were: 7.8 ± 0.8 and 7.0 ± 1.3 mmol/L (A15+15 in the morning); 6.2 ± 0.5 and 5.4 ± 0.5 mmol/L (A15+15 in the afternoon); and 6.4 ± 0.8 and 6.0 ± 0.8 mmol/L (A30). Significant main effects were observed for condition ($\chi^2 = 57.772$, $p < 0.001$) and time ($\chi^2 = 27.492$, $p < 0.001$), with no significant interaction ($p = 0.300$). CBG delta changes (after minus before CBG values) were also observed: -0.5 ± 1.3 mmol/L (A15+A15 in the morning), -0.7 ± 0.3 mmol/L (A15+A15 in the afternoon), and -0.5 ± 0.5 mmol/L (A30), with no differences among conditions ($p = 0.394$). Pooled pre- and post-exercise data revealed significantly higher CBG levels during the morning A15+15 session (7.4 ± 1.1 mmol/L) compared with both the afternoon A15+15 session (5.8 ± 0.5 mmol/L) and the A30 session (6.2 ± 0.8 mmol/L). In addition, reduced CBG levels (pooled data from A30, A15+15, REST) were found immediately after experimental conditions (6.1 ± 0.8 mmol/L) compared to those immediately before (6.7 ± 0.5 mmol/L). No hypoglycemic episodes (CBG ≤ 4.0 mmol/L) occurred immediately before, during, or immediately after exercise sessions, even among participants receiving insulin therapy. No exercise-related adverse events were reported. Mean HR values were similar ($p = 0.444$) across experimental conditions (~105 bpm; ~56% HR_{max}).

Figure 2 depicts mean CBG levels across D1 and D2 (pooled data from six time points each day). Mean CBG values observed on D1 and D2, respectively, were: 6.3 ± 0.6 and 6.2 ± 0.9 mmol/L (REST); 5.9 ± 0.5 and 5.5 ± 0.5 mmol/L (A15+15); and 6.0 ± 0.3 and



REST: resting condition; A15+15: 15 min of aerobic exercise in the morning and 15 min in the afternoon; A30: 30 min of aerobic exercise in the afternoon; D1: Day 1; D2: Day 2. A significant condition effect was observed between A15+15 and REST. *Statistical difference from REST.

Figure 2. Mean capillary blood glucose levels across both days (six measurements per day).

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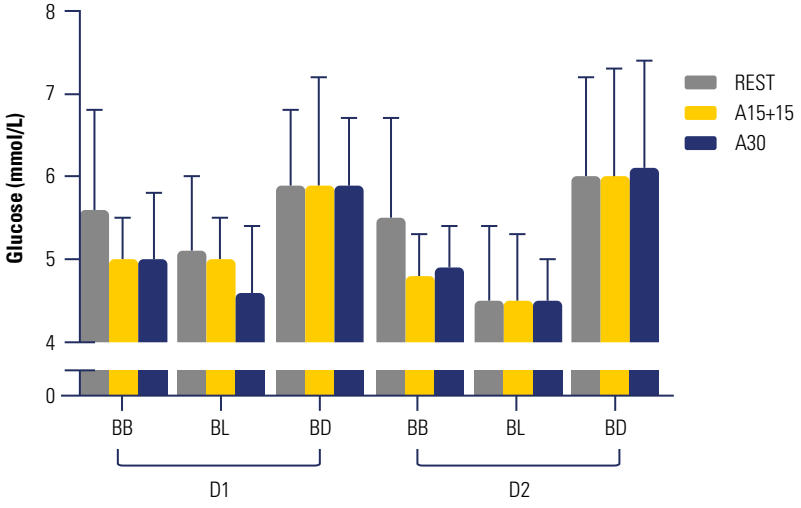
6.0 ± 0.3 mmol/L (A30). There were no differences in time/day (p = 0.09) or interaction (p = 0.320), albeit a condition effect was found (χ^2 = 15.078, p = 0.001). These results indicate that lower (~-9.5%) CBG levels (pooled data from D1 and D2, six measurements each day) were observed in A15+15 (5.7 ± 0.3 mmol/L) than in REST (6.3 ± 0.6 mmol/L).

Regarding preprandial CBG levels, GEE revealed a significant main effect of time (pooled data from six time points across D1 and D2; χ^2 = 78.260, p < 0.001), with no significant main effect of condition (p = 0.662) or interaction (p = 0.805). Pairwise comparisons of pooled data from the three experimental conditions showed significant differences between before breakfast (BB) on D1 and before lunch (BL) on D2 (p = 0.011). Preprandial CBG levels were significantly higher before dinner (BD) on D1 compared with BL on D1 (p = 0.004), BB on D2 (p = 0.003), and BL on D2 (p < 0.001). Similarly, CBG values were higher BD on D2 compared with BB (p = 0.023) and BL (p < 0.001) on D1, as well as BB (p = 0.001) and BL (p < 0.001) on D2. No significant differences were observed among the three experimental conditions when considering the same time points (**Figure 3**).

Analysis of postprandial CBG levels via GEE revealed a significant main effect of time (pooled data from six time points across D1 and D2; χ^2 = 52.678, p < 0.001), while condition (p = 0.613) and interaction (p = 0.816) effects were not observed. Pairwise comparisons of pooled data from the three experimental conditions indicated that CBG levels were significantly higher after breakfast (AB) on D1 compared with after lunch (AL) and after dinner (AD) on D1 (p < 0.001 for both), as well as AL and AD on D2 (p < 0.001 for both). Moreover, higher CBG values were observed AB on D2 compared with AD on D1 (p = 0.007) and AD on D2 (p = 0.047). Postprandial CBG levels peak after breakfast. Notably, no significant differences were observed among the three experimental conditions when considering the same time points (**Figure 4**).

DISCUSSION

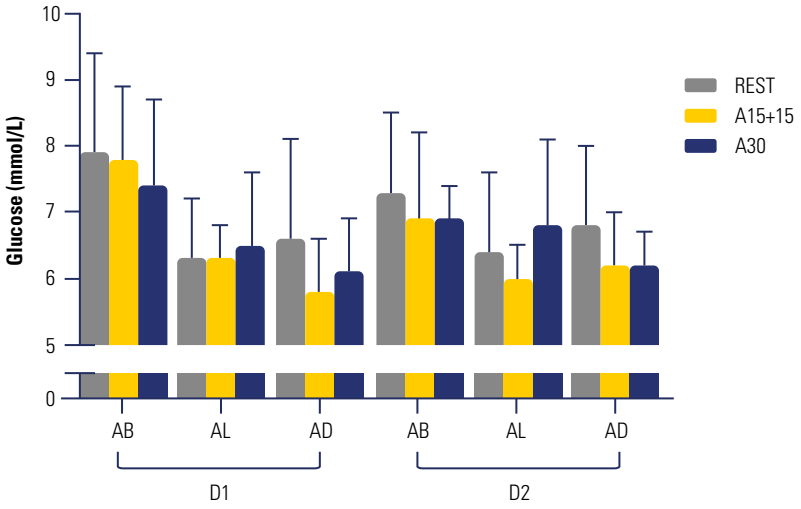
The main findings of this study include (1) lower CBG levels (pooled data from D1 and D2, representing the sum of pre- and postprandial glycemia each day) in the A15+15 condition compared with REST (~-9.5%), despite limited clinical relevance, and (2) no differences being found among the three experimental conditions at identical pre- and postprandial time points. To the best of our knowledge, this is the first study to compare the effects of low-intensity, relatively short-duration (30 min) CAE sessions, whether performed once (30 min) or divided into two 15-min bouts (15+15 min) on pre- and postprandial glycemia in hospitalized women with GDM.



REST: resting condition; A15+15: 15 min of aerobic exercise in the morning and 15 min in the afternoon; A30: 30 min of aerobic exercise in the afternoon; BB: before breakfast; BL: before lunch; BD: before dinner; D1: Day 1; D2: Day 2.

Figure 3. Preprandial capillary blood glucose levels under experimental conditions.

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REST: resting condition; A15+15: 15 min of aerobic exercise in the morning and 15 min in the afternoon; A30: 30 min of aerobic exercise in the afternoon; AB: after breakfast; AL: after lunch; AD: after dinner; D1: Day 1; D2: Day 2.

Figure 4. Postprandial capillary blood glucose levels under experimental conditions.

In the study by Andersen and cols. (3), following a 4-day period, the authors found lower glycemic excursions during diurnal hours in the first three days of the protocol in a sample comprising non-insulin treated GDM women completing 20-min moderate-intensity interval walking (alternating 3-min slow and fast intervals) three times a day (after each main meal, comprising 60 min/day) and with identical diets. Recently, Christie and cols. (4) and Brislane and cols. (5) compared the effects of three 10-min postprandial CAE walking sessions (totaling 30 min/day) with a single 30-min CAE walking session over 3-5 days. Neither study found differences in postprandial glycemia between the moderate-intensity exercise protocols and baseline conditions (4,5). However, women with GDM who completed the three 10-min postprandial walking sessions accumulated more daily minutes of prescribed physical activity than those in the 30-min CAE condition (4). Similarly, our study found no differences among the exercise conditions and REST at matched postprandial time points. We speculate that repeated performance of the A15+15 or A30 protocols over several days or weeks may be necessary to observe such changes in hospitalized women with GDM. The significance observed in the pooled data was likely a statistical artifact driven by the aggregation of non-matched data points that individually lacked sufficient statistical power. Indeed, in the case of our study, a larger sample size may have provided statistical differences from time-point data.

Previous work in participants with T2DM demonstrated that split exercise, specifically 20 min of moderate-intensity CAE before and 20 min after lunch, resulted in lower glycemia in the hours following lunch than 40 min of CAE performed after the same meal (17). Van Dijk and cols. (18) found that 15-min bouts of slow-paced strolling (~100 bpm) after each main meal (totaling 45 min/day) over three days lowered postprandial glycemia in non-insulin-treated patients with T2DM, compared with a sedentary control condition in which participants spent the day seated on a couch, talking, reading, or watching television. In this line, our findings showed interesting partial glycemic benefits from interrupting the sedentary behavior commonly observed during hospitalization (extended time spent lying or sitting). These benefits were achieved using simple, very short (two 15-min bouts/day), low-intensity CAE (~105 bpm, ~56% HR_{max}) in women with GDM. Moreover, because half of the participants used rapid-acting or intermediate-acting insulin, an additive effect between exogenous insulin and muscle contractility during the A15+15 protocol may have accelerated glucose uptake. Furthermore, pre-exercise CBG levels in the morning (~7.8 mmol/L) were higher than those before the afternoon exercise sessions (~6.3 mmol/L). This disparity was likely

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associated with exogenous insulin pharmacokinetics, specifically increased basal/bolus active insulin after lunch.

In the current study, eight of the nine participants with GDM exceeded the recommended upper fasting glucose limit of 5.1 mmol/L (14) in at least one experimental condition, although their glycated hemoglobin (HbA_{1c}) levels (~5.4% or 36 mmol/mol) indicated normal baseline glycemic control (**Table 1**). In addition, it is not surprising that CAE exercise did not induce hypoglycemic episodes in the time points measured even in women under insulin therapy, as the cycling sessions were relatively short, and meals and snacks were served at regular intervals throughout the participants' hospital stay.

Some limitations of our study must be acknowledged. First, the participant cohort was susceptible to selection bias, since the women who agreed to participate may have represented those with less complicated pregnancies, greater health awareness, or stronger commitment to lifestyle changes during their current pregnancy. Other relevant shortfalls of this pilot study include the relatively small sample size and the convenience sample, which partially limits its generalizability. Despite the possible bias, a finger pulse oximeter was used to monitor HR during exercise sessions due to its ease of cleaning during the COVID-19 pandemic. In this regard, a recent study found no significant differences between HR values recorded by a finger pulse oximeter and a 12-lead ECG during low-intensity exercise (19). The contemporary need to standardize sensor technologies in clinical and sports settings remains critical to ensure robust data validity (20). Therefore, future research should incorporate responsive devices to accurately evaluate recovery and physiological load (21) after exercise sessions in women with GDM, in addition to mitigating risks and enhancing performance (22).

Other critical limitations of this pilot study included a potential "period effect" (e.g., adaptation to the hospital environment, reduced stress over time, sleep normalization, or the stabilization of diet and medication). Because the REST condition was strictly performed first, the study design could not rule out an order effect. Another significant issue was the absence of a continuous glucose monitoring system. Although continuous glucose monitoring measures interstitial glucose levels every few minutes, detects subtle physiological excursions, and provides detailed and valuable monitoring of time-course glycemic changes (3,4), it entails substantial costs. In our study, glucose levels were measured using a hand-held glucose meter, since capillary tests require minimal blood volume, are highly cost-effective, and reflect the pragmatic methods utilized outside rigorous research settings (23,24) in the conventional management of diabetes.

Despite these limitations, the preliminary data provide novel insights for the research field and exercise prescription for women with GDM. This population is generally more likely to engage in low-intensity exercise than moderate intensity exercise. In addition to the relatively short-duration patterns, our study evidenced that low-intensity CAE sessions (especially A15+15) may partially modulate glycemia following routine everyday meals (and not oral glucose tolerance tests). Because these findings are limited to the acute and subacute effects of the exercise session(s), future research is required to investigate the impact of regular low-intensity, short-duration aerobic exercise training on the glycemic parameters in this population.

In conclusion, although the non-randomized allocation of the control condition remained a critical methodological flaw in this work, low-intensity and relatively short-duration (15 min) aerobic exercise sessions performed twice a day effectively attenuated mean daily (pre- and postprandial) CBG levels in hospitalized women with GDM compared to a complete sedentary pattern during a hospital stay, suggesting its potential in clinical practice. This attenuation was only observed in the pooled data, as specific postprandial time points showed no significant differences. No significant differences in glycemic parameters were observed between the A30 and A15+15 protocols. These findings hold relevance for endocrinology research and broader healthcare practice, as the A15+15 regimen aligns with established physical activity guidelines and offers novel clinical insights to inform exercise prescriptions.

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Data availability: the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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original

Quality of life outcomes in Brazilian adults with childhood-onset growth hormone deficiency: impact of transition-age growth hormone therapy

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ABSTRACT

Objective: This study evaluated the QoL of Brazilian adult patients with childhood-onset growth hormone deficiency GHD (CO-GHD), comparing those who continued GH replacement therapy (GHRT) after transition to adulthood with those who discontinued treatment and with healthy subjects. **Subjects and methods:** In this cross-sectional study, quality of life (QoL) was assessed using the Quality of Life Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA) questionnaire in 61 Brazilian adults aged 18-50 years. The sample included 31 patients with CO-GHD confirmed during the transition period, who continued or discontinued GHRT, and 30 matched controls. **Results:** GHD patients had poorer QoL, as indicated by higher QoL-AGHDA scores than controls (median 8 [IQR 5-14] vs 4 [IQR 2-8]; $p < 0.01$). Continued GHRT in adulthood did not impact QoL compared with discontinued GHRT (8 [IQR 6-16] vs. 9 [IQR 5-12], $p = 0.33$). Overall, women had lower QoL irrespective of GHD (9 [IQR 6-13] vs. 5 [IQR 2-8.5], $p = 0.01$, women and men, respectively). We found no association between QoL-AGHDA scores and education level ($p = 0.51$), short stature ($p = 0.78$), associated pituitary deficiencies ($p = 0.34$), or overweight/obesity ($p = 0.16$). **Conclusion:** GHD impairs quality of life in young adults; however, our cross-sectional cohort did not show significant improvements in quality of life associated with GH replacement therapy. A multidisciplinary approach combining individualized treatment and psychosocial interventions remains essential.

Keywords: GHD; GHRT; QoL-AGHDA; QoL

INTRODUCTION

The clinical presentation of hypopituitarism spans from isolated hormonal deficiency to complete absence of pituitary hormone production. Concomitant central nervous system abnormalities can be present in some cases (1,2). Growth hormone deficiency (GHD) is the most common isolated pituitary hormone deficiency (3,4). GHD leads to changes in body composition: higher body fat percentage and central obesity; lower lean mass and bone density; and higher risk for fracture. Patients with GHD have higher cardiovascular risk, higher total cholesterol, triglycerides, and low-density lipoproteins. Adults with GHD also report reduced physical capacity and decreased quality of life (QoL). These clinical manifestations vary with chronological age, age of onset of GHD, and other concomitant multiple pituitary hormone deficiencies (MPHD) (5-8).

QoL scores are important tools in research, clinical practice, and global health care policies (9). Compromised QoL is a main concern in adult patients with GHD, with concentration, memory, and tiredness being the most affected parameters (9-11).

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The Assessment of Quality of Life in Adults with Growth Hormone Deficiency (QoL-AGHDA) questionnaire is a specific tool and the most used questionnaire for GHD patients (12,13). It evaluates memory, concentration, fatigue, tension, social isolation, and self-confidence. QoL evaluation enables a cost-benefit analysis, which may help to determine the need for prolonged treatment with GH from childhood to adult life (14).

GH replacement therapy (GHRT) in adults with GHD improves body composition and bone density (5,15). However, the impact of GHRT on QoL remains controversial. Some studies demonstrate benefits in QoL in patients who received GHRT (9,11,16), while others show no improvements (17).

In Brazil, guidelines indicate which patients should receive GHRT (18). However, QoL is currently not part of the decision process. The establishment of QoL scores as validated tools to guide the management of patients with GHD may benefit clinical practice (19). The main goal of this study was to evaluate QoL in adult patients with childhood-onset GHD (CO-GHD) who received GHRT during longitudinal growth and continued treatment in adulthood, compared with those who discontinued GHRT after the transition to adult life, as well as in healthy controls. To our knowledge, this is the first study to compare the QoL of adult Brazilian GHD patients with and without GHRT, using a GHD-specific QoL score validated in the country.

SUBJECTS AND METHODS

Subjects

This is a cross-sectional study. The inclusion criteria were patients aged 18 to 50 years with CO-GHD, with isolated GHD or multiple pituitary hormone deficiencies (MPHD), who received GHRT for at least 3 years before the start of the study, and who had confirmed GHD after retesting during the transition period. The exclusion criteria were patients who presented with previous functioning pituitary tumors, malignant neoplasia, active inflammatory diseases, psychiatric illnesses, cognitive disabilities, diabetes mellitus, advanced kidney or liver failure, Turner Syndrome and other chromosomal anomalies, physical disabilities that prevented anthropometric measurements, and pregnancy.

GHD was confirmed by insulin tolerance test (ITT) with a cutoff of 5 μg/L in the transition period, and 3 μg/L in adulthood, or by glucagon stimulation test (GST), with a cutoff of 3 μg/L in eutrophic patients and 1 μg/L in overweight or obese individuals (5,20,21). In patients with MPHD and in those with genetic structural abnormalities of the hypothalamus or the pituitary gland, retesting was not necessary (20,21). MPHD was confirmed by hormone measurements, and adequate treatment was confirmed by normalization of values in the 6 months before the beginning of the study.

We evaluated 61 Brazilian adult patients aged 18 to 50 years. Of these, 31 were diagnosed with CO-GHD, including 15 patients who were undergoing GHRT treatment at the time of the study, 16 patients who had discontinued GHRT use more than two years before the study, and 30 controls matched for age, sex, and body mass index (BMI). This sample of patients represents the total population of eligible patients available at the tertiary center during the study period, between January 2024 and December 2025, who accepted to participate in this study, and 30 controls matched for age, sex, and body mass index (BMI). Therefore, a formal a priori sample size calculation was not performed. Thus, after adjusting the beta-binomial regression model, considering the main objective (comparison between GHRT-C and GHRT-I, we calculated the difference between means, already adjusted for BMI and GH use time, as well as the 95% confidence interval and the effect size, proposed by Cohen, adjusted for the same covariates, resulting from the same model. The difference between the adjusted means is 2.08 (95% CI: -2.94; 7.07), and the effect size (Cohen's d) is 0.34, indicating a small to medium effect. Among the 31 patients with CO-GHD had isolated GHD (n = 9) or MPHD (n = 22). Patients with MPHD were on replacement therapy with glucocorticoids (prednisone at doses ranging from 2.5 mg to 5 mg per day), thyroxin, gonadal steroids, and desmopressin. Patients in GHRT were using a fixed dose of GH of 0.3 mg/day. The criteria for maintaining or discontinuing growth hormone (GH) therapy during the transition period were not defined for this research protocol. The decision to maintain or discontinue GH therapy during the transition period and into adulthood was

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guided by the patient's own preference to continue GH replacement after reaching final height.

Anthropometric measures were evaluated using WHO growth curves, and short stature was defined as a Z-score of 2 standard deviation units or lower. Patients were followed in the Pediatric Endocrinology outpatient clinic until reaching 18-20 years of age and are now followed in the adult outpatient neuroendocrinology clinics at the São Paulo University Hospital of the Ribeirão Preto Medical School (HC-FMRP-USP), a tertiary referral center. Control patients were recruited among the families of patients and Hospital employees.

All patients signed an informed consent form, and the study was approved by the Clinics Hospital Research Ethics Committee (Approval number: CAAE 75126423.0.0000.5440).

Assessment of QoL

The QoL-AGHDA questionnaire was the instrument used in this study, after a license was obtained from Galen Research. QoL-AGHDA is a 25-question questionnaire scored as 1/0 (yes/no). The total score is calculated for all items, spanning from 0 to 25. Higher scores are associated with worse QoL (13); thus, in our study, a score of 11 or higher was considered poor QoL (22). The absence of 25 complete answers excluded the patient from the analyses. To compare scores across different subareas of the original questionnaire, we used the five areas defined by Koltowska (23), and the results are presented as the proportion of positive responses within each subarea. The questionnaire was administered during interviews supervised by a single author (KCO).

Anthropometric measurements

Anthropometric measurements were performed in all patients in the morning after a 12-hour fast. Body weight (kg) and height (m) were measured in a digital scale (Welmy W200A). BMI (kg/m²) was calculated, and patients were divided among groups: underweight (BMI < 18.5 kg/m²), eutrophic (≥ 18.5 and < 25 kg/m²), overweight (≥ 25 an < 30 kg/m²), and obese (≥ 30 kg/m²). Abdominal circumference was measured with a flexible and non-elastic metrical tape at the midpoint between the inferior costal margin and the iliac crest.

Statistical analysis

All numeric variables were examined for distribution using histograms, boxplots, and QQ-plots. The most appropriate central tendency measure was selected based on the distribution, and values are presented as means (± standard deviation, SD) or median (min-max or interquartile range [IQR]). Frequency tables for nominal categorical variables were compared using Fisher's Exact Test or Pearson's x^2 test. Proportions for binary variables were compared among ordinal categories using an ordinal logistic regression (proportional odds model). Normally distributed variables were compared between groups using the Student's t-test. Nonparametric variables were compared using the Mann-Whitney U Test. Pearson correlation was used to test correlations between numeric variables. To compare QoL-AGHDA scores among groups, we fitted a beta-binomial regression adjusted for sex, education level, sedentarism, alcoholism, presence of associated pituitary deficiencies, short stature, and obesity. All analyses and figures were made using open-source packages for R (v4.5.2): glmmTMB v1.1.14; emmeans v2.0.1 or Python (v3.13): matplotlib v3.10.8, numpy v2.4.2, pandas v3.0.1, scipy v1.17.0, seaborn v0.13.2, stats models v0.14.6.

RESULTS

The final study sample consisted of 31 patients with GHD and 30 controls, most of whom were males (22; 21, respectively), aged 18-47 and 19-49, respectively. The individual characteristics of each study participant are described in **Supplementary Table 1**. All of them completed the questionnaire in less than five minutes with no missing answers. Anthropometric, clinical, social, and demographic characteristics of the participants are described in **Table 1**. There were no differences in age, sex distribution, BMI, or marital status between GHD patients and controls. Patients with GHD were shorter, had a higher proportion of sedentary, and a lower proportion of alcohol consumption than controls. Moreover, the distributions of employment status and education levels differed between the groups, and controls were more likely to have completed 13 or more years of education. One patient from the control group was diagnosed with high blood pressure.

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Table 1. Anthropometric and demographic characteristics of the participants

	GHD (n = 31)	Controls (n = 30)	*p*
Age (years)	26 (18 – 48)	27 (20 – 49)	0.68
Males (n)	22 (71%)	21 (70%)	1.0
Height (SDS)	-1.3 (-3.3 – 0.7)	-0.3 (-1.9 – 3.0)	<0.01
BMI (kg/m²)	24.4 (15.4 – 39.3)	23.1 (17.7 – 39.4)	0.92
Overweight or obesity (n)	14 (45.2%)	14 (46.7%)	1.0
Smokers (n)	1 (3.2%)	3 (10%)	0.35
Alcoholism (n)	5 (16.1%)	13 (43.3%)	0.03
Sedentary (n)	23 (74.2%)	8 (26.7%)	<0.01
Marital status			0.11
Married	7 (22.6%)	13 (43.3%)	
Single	24 (77.4%)	17 (56.7%)	
Employment status			<0.01
Unemployed	8 (25.8%)	0	
Student	5 (16.1%)	15 (50%)	
Working	18 (58.1%)	15 (50%)	
Education level			<0.01
5 years or less	3 (9.7%)	1 (3.3%)	
6 – 9 years	1 (3.2%)	0	
10 – 12 years	20 (64.5%)	3 (10%)	
13 years or more	7 (22.6%)	26 (86.7%)	

GHD: growth hormone deficiency; BMI: body mass index; SDS: standard deviation score. Data are presented as medians (min – max), or absolute counts (proportion).

The clinical characteristics of participants with GHD are described in **Table 2** and are divided between those who stopped and those who continued receiving GHRT after the transition period. The median time between interruption of treatment from the transitional period and the evaluation was 6.1 years (SD ± 2.4). Participants who interrupted GHRT had a lower BMI and a lower total duration of treatment since childhood. The distribution of GHD etiologies across subgroups was similar, as were the proportions of multiple pituitary deficiencies (**Table 2**). Most patients had congenital idiopathic GHD, and more than two-thirds had at least one other pituitary hormone deficiency.

The QoL-AGHDA scores of the patients with GHD were higher than the scores of matched controls (median 8 [IQR 5 – 14] vs 4 [IQR 2 – 8]; $p < 0.01$), adjusted by sex, education level, sedentary lifestyle, alcoholism, smoking, presence of other pituitary deficiencies, short stature, and obesity. There were no differences between participants using GHRT and those who interrupted GHRT (8 [IQR 6 – 16] vs. 9 [IQR 5 – 12], respectively, $p = 0.33$). These results are shown in **Figure 1**.

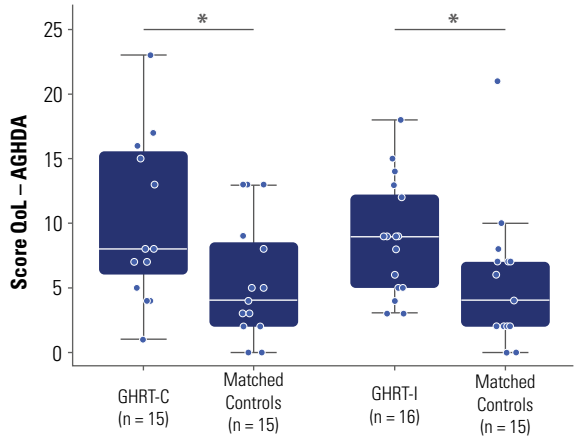
There were no predominant sub-areas across the three groups, except for the patients who discontinued GHRT, who presented higher scores in memory and concentration than in fatigue ($p = 0.02$). These results are shown in **Figure 2**. In the analysis of each specific domain of the QoL-AGHDA, it was observed that both groups with GHD showed higher proportions of positive responses than the controls for memory and concentration ($p = 0.02$ for both), but no difference was found between the groups with GH deficiency ($p = 0.85$). Regarding social isolation, there was a difference between the continued GHRT group vs Control ($p = 0.01$) and between the discontinued GHRT group vs. Control ($p = 0.03$). In terms of self-confidence, the discontinued GHRT group had higher scores than controls ($p = 0.02$). The distribution of positive answers for tiredness and tenseness did not differ among patients in the continued GHRT, discontinued GHRT, and control groups (**Figure 2**).

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Table 2. Clinical characteristics, distribution etiologies and distribution of associated pituitary deficiencies in the patients with growth hormone deficiency divided by treatment interruption or maintenance during the transition period

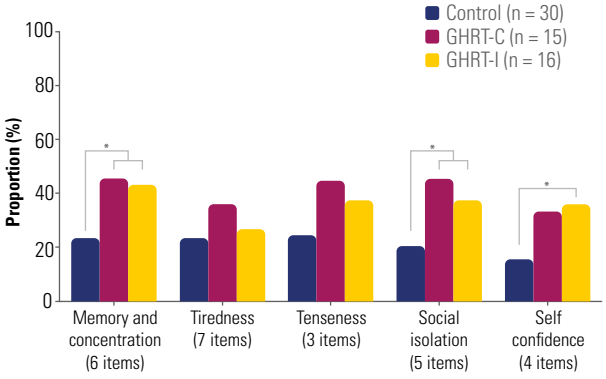
	GHRT-C (n = 15)	GHRT-I (n = 16)	*p*
Males (n)	11 (73%)	11 (69%)	1.0
Age at diagnosis (years)	8 [6.5; 9.5]	12 [8; 14]	0.11
BMI (kg/m²)	27.6 [22.4; 33.1]	21.7 [19.6; 25]	0.03
Height (SDS)	-0.6 (-2.3; 0.5)	-1.5 (-3.3; 0.7)	0.11
Total treatment (years)	17 (12 - 30)	6 (3 – 20)	< 0.01
GHD etiology			1.0
Congenital GHD	12 (80%)	13 (81%)	
Craniopharyngioma[§]	1 (6.7%)	2 (12.5%)	
Non-functional pituitary adenoma	1 (6.7%)	0	
Langerhans cell histiocytosis	0	1 (6.2%)	
Astrocytoma	1 (6.7%)	0	
Other pituitary deficiencies			
None	4 (26.7%)	5 (31.2%)	1.0
TSH	11 (73,3%)	11 (68.8%)	1.0
AVP	2 (13.3%)	4 (25%)	0.65
ACTH	10 (66,7%)	8 (50%)	0.47
LH/FSH	10 (66.7%)	7 (43.8%)	0.29

Data are presented as medians (min-max), median [interquartile range, IQR] or absolute counts (proportions, %). [§]Two patients received cranial irradiation. GHRT-C: GHD patients who continued growth hormone replacement therapy in the transition period; GHRT-I: GHD patients who interrupted growth hormone replacement therapy in the transition period; BMI: body mass index; SDS: standard deviation score; GHD: growth hormone deficiency; TSH: thyroid-stimulating hormone; AVP: arginine vasopressin; ACTH: adrenocorticotropic hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone.



QoL-AGHDA: quality of life assessment of growth hormone deficiency (GHD) in adults; GHRT-C: GHD patients who continued growth hormone replacement therapy in the transition period; GHRT-I: GHD patients who interrupted growth hormone replacement therapy in the transition period. *$p < 0.05$.

Figure 1. Distribution of QoL-AGHDA scores for each group

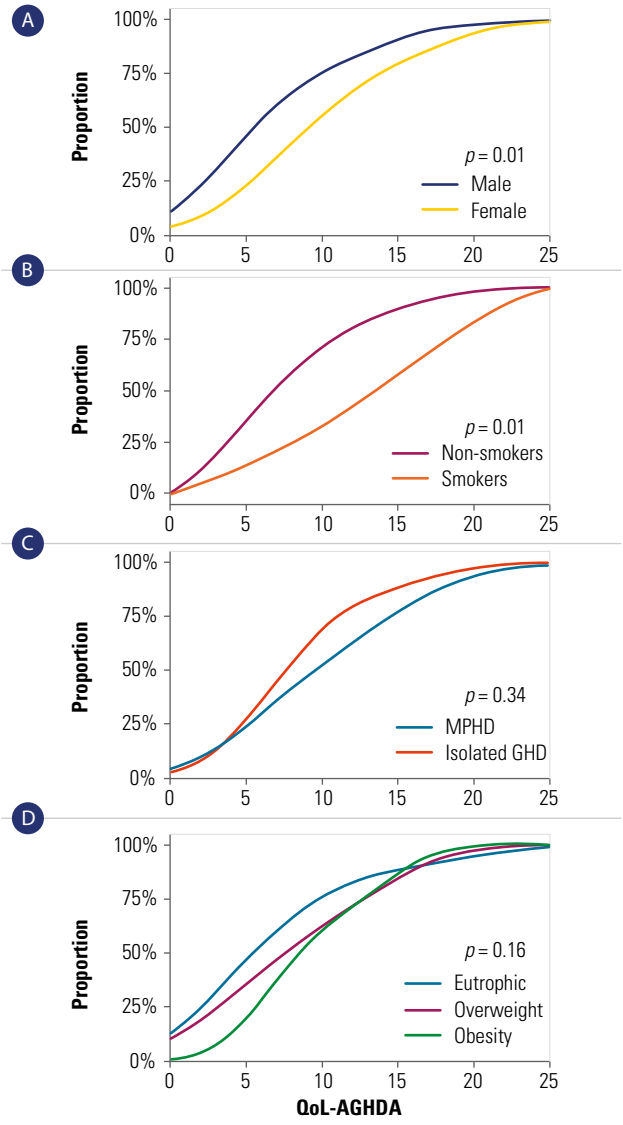


QoL-AGHDA: quality of life assessment of GHD in adults; GHRT-C: GHD patients who continued growth hormone replacement therapy in the transition period; GHRT-I: GHD patients who interrupted growth hormone replacement therapy in the transition period. [†]positive represents the presence of such a parameter or an impaired parameter; *$p < 0,05$.

Figure 2. Average proportion of positive[†] answers for each group across subareas of the QoL-AGHDA questionnaire

The QoL-AGHDA scores were higher in women than in men (9 [IQR 6 – 13] vs. 5 [IQR 2 – 8.5], $p = 0.01$, **Figure 3A**), independently of the diagnosis of GHD. Smokers also had higher scores than non-smokers (14 [IQR 10 – 17] vs. 7 [IQR 3 – 9], $p = 0.01$, **Figure 3B**). We found no association between QoL-AGHDA scores and education level ($p = 0.51$), sedentary lifestyle ($p = 0.70$), alcoholism ($p = 0.45$), short stature ($p = 0.78$), presence of multiple pituitary deficiencies ($p = 0.34$, **Figure 3C**), or overweight/obesity ($p = 0.16$, **Figure 3D**). Among patients with GHD, the mean difference in scores between those younger than 30 years and those older than 30 years was -1.40 (95%CI: -5.2; 3.2).

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QoL-AGHDA: quality of life assessment of GHD in adult questionnaire; BMI: body mass index; MPHD: multiple pituitary hormone deficiencies; GHD: growth hormone deficiency.

Figure 3. Cumulative distribution of QoL-AGHDA scores according to group, gender, smoking, presence of associated pituitary deficiencies, or BMI category. Cumulative distribution of QoL-AGHDA scores by: (**A**) gender (n = 61); (**B**) smoking (n = 61); (**C**) the presence of associated pituitary deficiencies (n = 31); (**D**) BMI category (n = 61)

The mean difference in QoL-AGHDA scores between GHD patients with treatment interruption for less than five years and those who had interruption for five years or more was 1.2 (95%IC -2.9; 6.8). There was no correlation between interruption time and QoL-AGHDA scores (p = 0.7; r = 0.1).

There was no correlation between QoL-AGHDA scores and BMI (p = 0.38; r = 0.11), height SDS (p = 0.16; r = -0.18), or IGF-1 levels (p = 0.03, r = -0.28). A beta-binomial regression model was fitted, including BMI and treatment duration as covariates. The mean difference was 2.08 (95% CI: -2.94 to 7.07; p = 0.41), indicating that even after accounting for potential inter-group discrepancies regarding these covariates, no significant difference in quality of life was observed between the groups.

DISCUSSION

In the present study, we applied QoL-AGHDA, a Brazilian Portuguese validated instrument (13,19), to assess QoL in patients with childhood-onset GHD confirmed during the adolescence to adult transition period, who continued or discontinued GHRT and matched controls. Untreated patients typically exhibit poorer QoL, while GHRT improves outcomes toward normative values (23). Current guidelines recommend continuation of GHRT until peak somatic development, with restricted use thereafter based on severity and QoL impairment (22).

Our study showed that patients with GHD were shorter than controls with no differences in age, sex distribution, BMI, or marital status. Many of these similar characteristics between the groups are important to strengthen the differences, such as GHD patients had a higher proportion of sedentarism, more unemployment status, and were more likely to have fewer years of education, which implicates a putative low QoL. On the other hand, adults with childhood-onset-GHD present lower QoL than controls, which is consistent with previous reports indicating poorer outcomes observed in patients with GHD (24,25). This finding can be attributable to GHD, besides a multifactorial burden, involving adverse body composition, reduced physical performance, metabolic disturbances, and neuroendocrine alterations, all of which negatively impact cognitive function and psychological well-being (7,11,26). Thus, both disease-specific factors, socioeconomic and genetic determinants, may collectively explain the observed QoL outcome. It has already been recognized that prevalent comorbidities, such as anxiety and depression, are likely involved in the complex interplay of genetic and psychosocial determinants (4), alongside GH/IGF-1 axis dysregulation (27). Of interest, GHD patients had a lower proportion of alcohol consumption than controls. Studies have suggested better adaptation of childhood-onset GHD

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patients to their functional limitations compared to adult-onset GHD cohorts, who perceive a more acute decline in QoL (11,13).

Although GHRT leads to progressive improvement across QoL domains, earlier in social functioning and later in cognitive aspects (9,11,16), our study found no differences between childhood-onset GHD patients who early stopped GHRT at the transitional period (6.1 years before the study) and those who continued receiving GHRT after the adolescence to adult transition period. This may be explained by the young age of the cohort, adaptive mechanisms, and limited statistical power. As expected, patients who interrupted GHRT during the transitional period had a lower total duration of treatment since childhood, whereas both groups presented similar GHD etiologies as well as the proportions of multiple pituitary deficiencies, age at diagnosis, and proportion by gender, which enable us to appropriately compare these groups regarding metabolic parameters and QoL.

While the physiological efficacy of GHRT on body composition, linear growth, and bone mineral density is unequivocally established, the effect of maintenance of GH treatment throughout the adolescence to adult transitional period on QoL remains a subject of debate (5,7,15,28). While some authors show that most patients with GHD and other pituitary hormone deficiencies show a long-lasting improvement of QoL after GHRT (9,11,16,26), others show no difference in those who received or did not receive GH treatment throughout adolescence to the adult transitional period (17).

Our data showed no differences between adult patients on GHRT and those who interrupted GHRT in accordance with previous studies (4,14). One study also demonstrated that children and adolescents with GHD treated with GH exhibit health-related QoL comparable to that of healthy controls (29). Although the overall quality of life may not decline immediately after discontinuation of GHRT upon attainment of adult height, patients with childhood-onset GHD may experience significant age-specific psychosocial impairments, including disturbances in body image. These deficits can be alleviated by continued GH therapy; therefore, GHRT should not be discontinued solely after achieving adult height, to prevent progressive psychosocial distress in adulthood and to preserve optimal body composition (30).

Interestingly, the duration of GHRT itself does not seem to significantly influence QoL or mood states, as comparable outcomes have been observed between patients treated for shorter versus longer periods. Consistent with these observations, in our cohort, patients who had received long-term GHRT (mean duration 17.5 years) did not differ in QoL scores from those who had discontinued treatment and had remained off therapy for a mean of 6.1 years. These data are also in agreement with a recent study that showed no significant differences in QoL or mood states between patients treated for less than 5 years and those receiving therapy for 5 years or longer (4). The improvement in QoL in patients receiving GHRT usually occurs within the first year of treatment and persists for up to 10 years (26).

Favorable responses to GHRT have been associated with the absence of depression, lower BMI, absence of visual impairment, worse baseline QoL, isolated GHD, and childhood-onset GHD (11,26). In contrast, GH dose and IGF-1 levels do not appear to predict QoL outcomes (4,14,26). Additionally, after discontinuation of GHRT, mood states have been reported to correlate negatively with height and age at treatment cessation and positively with BMI (4). However, in our study, IGF-1 levels, BMI, and height were not associated with QoL-AGHDA scores.

The QoL-AGHDA captures key domains affected in GHD, such as cognition, fatigue, self-confidence, tension, and social isolation. GHRT leads to progressive improvement, with earlier gains in less affected domains such as social isolation and later recovery in cognitive domains (9,11,23). Long-term data confirm sustained QoL benefits, with fatigue improving within the first year and memory over subsequent years (16).

In our sample, there was no predominant compromised subarea in the GHD patients; this contrasts with previous studies that showed concentration, memory, and tiredness to be the parameters most affected (9-11). These findings may be attributed to the fact that our cohort is substantially young, and it is plausible that age-related cognitive and physical demands are not yet sufficiently pronounced to demonstrate

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the protective effects of ongoing GHRT. However, when patients with GHD were compared to matched controls, both GHD groups' scores were higher than those of controls for memory and concentration and social isolation, while tiredness, tenseness, and self-confidence were not significantly different. Since this was a secondary analysis for which the sample size was not optimized, the sample power for this may not have been sufficient to detect smaller differences.

Overall, in our cohort, QoL was influenced by sex, with women reporting significantly lower QoL than men, while no associations were observed with education level, short stature, presence of additional pituitary deficiencies, or overweight/obesity. Actually, women tend to report poorer QoL than men (31). Women using oral estrogen as replacement therapy or for contraceptive purposes are more GH-resistant than men because estrogen attenuates GH action on the liver, the principal site of IGF-1 synthesis, resulting in lower IGF-1 generation (21), which could exacerbate the decline in quality of life observed in this subgroup. In the general population, QoL-AGHDA normative scores average 4.6, with slightly higher values in women and older individuals (12). In our cohort, patients younger than 30 years reported lower QoL than older individuals, although this difference was not statistically significant. Additionally, childhood-onset hypopituitarism has been associated with adverse socioeconomic outcomes, including lower educational attainment, higher unemployment, and fewer relationships (25), findings that are consistent with the higher unemployment and lower education levels observed in our GHD cohort compared with controls.

Compared with a previously reported cohort of untreated Brazilian adults with GHD (mean age 46.3 years, predominantly female; median QoL-AGHDA score 11) (19), our patients exhibited lower median QoL-AGHDA scores regardless of GHRT status during transition. However, this comparison should be interpreted cautiously, as our cohort was substantially younger (mean age 27.2 years) and predominantly male (71%). Consistent with normative population data showing poorer QoL among women and older individuals (12), these demographic differences likely account for the more favorable QoL observed in our study.

Several limitations should be considered when interpreting these findings. First, the relatively small sample size may limit generalizability to the broader adult GHD population. Second, unreported comorbidities and psychosocial factors may have introduced residual confounding in QoL assessments. Although concomitant pituitary deficiencies were appropriately treated, their potential impact on perceived health cannot be entirely excluded. In addition, the cohort showed a skewed gender distribution, with male predominance. Another important point to consider is that the decision to continue or discontinue GH therapy was based on patient preference and not randomization. This non-randomized approach may introduce selection bias, since patients who did not perceive a significant benefit from treatment may have been more likely to discontinue treatment. However, worse quality of life scores were predicted in the discontinuation group due to this potential bias; however, this expected trend was not observed in our cohort, which showed no significant difference in quality-of-life scores between the groups.

Despite these limitations, this study has important strengths. To our knowledge, it is the first to evaluate QoL in Brazilian adults with childhood-onset GHD receiving GHRT, in comparison with childhood-onset GHD who early stopped GHRT at the transitional period and healthy controls, using a rigorously validated, disease-specific instrument adapted for the local population.

In conclusion, although GHD is known to impair quality of life in young adults, our cross-sectional data did not demonstrate a significant association between GHRT and QoL improvements. Future longitudinal research is required to determine the definitive clinical impact of GH replacement. In the meantime, therapy must remain individualized and combined with psychosocial interventions to maximize patients' overall health.

Author contributions: conceptualization: K.C.O, S.R.A; literature review: K.C.O.; patient selection and data collection: K.C.O., R.L.M.S.; R.S.P.; T.M.L; statistical analysis: D.C.A, L.E.C; writing original draft preparation: K.C.O, M.P.H., L.E.C.; reviewed and edited the manuscript: K.C.O, P.C.L.E, M.C, and S.R.A. All authors reviewed and approved the final draft.

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Data availability: the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Characteristics of all individual participants of the study

	Age (years)	Sex	Etiology	Height (SDS)	Weight (kg)	BMI (kg/m²)	Age of diagnosis (years)	IGF-1 (SDS)	Current Treatment	Total GHRT (years)	Score QoL-AGHD
GHD patients who continued growth hormone replacement therapy in the transition period											
Pt 1	42	M	Pituitary hypoplasia	-0.62	82	27.72	12	0.05	P-T4-T-GH	30	4
Pt 2	30	M	Pituitary hypoplasia	0.47	81.9	25.28	8	-1.07	P-T-GH	23	13
Pt 3	26	M	Pituitary hypoplasia	-0.62	111	37.52	8	2.34	GH	18	5
Pt 4	27	M	Pituitary hypoplasia	-0.01	64.8	20.8	10	0.12	P-T4-T-GH	17	23
Pt 5	21	M	Pituitary hypoplasia	-0.21	94	30.69	6	-0.29	T4-GH	15	7
Pt 6	38	M	Mutation GHRH	-1.86	73.2	27.55	8	-0.02	GH	18	8
Pt 7	39	F	Mutation GHRH	-0.79	59	23.63	9	-0.38	GH	18	7
Pt 8	25	M	Craniopharyngioma	0.47	87.5	27.01	7	-1.07	P-T4-T-GH-D	17	17
Pt 9	26	F	Pituitary hypoplasia	-1.55	91	38.87	9	-0.03	P-T4-E/P-GH	17	16
Pt 10	26	M	Pituitary hypoplasia	-0.21	65	21.22	8	0.28	P-T4-T-GH	17	4
Pt 11	25	F	Pituitary hypoplasia	-0.48	72	28.12	6	-2.62	P-T4-E/P-GH	19	17
Pt 12	18	M	Pituitary hypoplasia	-0.2	54	17.63	6	-1.29	GH	12	8
Pt 13	20	M	Idiopathic	-2.27	51.4	20.08	5	-0.14	T-GH	13	1
Pt 14	29	M	Nonfunctioning macroadenoma	-2.27	92	35.94	14	-0.79	P-T4-T-GH	15	15
Pt 15	28	F	Astrocytoma	-2.01	80	35.56	14	-2.18	P-T4-E/P-GH	14	8
GHD patients who interrupted growth hormone replacement therapy in the transition period											
Pt 16	18	F	Pituitary hypoplasia	-1.5	46.5	19.86	9	-1.57	---	7	18
Pt 17	47	M	Pituitary hypoplasia	-2.13	66.3	25.58	14	-2.33	P-T4-D	3	14
Pt 18	19	F	Histiocytosis	-0.48	48.3	18.87	12	-1.44	T4-D	3	4
Pt 19	22	M	Craniopharyngioma	-0.01	69.1	22.18	12	-4.46	P-T4-T-D	4	13
Pt 20	22	F	Pituitary hypoplasia	-2.16	34.1	15.36	12	-3.5	P-T4-E/P	3	9
Pt 21	18	F	Pituitary hypoplasia	-1.51	65	27.77	13	-0.44	---	3	9
Pt 22	20	M	Craniopharyngioma	-1.99	103	39.25	14	-3.32	P-T4-T- D	3	6
Pt 23	35	M	Pituitary hypoplasia	-1.58	43.6	16.01	8	-1.99	P-T4-T	18	9
Pt 24	29	M	Pituitary hypoplasia	0.75	82	24.76	6	-3.18	P-T4-T	17	9
Pt 25	22	M	Pituitary hypoplasia	-2.4	50	19.78	8	-1.1	---	7	3
Pt 26	44	M	Pituitary hypoplasia	-1.72	72	26.77	15	-1.83	P-T4-T	20	5
Pt 27	18	M	Pituitary hypoplasia	-3.33	40	17.31	4	-5.42	P-T4-T	11	8
Pt 28	25	M	Pituitary hypoplasia	-0.21	64	20.9	6	-0.78	---	13	5
Pt 29	24	M	Idiopathic	-1.31	68	24.38	10	-3.17	T4	10	3
Pt 30	29	M	Pituitary hypoplasia	-0.62	63	21.3	16	-1.11	T4	4	15
Pt 31	32	F	Pituitary hypoplasia	-2.32	52	23.74	16	-1.66	---	5	12
Controls											
Pt 32	49	M	---	1.57	82	23.2	---	1.18	---	---	0
Pt 33	29	M	---	-0.07	66	21.31	---	1.62	---	---	2
Pt 34	21	M	---	0.54	74.8	22.96	---	-0.42	---	---	2
Pt 35	27	M	---	1.16	135	39.44	---	-1.73	---	---	5
Pt 36	22	F	---	1.66	60	19.82	---	0.71	---	---	4
Pt 37	21	F	---	-1.55	47	20.08	---	0.32	---	---	6
Pt 38	21	F	---	-0.94	63	25.56	---	0.51	---	---	10

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	Age (years)	Sex	Etiology	Height (SDS)	Weight (kg)	BMI (kg/m2)	Age of diagnosis (years)	IGF-1 (SDS)	Current Treatment	Total GHRT (years)	Score QoL-AGHD
Pt 39	36	F	---	3.03	74.7	22.31	---	0.32	---	---	3
Pt 40	21	M	---	1.16	103	30.09	---	1.82	---	---	5
Pt 41	21	M	---	0.88	70	20.9	---	0.33	---	---	7
Pt 42	23	M	---	-1.44	73	26.49	---	-0.83	---	---	0
Pt 43	29	M	---	0.88	85	25.38	---	0.95	---	---	4
Pt 44	27	M	---	-0.49	77.5	25.89	---	0.7	---	---	2
Pt 45	27	M	---	-0.35	76	25.1	---	-0.08	---	---	8
Pt 46	24	M	---	0.13	66	20.95	---	0.69	---	---	2
Pt 47	36	M	---	-1.03	86.5	30.29	---	-0.67	---	---	13
Pt 48	43	M	---	2.39	103	27.37	---	1.27	---	---	2
Pt 49	23	M	---	-1.86	60	22.58	---	0.78	---	---	0
Pt 50	34	F	---	-0.48	56.5	22.07	---	1.11	---	---	2
Pt 51	24	F	---	-0.79	64	25.64	---	1.02	---	---	13
Pt 52	24	M	---	-1.31	62	22.23	---	2,76	---	---	0
Pt 53	21	M	---	-0.62	56	18.93	---	1,48	---	---	2
Pt 54	47	M	---	1.3	93	26.88	---	0.29	---	---	2
Pt 55	19	M	---	-1.03	59	20.66	---	-0.04	---	---	3
Pt 56	27	M	---	0.47	74	22.84	---	0.67	---	---	7
Pt 57	23	F	---	-1.4	42	17.71	---	2.05	---	---	21
Pt 58	32	F	---	-1.09	88	36.16	---	1.66	---	---	8
Pt 59	26	F	---	-0.18	102	38.87	---	0.54	---	---	9
Pt 60	32	M	---	0.47	107	33.02	---	-0.18	---	---	13
Pt 61	37	M	---	-1.58	52	19.1	--	-0.02	---	---	7

Pt: patient; M: male; F: female; BMI: body mass index; P: prednisone; T4: levothyroxine; E/P: estrogen/progesterone; D: desmopressin; GHRT: growth hormone replacement therapy; QoL-AGHDA: quality of life assessment of GHD in adult questionnaire; SDS: standard deviation score.

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original

Analysis of differences in serological indicators between Graves' disease and transient thyrotoxicosis during pregnancy

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ABSTRACT

Objective: To investigate serological differences between gestational Graves' disease (GGD) and gestational transient thyrotoxicosis (GTT), and to establish practical cutoff values for differential diagnosis. **Subjects and methods:** We enrolled 97 first-trimester patients with gestational thyrotoxicosis (63 newly diagnosed GGD, 34 GTT) and 57 age- and gestational-age-matched healthy pregnant controls at Lanzhou University Second Hospital (China) between January 2020 and December 2024. We measured serum levels of TSH, FT4, FT3, TT4, TT3, TRAb, TgAb, TPOAb, and hCG and used ROC curve analysis to determine optimal diagnostic cutoff values and multivariable logistic regression to identify independent predictors. **Results:** Compared to the GTT group, the GGD group showed significantly higher TT3 ($p = 0.033$), TRAb ($p < 0.001$), and TPOAb ($p = 0.002$) levels, along with significantly lower hCG levels ($p < 0.001$). Compared to the healthy controls, the GGD group exhibited markedly suppressed TSH and elevated FT4, FT3, TT3, and TT4 (all $p < 0.001$). ROC analysis established the following optimal cutoffs: hCG > 63,518.50 IU/L (sensitivity 85.29%, specificity 53.97%), TT3 > 24.05 nmol/L (sensitivity 82.35%, specificity 57.14%), and WBC > 9.37×10^9/L (sensitivity 70.59%, specificity 61.9%). Multivariable logistic regression identified hCG, TT3, TPOAb, and WBC as independent predictors distinguishing GGD from GTT. **Conclusion:** Our findings showed that TT3, TRAb, TPOAb, hCG, and WBC differed significantly between GGD and GTT and that the hCG cutoff of 63,518.50 IU/L provides practical diagnostic guidance that complements TRAb measurements.

Keywords: Graves' disease; gestational transient thyrotoxicosis; human chorionic gonadotropin; thyrotropin receptor antibodies; pregnancy

INTRODUCTION

Gestational thyrotoxicosis refers to a series of hypermetabolic symptoms caused by excessive secretion of thyroid hormones during pregnancy. The primary etiologies are gestational Graves' disease (GGD) and gestational transient thyrotoxicosis (GTT). GGD is an autoimmune condition characterized by persistent activation of the thyroid-stimulating hormone (TSH) receptor by thyrotropin receptor antibodies (TRAb), independent of physiological negative feedback regulation (1,2). In contrast, GTT is typically associated with elevated human chorionic gonadotropin (hCG) levels in early pregnancy. Due to the structural similarity between hCG and TSH, hCG can weakly activate the TSH receptor, leading to a transient and mild increase in thyroid hormone levels, which remains partially regulated by negative feedback mechanisms (3,4). TRAb-mediated stimulation represents an

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uncontrolled pathological autoimmune attack, whereas hCG-mediated activation is a self-limiting physiological phenomenon. These two conditions therefore differ fundamentally in etiology, intensity, duration, and clinical significance (5,6).

Given their distinct pathogenic and pathophysiological mechanisms, clinical management and treatment strategies also differ significantly: GGD often requires antithyroid drug therapy and close monitoring, whereas GTT is primarily managed with supportive care (7–9). Consequently, accurate differential diagnosis between these conditions during early pregnancy is crucial for developing individualized treatment plans and improving maternal and fetal outcomes. This study aimed to systematically compare thyroid-related serological markers between patients with GGD and GTT, thereby providing a theoretical basis for precise clinical diagnosis and informed treatment decision-making.

SUBJECTS AND METHODS

Patients

A total of 97 first-trimester patients (< 14 weeks' gestation) with gestational thyrotoxicosis who presented to Lanzhou University Second Hospital between January 2020 and December 2024 were enrolled. Patients were divided into a GGD group (n = 63) and a GTT group (n = 34). The diagnostic criteria for GGD included positive TRAb, elevated free thyroxine (FT4), decreased TSH, and no prior history of hyperthyroidism prior to pregnancy. GTT was diagnostic criteria for GTT were negative TRAb, elevated FT4, decreased TSH, a self-limiting clinical course during follow-up, and no history of hyperthyroidism. Additionally, 57 healthy pregnant women, matched for age and gestational week and with normal thyroid function, were enrolled as control to provide a physiological reference baseline for pregnancy-related serological indicators.

Inclusion and exclusion criteria

Eligible participants were first-trimester women with singleton pregnancies, decreased TSH, and elevated FT4 levels. Exclusion criteria were severe cardiovascular or cerebrovascular disease, significant autoimmune disorders, multiple gestation, conception via assisted reproductive technology, incomplete clinical data, or history of anti-thyroid drug therapy.

Data collection

Comprehensive clinical data were gathered for all participants, including: (1) Baseline characteristics: age, gravidity, gestational age at delivery; (2) Laboratory parameters: white blood cell count (WBC), red blood cell count, hemoglobin, platelet count, neutrophil count, absolute neutrophil count, alanine aminotransferase, aspartate aminotransferase, total bilirubin, direct bilirubin, indirect bilirubin, albumin, triglycerides, total cholesterol, low-density lipoprotein cholesterol, blood urea nitrogen, creatinine, and uric acid; (3) Thyroid function tests: TSH, FT4, free triiodothyronine (FT3), total thyroxine (TT4), total triiodothyronine (TT3), TRAb, thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb); (4) Pregnancy-related hormone—hCG.

Serological testing

Fasting venous blood samples (5 mL) were collected from all participants in the morning. Samples were centrifuged at 4000 rpm to separate serum, which was stored at -30 ℃ until analysis. Serum levels of TSH, FT3, FT4, TT3, TT4, TRAb, TPOAb, TgAb, and hCG were measured using an electrochemiluminescence immunoanalyzer (Cobas E602, Roche Diagnostics, Switzerland). Complete blood counts were analyzedz with an automated hematology analyzer (XN-9000, Sysmex, Japan), and biochemical parameters were assessed using an automated biochemistry analyzer (Cobas C701, Roche Diagnostics, Switzerland). The reference ranges for thyroid function tests are shown in **Table 1**.

Table 1. The reference range of thyroid function

Thyroid function indices (units)	Reference range
TT3, nmol/L	1.14–3.02
TT4, nmol/L	75.45–148.18
TSH, mIU/L	0.31–5.30
FT4, pmol/L	9.8–22.0
FT3, pmol/L	3.1–6.8
TRAb, IU/L	0–1.5
TPOAb, IU/mL	0–34.0
TgAb, IU/mL	0–115.0

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Statistical analysis

All statistical analyses were conducted using SPSS version 27.0. The normality of continuous variables was assessed with the Shapiro-Wilk test. Normally distributed data are presented as mean ± standard deviation and were compared between groups using independent samples t-tests. Non-normally distributed data are presented as median (interquartile range) and were compared using Mann-Whitney U tests. Categorical variables are expressed as counts (percentages) and were compared using Chi-square tests. Effect sizes were calculated using the non-parametric r metric derived from the Mann-Whitney U test ($r = Z/\sqrt{N}$). Optimal diagnostic cutoff values for hCG, WBC, and TT3 were determined by receiver operating characteristic (ROC) curve analysis, from which the corresponding sensitivity and specificity were obtained. A two-tailed *p*-value < 0.05 was considered statistically significant. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test, and Nagelkerke R^2 was calculated to estimate the proportion of variance explained by the model.

Ethical aspects

This research was approved by the Ethics Committee of The Second Hospital of Lanzhou University (no. 2025A-1286); all participants signed an informed consent form.

RESULTS

Comparison of baseline characteristics between the two groups

A total of 97 patients with gestational thyrotoxicosis were enrolled, comprising 34 in the GTT group and 63 in the GGD group, along with a control group of 57 healthy pregnant women with normal thyroid function (Table 2). No significant differences were observed between the GTT and GGD groups in baseline

Table 2. Demographic and laboratory characteristics of the study subjects

Parameter	GTT (n = 34)	GGD (n = 63)	Control (n = 57)	t/z	*p*
RBC, $\times10^{12}$/L	4.71 ± 0.54	4.85 ± 0.47	4.92 ± 0.39	-1.31	0.195
PLT, $\times10^{9}$/L	264.06 ± 78.95	262.11 ± 72.31	264.79 ± 77.35	0.12	0.903
NEU, %	78.83 ± 5.03	79.34 ± 4.61	78.60 ± 7.06	-0.51	0.615
ANC, $\times10^{9}$/L	7.76 ± 1.60	7.89 ± 1.34	8.14 ± 1.46	-0.42	0.676
ALB, g/L	44.69 ± 3.05	44.86 ± 4.16	44.14 ± 3.47	-0.21	0.834
DBIL, μmol/L	4.32 ± 1.61	4.28 ± 1.95	4.83 ± 1.90	0.09	0.928
TG, mmol/L	3.69 ± 1.64	3.46 ± 1.60	2.81 ± 1.31	0.66	0.513
LDL, mmol/L	2.89 ± 1.01	2.89 ± 1.12	2.62 ± 0.90	-0.00	0.997
TC, mmol/L	5.66 ± 1.47	5.56 ± 1.21	5.11 ± 1.10	0.37	0.713
Cr, μmol/L	48.82 ± 9.10	48.08 ± 13.33	46.91 ± 10.12	0.29	0.773
UA, μmol/L	297.94 ± 79.85	304.68 ± 76.04	299.98 ± 63.32	-0.41	0.683
Age, years	30.00 (28.00, 33.00)	30.00 (28.00, 32.00)	30.00 (28.00, 33.00)	-0.42	0.673
Gravidity	2.00 (1.00, 2.00)	1.00 (1.00, 2.00)	1.00 (1.00, 2.00)	-0.92	0.358
WBC, $\times10^{9}$/L	9.49 (9.22, 9.71)	9.16 (8.53, 9.68)	9.22 (8.75, 9.55)	-2.18	0.029*
HGB, g/L	134.50 (96.50, 145.75)	138.00 (98.00, 144.00)	138.00 (117.00, 147.00)	-0.48	0.634
ALT, U/L	9.40 (8.30, 19.40)	9.40 (8.05, 13.80)	9.80 (8.40, 20.80)	-0.51	0.607
AST, U/L	20.85 (18.38, 30.23)	19.60 (15.45, 27.65)	21.30 (18.10, 28.10)	-1.02	0.307
TBIL, μmol/L	9.30 (8.53, 16.20)	9.30 (7.80, 14.15)	9.50 (7.90, 11.60)	-0.60	0.548
IBIL, μmol/L	8.50 (7.12, 9.28)	8.15 (7.10, 9.30)	8.70 (6.10, 9.30)	-0.27	0.789
BUN, mmol/L	3.90 (3.00, 4.30)	3.80 (3.20, 4.55)	3.70 (3.10, 4.30)	-0.24	0.812
Gestational age at delivery	39.00 (38.00, 40.00)	39.00 (38.00, 40.00)	39.00 (38.00, 39.00)	-0.53	0.594
Preterm, n (%)	4 (11.76%)	5 (7.94%)	6 (10.52%)	0.38	0.535

Normally distributed continuous data are expressed as mean ± standard deviation; non-normally distributed continuous data are expressed as median and interquartile range (Q1, Q3); Categorical data are expressed as n (%) and compared using Chi-square tests. *$p < 0.05$ is considered statistically significant. Comparisons (P) provided are between GTT and GGD. ALB: albumin; ALT: alanine aminotransferase; ANC: absolute neutrophil count; AST: aspartate aminotransferase; BUN: blood urea nitrogen; Cr: creatinine; DBIL: direct bilirubin; HGB: hemoglobin; IBIL: indirect bilirubin; LDL: low-density lipoprotein cholesterol; NEU: neutrophil count; PLT: platelet count; RBC: red blood cell count; TBIL: total bilirubin; TC: total cholesterol; TG: triglycerides; UA: uric acid.

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characteristics, including maternal age, gravidity and parity, mode of delivery, rate of preterm birth, complete blood count, liver and renal function, and lipid profiles (all $p > 0.05$), indicating that the groups were comparable. However, the WBC count was significantly higher in the GTT group compared to the GGD group ($Z = -2.18$, $p = 0.029$).

Comparison of serum hCG, thyroid function, and autoantibody profiles among the three groups

Compared to the GTT group, the GGD group showed significantly higher levels of TT3 ($Z = -2.13$, $p = 0.033$), TRAb ($Z = -8.10$, $p < 0.001$), and TPOAb ($Z = -3.10$, $p = 0.002$), whereas hCG was significantly lower ($Z = -4.42$, $p < 0.001$) (Table 3). No significant differences were observed between the two groups in TT4, TSH, FT4, FT3, or TgAb (all $p > 0.05$). Compared to healthy controls, the GGD group exhibited markedly suppressed TSH ($Z = -8.35$, $p < 0.001$) and significantly elevated FT4 ($Z = -8.35$, $p < 0.001$), FT3 ($Z = -9.42$, $p < 0.001$), TT3 ($Z = -7.43$, $p < 0.001$), and TT4 ($t = 9.41$, $p < 0.001$). TRAb, TPOAb, and TgAb were also significantly higher in the GGD group than in controls (all $p < 0.001$). Notably, hCG levels did not differ between GGD patients and controls ($p = 0.787$).

Effect sizes and bootstrap confidence intervals

TRAb demonstrated a large effect size ($r = -0.82$), while hCG and TPOAb showed medium effect sizes ($r = -0.45$ and $r = -0.31$, respectively) (Table 4). TT3 and WBC showed small-to-medium effect sizes ($r = -0.22$ for both). Bootstrap analysis with 1,000 resamples provided 95% confidence intervals for medians.

Diagnostic performance of serological parameters in differentiating new-onset GGD from GTT

The optimal cutoff values for TT3, WBC, and hCG in distinguishing GGD from GTT were determined using ROC curves, along with their corresponding areas under the curves (AUCs) (Table 4, Figure 1). For hCG, the optimal cutoff value was 63,518.50 (95% CI: 0.6794–0.8668), with a sensitivity of 85.29% and a specificity of 53.97%. For WBC, the optimal cutoff value was 9.37 (95% CI: 0.5218–0.7481), with a sensitivity of 70.59% and a specificity of 61.90%. For TT3, the optimal cutoff value was 24.05 (95% CI: 0.5847–0.7948), with a sensitivity of 82.35% and a specificity of 57.14%.

Multivariable logistic regression analysis

To adjust for potential confounding factors, we conducted a multivariable logistic regression analysis with group assignment (GGD = 1, GTT = 0) as the dependent variable. The following variables were entered as covariates: hCG, TT3, TPOAb, and WBC. The Hosmer-Lemeshow test indicated good model fit ($\chi^2 = 8.180$, df = 8, $p = 0.416$), and the Nagelkerke R^2 was 0.502, indicating that the model explained 50.2% of the variance in the outcome. Moreover, hCG, TT3, TPOAb, and WBC were all independent predictors of

Table 3. Comparison of hCG levels, thyroid function, and autoantibody titers in the study subjects

Parameter	GTT (n = 34)	GGD (n = 63)	Control (n = 57)	t/z (GTT vs. GGD)	P(GTT vs. GGD)	P(GGD vs. control)
TT4, nmol/L	199.54 ± 60.56	204.43 ± 75.65	106.29 ± 22.99	-0.32	0.746	<0.001*
hCG, mIU/L	125127.00 (74710.65, 200000.00)	60771.00 (18482.50, 102788.00)	50246.00 (24674.00, 88075.00)	-4.42	<0.001*	0.787
TSH, mIU/L	0.01 (0.01, 0.02)	0.01 (0.01, 0.04)	2.31 (1.68, 2.98)	-1.49	0.137	<0.001*
FT4, pmol/L	34.05 (28.32, 51.60)	31.80 (25.50, 42.30)	16.30 (11.70, 19.50)	-0.79	0.429	<0.001*
FT3, pmol/L	7.96 (6.63, 8.74)	7.61 (6.48, 9.20)	4.60 (3.30, 5.51)	-0.26	0.794	<0.001*
TT3, nmol/L	3.42 (3.16, 3.71)	3.95 (3.21, 5.39)	2.11 (1.47, 2.73)	-2.13	0.033*	<0.001*
TRAb, IU/L	1.00 (0.80, 1.12)	4.43 (2.40, 6.93)	-	-8.10	<0.001*	<0.001*
TPOAb, IU/mL	9.66 (9.00, 23.04)	34.40 (9.65, 98.05)	-	-3.10	0.002*	<0.001*
TgAb, IU/mL	34.50 (18.40, 137.62)	23.70 (16.25, 139.50)	-	-0.73	0.468	<0.001*

Normally distributed data are expressed as mean ± standard deviation, and non-normally distributed data are expressed as median (Q1, Q3). *$p < 0.05$ is considered statistically significant.

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Table 4. Effect sizes and bootstrap 95% confidence intervals for medians

Parameter	Group	Median	95% CI (lower, upper)	Effect size (r)	Interpretation
hCG	GTT	125,127.00	(85,823.25, 163,864.00)	-0.45	Medium
	GGD	60,771.00	(25,933.60, 83,839.16)		
TRAb	GTT	1.00	(0.81, 1.10)	-0.82	Large
	GGD	4.43	(3.29, 6.00)		
TPOAb	GTT	9.66	(9.00, 19.80)	-0.31	Medium
	GGD	34.40	(19.85, 56.10)		
TT3	GTT	3.42	(3.26, 3.65)	-0.22	Small-to-medium
	GGD	3.95	(3.47, 4.46)		
WBC	GTT	9.49	(9.40, 9.62)	-0.22	Small-to-medium
	GGD	9.16	(8.85, 9.40)		

Effect sizes were calculated using the r metric ($r = Z/\sqrt{N}$). TRAb demonstrated a large effect size (r = -0.82), indicating a substantial difference between the GGD and GTT groups. Both hCG and TPOAb showed medium effect sizes (r = -0.45 and r = -0.31, respectively), whereas TT3 and WBC showed small-to-medium effect sizes (r = -0.22 for both).

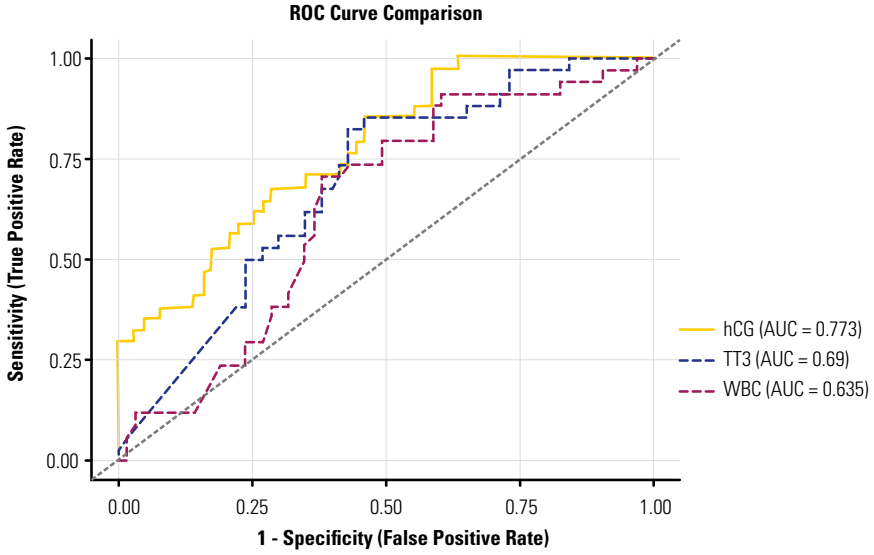


Figure 1. ROC curves of hCG, TT3, and WBC for differentiating between GGD and GTT patients.

Table 5. ROC curves and cutoff values of hCG, WBC, and TT3 for differentiating GGD from GTT

Variables	Cutoff value	AUC	95% CI	Sensitivity (%)	Specificity (%)
hCG	63518.50	0.773	0.6794–0.8668	85.29	53.97
WBC	9.37	0.635	0.5218–0.7481	70.59	61.9
TT3	24.05	0.690	0.5847–0.7948	82.35	57.14

GGD vs. GTT (Table 5). Higher hCG levels were associated with lower odds of GGD (OR = 1.000 per IU/L increase, 95% CI: 1.000–1.000, $p < 0.001$), reflecting the markedly elevated hCG levels in GTT patients. Higher TT3 levels were associated with increased odds of GGD (OR = 1.569, 95% CI: 1.051–2.344, $p = 0.028$). TPOAb positivity was associated with increased odds of GGD (OR = 1.010, 95% CI: 1.001–1.019, $p = 0.026$). In contrast, higher WBC count was associated with lower odds of GGD (OR = 0.559, 95% CI: 0.321–0.973, $p = 0.040$), consistent with the observation that GTT patients had significantly higher WBC counts in univariate analysis. The results of the multivariable logistic regression analysis are presented in Table 6.

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Table 6. Multivariable logistic regression analysis for predicting GGD (vs. GTT)

Variable	B	SE	Wald	*p*	OR	95% CI for OR	
						Lower	Upper
hCG	0.000	0.000	15.110	<0.001	1.000	1.000	1.000
TT3	0.451	0.205	4.852	0.028	1.569	1.051	2.344
TPOAb	0.010	0.004	4.931	0.026	1.010	1.001	1.019
WBC	-0.581	0.283	4.226	0.040	0.559	0.321	0.973
Constant	5.377	2.741	3.847	0.050	216.342	-	-

The variables entered were hCG, TT3, TPOAb, and WBC. TRAb was not included due to complete separation between groups (positive in all GGD and negative in all GTT). Model fit: Hosmer-Lemeshow $\chi^2 = 8.180$, df = 8, $p = 0.416$; Nagelkerke $R^2 = 0.502$. CI: confidence interval; OR: odds ratio; SE: standard error.

DISCUSSION

Accurate differentiation between GGD and GTT is central to the clinical management of gestational thyrotoxicosis. Among currently available serological markers, substantial evidence has established the critical role of TRAb in the differential diagnosis of GGD and GTT (1,2,5). However, the diagnostic utility of other serum parameters warrants further investigation (10,11). Our findings confirmed the critical role of TRAb in distinguishing GGD from GTT and further demonstrates the significant contributions of TT3, TPOAb, WBC, and hCG to this diagnostic distinction.

TRAb is a group of polyclonal antibodies that directly target the TSH receptor on the membrane of thyroid follicular cells and belong to the immunoglobulin G class. Based on their functional effects on the TSH receptor, TRAb is mainly classified into three subtypes: Stimulating antibodies, which mimic the biological activity of TSH and, upon binding to the TSH receptor, activate the cAMP signaling pathway, continuously stimulating thyroid follicular cell proliferation and the synthesis and secretion of thyroid hormones. This is the primary pathogenic antibody responsible for hyperthyroidism in Graves' disease (12). Blocking antibodies bind to the TSH receptor but do not activate the cAMP pathway; rather, they block the binding of TSH or stimulating antibodies to the receptor, exerting an antagonistic effect that can result in hypothyroidism. Neutral antibodies can bind the receptor but do not affect its function and do not directly impact thyroid activity (1).

International guidelines recommend measuring TRAb when suppressed serum TSH is detected in the first trimester to clarify the etiology of thyrotoxicosis (6,7). Timely TRAb testing not only aids in confirming the diagnosis of newly onset Graves' disease during pregnancy but also guides medication management, assesses treatment response, and evaluates the risk of fetal and neonatal hyperthyroidism, thereby enabling targeted monitoring of high-risk pregnancies (13–15). Danda and cols. (16) reported that serum TRAb levels were significantly higher in the GGD group than in the GTT group ($p < 0.0001$), further supporting the utility of TRAb as a discriminatory biomarker in the differential diagnosis of pregnancy-related thyrotoxicosis.

In our investigation, serum TRAb levels were significantly elevated in the GGD group relative to both the GTT group ($Z = -8.10$, $p < 0.001$) and the healthy control cohort ($p < 0.001$). Drawing on the most current clinical data, our results not only corroborate established guidelines and prior literature regarding the robust discriminatory capacity of TRAb in distinguishing GGD from GTT and healthy controls but also provide a solid evidence-based foundation for its clinical application in differential diagnosis. Moreover, the marked divergence of TRAb levels in GGD patients compared to normophysiological controls in this real-world dataset emphasizes the substantial association between TRAb dynamics and the pathophysiology of GGD.

In terms of thyroid autoantibodies, our study found that TPOAb levels were significantly higher in the GGD group than in the GTT group and the healthy control group. Although TPOAb is traditionally associated with Hashimoto's thyroiditis, it is frequently positive in patients with Graves' disease, reflecting a broader autoimmune diathesis involving the thyroid gland (17). In pregnancy, a state characterized by complex immunological adaptation, the presence of TPOAb may further support an underlying

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autoimmune etiology (18), thereby aiding differentiation from GTT, which is driven primarily by hCG-mediated thyroid stimulation rather than autoimmunity (19). Moreover, TPOAb positivity in pregnant women with Graves' disease has been linked to an increased risk of postpartum thyroid dysfunction, warranting continued follow-up after delivery (20,21).

The more pronounced TT3 elevation observed in GGD patients compared to those with GTT reflects fundamental differences in thyroid hormone metabolism between these two conditions. In Graves' disease, sustained activation of the TSH receptor by TRAb not only increases thyroid hormone synthesis but also upregulates type 1 iodothyronine deiodinase (DIO1) within the thyroid gland. DIO1 catalyzes the outer-ring deiodination of thyroxine (T4) to the biologically active triiodothyronine (T3), resulting in preferential T3 secretion and an elevated T3:T4 ratio (22). In contrast, GTT patients typically exhibit elevated serum T4 levels without a proportional increase in T3, as GTT is caused by stimulation of the thyroid TSH receptor by high levels of hCG in maternal circulation (23). The thyroid hormone secretion pattern in GTT is characterized by a predominant increase in T4, with only a mild or disproportionate elevation of T3 (24). The distinct mechanisms of TT3 elevation in GTT and GGD are summarized in **Table 7**.

hCG is a hormone produced by the placenta during pregnancy. Both hCG and TSH belong to the glycoprotein hormone family, which also includes luteinizing hormone and follicle-stimulating hormone. These hormones share a common alpha subunit and possess structurally similar beta subunits that confer receptor specificity (25). Their receptors (i.e., TSH and luteinizing hormone/chorionic gonadotropin) also exhibit high homology, particularly in the extracellular domains, allowing hCG to possess weak thyroid-stimulating activity (26). Elevated hCG levels tend to be associated with hyperemesis gravidarum (10). Studies have shown a positive correlation between hCG and free T4 levels in patients tested at 8–11 weeks of gestation (27). Therefore, in early pregnancy, clinicians should first suspect GTT in cases of thyrotoxicosis with extremely high hCG, elevated T4, and hyperemesis gravidarum. Conversely, a more pronounced elevation in T3 should strongly suggest GDD (11).

This study identified a significantly higher WBC count in the GTT group compared to the GGD group at baseline. Physiologic leukocytosis during pregnancy is a recognized phenomenon, attributed to enhanced inflammatory responses. This adaptation may result from a complex interplay of selective immune tolerance, immunomodulation, and immunosuppressive mechanisms necessary to sustain a successful pregnancy (28,29). The observed elevation in the GTT group may reflect a more pronounced systemic inflammatory or stress state facilitated by markedly high hCG levels.

We evaluated the diagnostic value of hCG, WBC, and TT3 in differentiating GGD from GTT using ROC curve analysis. The results showed that hCG demonstrated superior diagnostic performance (AUC = 0.852), with an optimal cutoff value of 63,518.5 IU/L and a sensitivity of 85.29%, indicating strong discriminatory ability in screening for GTT. In contrast, WBC and TT3 exhibited moderate diagnostic utility (AUC = 0.712 and 0.698, respectively), with relatively lower specificities (61.9% and 57.14%). This can be explained by the limited diagnostic specificity of both markers: although WBC may be elevated in Graves' disease, it is

Table 7. Distinct mechanisms of TT3 elevation in GTT vs. GGD

Parameter	GTT	GGD
Stimulus	hCG (placental hormone)	TRAb (autoantibody)
Duration	Transient (first trimester)	Persistent throughout pregnancy
TSH Receptor activation	Weak, transient	Strong, sustained
DIO1 Activity	Mildly increased or unchanged	Markedly upregulated
T4-to-T3 Conversion	Minimal enhancement	Enhanced (outer-ring deiodination)
T3 Secretion pattern	Proportional to T4 (normal T3:T4 ratio)	Preferential (high T3:T4 ratio)
Clinical correlation	Relatively lower TT3 levels	Higher TT3 levels

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a nonspecific marker of inflammation, and total triiodothyronine can be elevated in both conditions due to the hyperthyroid state itself, limiting their ability to effectively distinguish between them (24,30). In clinical practice, when evaluating pregnant patients with thyrotoxicosis, an hCG level markedly above this cutoff, in the context of negative or low-titer TRAb, strongly suggests GTT. This approach helps to avoid premature initiation of antithyroid drug therapy and reduces potential risks to the fetus (31). However, due to the diagnostic limitations of WBC and TT3, reliance on either parameter alone is not recommended. Instead, a comprehensive assessment based on a combination of laboratory parameters and clinical findings, especially TRAb levels, as well as the symptoms presented by the patient.

This study has several limitations that should be acknowledged. First, as a single-center retrospective study, it may be subject to selection bias, and the generalizability of the findings is limited. The proposed diagnostic cutoff values should be externally validated before being applied to other populations or to different medical settings. Second, the relatively small sample size, especially the small number of GTT cases, may have influenced statistical power and the detection of subtle group differences. Third, although all subjects were in the first trimester, gestational age at blood collection was not completely uniform, raising the possibility of residual confounding due to differences in pregnancy stage. Lastly, the lack of follow-up data on postpartum thyroid function and long-term maternal and neonatal outcomes precludes assessment of the predictive value of maternal serologic markers for long-term outcomes. Future research should involve multicenter, prospective, large-scale cohort studies with systematic postpartum follow-up to further validate these results and their clinical applicability.

In conclusion, this study further confirms that TRAb, as a key test recommended by current guidelines, holds core diagnostic value in identifying the etiology of gestational thyrotoxicosis. The observed differences in TPOAb, hCG, and TT3 levels provide valuable ancillary evidence to help determine the underlying cause. For patients with thyrotoxicosis of unknown origin, TRAb testing should be prioritized. A positive TRAb result, especially in conjunction with positive TPOAb, strongly suggests GGD and warrants the initiation of antithyroid drug therapy and close monitoring. In contrast, a negative TRAb result combined with significantly elevated hCG levels supports a diagnosis of GTT, for which symptomatic management and vigilant observation represent a reasonable approach. This serology-based diagnostic algorithm facilitates timely intervention in GGD while helping to avoid unnecessary antithyroid treatment in GTT, thereby offering a strategy to optimize pregnancy management. Its ultimate impact on maternal and neonatal outcomes requires further validation in prospective studies.

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Data availability: the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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original

Assessment of dehydroepiandrosterone sulfate (DHEA-S) suppression following the 1 mg overnight dexamethasone suppression test in women with hirsutism and elevated DHEA-S

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ABSTRACT

Objective: The utility of the 1-mg overnight dexamethasone suppression test (DST) for assessing dehydroepiandrosterone sulfate (DHEA-S) suppressibility is not well defined. Hence, this study aimed to assess DHEA-S response and identify predictors of non-suppression in hirsute women with elevated DHEA-S levels. **Subjects and methods:** This retrospective study included 362 hirsute women with elevated DHEA-S. Suppression was defined as a post-DST DHEA-S level normalized to the age-specific upper limit of normal (ULN) or a decrease of 50% from baseline. Patients were classified as suppressed (Group 1) or non-suppressed (Group 2). Logistic regression and receiver operating characteristic curve analysis were used to identify predictors of suppression, including the "percent excess" (% excess) above the ULN. **Results:** DHEA-S was suppressed in 65.5% of patients (Group 1), while 34.5% was not (Group 2). The non-suppressed group had significantly higher levels of total testosterone, a greater frequency of total testosterone >ULN, and higher 17-hydroxyprogesterone (17-OHP) levels. No differences were observed in luteinizing hormone, follicle-stimulating hormone, or homeostatic model assessment of insulin resistance. Logistic regression confirmed that both % excess (odds ratio [OR] = 0.947) and DHEA-S levels >700 µg/dL (OR = 0.069) were inversely associated with suppression. Receiver operating characteristic analysis for % excess showed good predictive performance (area under the curve = 0.827), with an optimal cut-off of 32.22%. **Conclusion:** Hirsute women with elevated DHEA-S, 34.5% exhibited a lack of DHEA-S suppression on the 1-mg DST. The % excess (cut-off >32.22%) and an absolute DHEA-S threshold of >700 µg/dL may help identify this subgroup, which was associated with higher total testosterone and 17-OHP levels despite similar luteinizing hormone levels. Overall, the lack of DHEA-S suppression on the 1-mg DST, together with higher total testosterone and 17-OHP despite similar luteinizing hormone, suggests a possible functional adrenal contribution to the hyperandrogenic phenotype.

Keywords: Hirsutism; DHEA-S; dexamethasone suppression test; adrenal androgen autonomy; functional adrenal hyperandrogenism; PMOS

INTRODUCTION

Hirsutism is a well-known clinical manifestation of hyperandrogenism that requires a thorough differential diagnosis to determine the underlying etiology (1). Hirsutism, characterized by the excessive growth of terminal hair in a male-pattern distribution (e.g., face, chest, back), is a common clinical condition that can significantly impair quality of life in women (1,2). In most cases, the underlying cause is hyperandrogenism, defined by the overproduction of androgens or increased tissue sensitivity to them (1,2). The most frequent cause of hyperandrogenism is polyendocrine metabolic ovarian syndrome (PMOS), which affects a substantial proportion of women of reproductive age (1,3).

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The source of hyperandrogenism is typically ovarian or adrenal. While total testosterone reflects androgen production from the ovaries and adrenal glands, dehydroepiandrosterone sulfate (DHEA-S) is secreted almost exclusively by the adrenal glands (2,4). Therefore, serum DHEA-S is considered a specific biochemical marker of adrenal androgen production. In a patient presenting with hirsutism and hyperandrogenism, elevated DHEA-S levels suggest that the underlying pathology may be of adrenal origin (1,3,5). The differential diagnosis for elevated DHEA-S includes PMOS (with an adrenal component), late-onset congenital adrenal hyperplasia (LOCAH), Cushing's syndrome (CS), or, rarely, androgen-secreting adrenal tumors (1,3). Because the clinical management and prognosis of these conditions differ significantly, accurate identification of the etiology is critical (1,3).

The 1 mg overnight dexamethasone suppression test (DST) is a standard diagnostic tool widely used to evaluate the hypothalamic-pituitary-adrenal axis, primarily for screening for CS (5). The purpose of this test is to determine whether adequate suppression of serum cortisol occurs following dexamethasone administration, which inhibits the release of adrenocorticotropic hormone (ACTH) from the pituitary gland (3,5). DHEA-S is also secreted by the adrenal cortex under ACTH regulation (3,5). However, the role and clinical utility of the 1 mg DST in assessing suppression of adrenal androgens, particularly DHEA-S, are not as clear as for cortisol evaluation. Although CS is a rare cause of hirsutism, the 1-mg overnight DST may be performed in selected patients when clinical features or biochemical findings raise suspicion for hypercortisolism (5).

In our clinical practice, when the 1-mg DST was performed as part of the evaluation of women with hirsutism, post-dexamethasone DHEA-S levels were also assessed in those with elevated baseline DHEA-S. Limited data exist on whether changes in elevated DHEA-S levels after DST could serve as a diagnostic tool for distinguishing adrenal-origin hyperandrogenism (6–8). Nevertheless, given the prolonged serum half-life of DHEA-S (~10–16 h) and reports that longer dexamethasone regimens may be required to reliably suppress DHEA-S, the discriminatory performance of a 1 mg overnight DST protocol for DHEA-S must be interpreted with caution. Some studies suggest that even among patients who achieve adequate cortisol suppression, DHEA-S responses may vary, possibly indicating partial or selective adrenal androgen autonomy (6–8).

Given the above, we aimed to evaluate the DHEA-S response to the 1 mg overnight DST in women with hirsutism and baseline DHEA-S levels above age-specific reference ranges. Because DHEA-S levels decline with age at similar rates in both women with PMOS and healthy controls, age-adjusted analyses were performed to identify predictors of DHEA-S suppression using the percent excess (% excess) of baseline DHEA-S above the age-specific upper limit of normal (ULN).

SUBJECTS AND METHODS

This retrospective study was conducted in accordance with the Declaration of Helsinki and consisted of reviewing the medical records of women with hirsutism and serum DHEA-S levels exceeding age-specific reference ranges between January 2018 and December 2024. A total of 362 patients who underwent the 1-mg overnight DST were included.

Exclusion criteria included age <18 years; pregnancy or postmenopausal status; current use of corticosteroids, oral contraceptives, or antiandrogenic medications; known adrenal or ovarian tumors or prior diagnosis of CS; presence of other endocrinopathies (e.g., hyperprolactinemia, hypothyroidism); chronic systemic illnesses (e.g., hepatic or renal failure, malignancy); incomplete hormonal data; and failure to suppress cortisol below 1.8 µg/dL after the 1 mg overnight DST.

All patients underwent comprehensive evaluation by experienced endocrinologists and gynecologists. Hirsutism was defined by a Ferriman-Gallwey score ≥9 (3). All patients had a confirmed diagnosis of hirsutism and available serum DHEA-S measured at both baseline and after the 1 mg DST. Blood samples were collected during the early follicular phase of the menstrual cycle (days 2–5) following an overnight fast. The baseline hormonal panel included fasting glucose and insulin, thyroid-stimulating hormone (TSH), luteinizing hormone (LH) and follicle-stimulating hormone

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(FSH), estradiol and prolactin, total testosterone and 17-hydroxyprogesterone (17-OHP), and DHEA-S.

All DHEA-S measurements were performed in the same institutional laboratory using a single immunoassay platform (Access DHEA-S, Beckman Coulter, Inc.). Age-specific ULNs were defined according to the 95th percentile reference intervals provided in the manufacturer's instructions for use. For premenopausal women, the ULN values used were: 321 µg/dL (ages 18–20), 391 µg/dL (ages 21–30), 266 µg/dL (ages 31–40), and 231 µg/dL (ages 41–50).

To exclude CS as part of the differential diagnosis of hirsutism, all patients underwent the 1 mg overnight DST. Since elevated DHEA-S levels were also present in our study population, the 1 mg overnight DST was also utilized to assess adrenal androgen suppression. Patients were informed to take 1 mg of oral dexamethasone at 11:00 pm, and blood samples for serum cortisol and DHEA-S were collected the following morning between 8:00 and 9:00 am after an overnight fast. Following the test, serum cortisol and DHEA-S levels were recorded. Adequate suppression was defined as a morning serum cortisol level <1.8 µg/dL (50 nmol/L).

Patients with a baseline 17-OHP level above 2 ng/mL underwent a 250 µg ACTH stimulation test to rule out LOCAH. In patients with a baseline DHEA-S level exceeding 700 µg/dL, non-contrast adrenal magnetic resonance imaging was performed to rule out adrenal masses. Menstrual irregularity was defined according to the 2023 guidelines as fewer than eight menstrual cycles per year or cycle lengths (<21 or >35 days) (3). PMOS was diagnosed according to the same 2023 evidence-based guidelines, requiring at least two of the following three features after exclusion of other etiologies: (i) oligo/anovulation, (ii) clinical and/or biochemical hyperandrogenism, and (iii) polycystic ovarian morphology (PCOM) on ultrasound (3); PCOM was defined as ≥20 follicles per ovary or an ovarian volume >10 mL (3).

Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as [glucose (mg/dL) × insulin (µU/mL)] / 405. The LH/FSH ratio was also recorded. A HOMA-IR value above ~2.5 was considered consistent with insulin resistance. The % excess of baseline DHEA-S above the age-specific ULN (%) and the % change in DHEA-S after the 1 mg DST (%) were calculated using Equations 1 and 2, respectively:

$$\% \text{ excess} = [(\text{baseline DHEA-S} - \text{age-specific ULN for DHEA-S}) / (\text{age-specific ULN for DHEA-S})] \times 100 \quad (1)$$

$$\% \text{ change} = [(\text{baseline DHEA-S} - \text{DHEA-S levels after 1 mg DST}) / (\text{baseline DHEA-S})] \times 100 \quad (2)$$

Patients were classified into two groups based on the DHEA-S response to the DST; Group 1 (DHEA-S suppressed) comprised patients whose post-DST DHEA-S level either normalized to the age-specific ULN or decreased by 50% from baseline and Group 2 (DHEA-S non-suppressed) comprised patients who did not meet the criteria for suppression. Group comparisons were made based on demographic, clinical, and biochemical parameters. Logistic regression analysis was conducted to evaluate variables potentially associated with DHEA-S suppression following the 1 mg DST.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (v. 27). Categorical variables were presented as frequencies and percentages; continuous variables as mean ± standard deviation. The Kolmogorov-Smirnov test was used to assess the normality of distribution. For independent-group comparisons, Student's t-test was applied to normally distributed variables, and the Mann-Whitney U test to non-normally distributed variables. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Bivariate correlations were assessed using Pearson's correlation for normally distributed variables and Spearman's rank correlation otherwise. Binary logistic regression analysis was performed to evaluate variables potentially associated with DHEA-S suppression after the 1 mg DST. Results were presented as odds ratios (ORs) with 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curve analysis was performed to assess the ability of baseline DHEA-S, expressed as % excess over the age-specific ULN, to predict suppression after the 1-mg DST. The optimal cut-off value was determined according to the Youden index. Statistical significance was defined as $p < 0.05$.

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RESULTS

A total of 362 female patients with hirsutism and elevated baseline serum DHEA-S were included in the study. The mean age of the study cohort was 24.44 ± 6.30 years, and the mean baseline DHEA-S level was 497.16 ± 119.58 μg/dL. Following administration of the 1-mg overnight DST, DHEA-S levels decreased in all participants. The mean post-DST DHEA-S level was 340.13 ± 91.93 μg/dL, corresponding to a mean absolute reduction of 157.03 μg/dL and a mean % change of 30.67 ± 15.34%. After the DST, DHEA-S levels normalized in 235 patients (64.9%). Thirty-one patients (8.6%) showed a ≥50% reduction from baseline; however, 29 of these patients were also among those who achieved normalization. Thus, only two additional patients met the suppression criterion based solely on a ≥50% reduction, resulting in a total of 237 patients (65.5%) classified as having DHEA-S suppression.

Based on predefined criteria, 237 patients (65.5%) were classified as Group 1 (DHEA-S suppressed), and 125 patients (34.5%) as Group 2 (DHEA-S non-suppressed). As expected, Group 2 exhibited significantly higher baseline DHEA-S levels, higher % excess of baseline DHEA-S above the ULN, and higher post-DST DHEA-S levels compared to Group 1 ($p < 0.001$). Conversely, the % change in DHEA-S was significantly greater in Group 1 ($p < 0.001$). Total testosterone and 17-OHP levels were statistically higher in Group 2 than in Group 1 ($p = 0.006$ and $p = 0.039$, respectively). Elevated total testosterone (>ULN) was more frequently observed in Group 2 ($p = 0.026$). The two groups did not differ significantly in age, glucose, insulin, HOMA-IR, TSH, LH, FSH, estradiol, or prolactin. Detailed demographic and hormonal comparisons between groups are presented in Table 1.

Table 1. Comparison of clinical and laboratory findings between patients with and without DHEA-S suppression (n = 362) after the 1-mg overnight dexamethasone suppression test

Variable	DHEA-S suppression (n = 237)	DHEA-S non-suppression (n = 125)	*p*-Value
Age, years	24.37 ± 5.98	24.58 ± 6.88	ns
PCOM on ultrasonography, n (%)	136 (57)	70 (56)	ns
Glucose, mg/dL	89.99 ± 8.58	90.41 ± 10.29	ns
Insulin, μU/mL	12.85 ± 12.57	12.63 ± 10.61	ns
HOMA-IR*	5.20 ± 3.47	4.44 ± 2.09	ns
TSH, mIU/L	2.21 ± 1.39	2.10 ± 1.14	ns
LH, IU/L	7.08 ± 3.87	6.57 ± 3.51	ns
FSH, IU/L	6.06 ± 1.56	6.12 ± 1.82	ns
LH/FSH ratio	1.24 ± 0.75	1.25 ± 1.02	ns
Estradiol, pg/mL	44.37 ± 36.64	44.85 ± 31.21	ns
Prolactin, ng/mL	20.07 ± 12.91	20.24 ± 10.95	ns
Total testosterone, ng/mL	0.60 ± 0.24	0.68 ± 0.27	0.006‡
Total testosterone above the age-specific ULN, n (%)	6 (2)	10 (8)	0.026‡
17-OHP, ng/mL	1.84 ± 1.46	2.12 ± 1.30	0.039‡
DHEA-S (baseline), μg/dL	465.18 ± 107.37	557.80 ± 118.43	<0.001‡
% excess DHEA-S (baseline) above the age-specific ULN, %**	27.50 ± 28.21	57.57 ± 29.84	<0.001‡
DHEA-S >700 μg/dL, n (%)	9 (4)	15 (12)	0.006‡
DHEA-S (after 1-mg DST), μg/dL	292.78 ± 57.84	429.55 ± 76.60	<0.001‡
Cortisol (after 1-mg DST), μg/dL	0.67 ± 0.27	0.67 ± 0.23	ns
Percent change in DHEA-S after the 1-mg DST (%)***	35.52 ± 14.67	21.48 ± 12.08	<0.001‡

Data are given as mean ± standard deviation. ‡Statistically significant ($p < 0.05$). *HOMA-IR: [(glucose × insulin) / 405]; **% excess: [(baseline DHEA-S - age-specific ULN for DHEA-S)/(age-specific ULN for DHEA-S)] × 100; ***percent change: [(baseline DHEA-S - DHEA-S levels after 1 mg DST)/(baseline DHEA-S)] × 100. ns: No statistical significance; DHEA-S: dehydroepiandrosterone sulfate; DST: dexamethasone suppression test; FSH: follicle stimulating hormone; HOMA-IR: homeostatic model assessment of insulin resistance; LH: luteinizing hormone; TSH: thyroid stimulating hormone; ULN: upper limit of normal; PCOM: polycystic ovary morphology; 17-OHP: 17-hydroxyprogesterone.

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Twenty-four patients (6.6%) had baseline DHEA-S levels of >700 μg/dL. In this specific subgroup (n = 24), the mean baseline DHEA-S was 802.17 ± 99.53 μg/dL. Following the 1-mg DST, the mean post-DST DHEA-S remained high at 443.33 ± 123.74 μg/dL, corresponding to a mean % change of 43.39% ± 19.07%. Within this subgroup, six patients (25.0%) achieved normalization of DHEA-S to the age-specific ULN, and an additional three patients (12.5%) had a reduction of 50% from baseline. Magnetic resonance imaging was performed in 20 patients; no adrenal masses or hyperplasia were identified. Among patients with baseline 17-OHP levels above 2 ng/mL (n = 45), an ACTH stimulation test was performed; two patients (4.4%) were diagnosed with LOCAH. Both were classified in the DHEA-S suppressed group after the DST. The first patient had a baseline DHEA-S of 525 μg/dL, which decreased to 257 μg/dL after DST; the second had a baseline DHEA-S of 413 μg/dL, decreasing to 341 μg/dL after DST.

Menstrual irregularities were observed in 37% of the patients (n = 134), and PCOM was present in 57% (n = 206). PMOS was diagnosed in 79% (n = 287) of patients. In non-parametric correlation analysis assessing sources of hyperandrogenism, the % excess of baseline DHEA-S showed statistically significant, positive, and weak associations with total testosterone ($r = 0.221$, $p < 0.001$) and 17-OHP ($r = 0.133$, $p = 0.025$). Multivariable binary logistic regression identified factors associated with DHEA-S suppression after the 1-mg DST (i.e., Group 1 assignment). Both the % excess of baseline DHEA-S above the age-specific ULN and DHEA-S >700 μg/dL were inversely associated with suppression (OR = 0.947, 95% CI: 0.923–0.972, $p < 0.001$; OR = 0.069, 95% CI: 0.013–0.352, $p = 0.001$, respectively). Patients with DHEA-S >700 μg/dL were 14.5 times more likely to exhibit non-suppression. Age, total testosterone, 17-OHP, and baseline DHEA-S were not significantly associated with suppression ($p > 0.05$). Full regression analysis results are provided in **Table 2**.

ROC curve analysis (**Figure 1**) of the % excess of baseline DHEA-S to predict suppression showed good discriminative performance, with an area under the curve of 0.827 (95% CI: 0.785–0.870, $p < 0.001$). The optimal cut-off for % excess of baseline DHEA-S was 32.22%, yielding a sensitivity of 72.3% and specificity of 82.7% for predicting suppression.

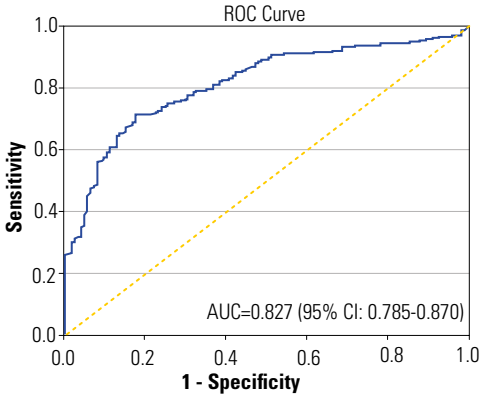


Figure 1. ROC curve demonstrating the diagnostic performance of basal DHEA-S expressed as percent excess over the age-specific upper limit of normal in predicting DHEA-S suppression after the 1-mg overnight dexamethasone suppression test.

DISCUSSION

This study evaluated the DHEA-S response to a 1-mg overnight DST in 362 hirsute women with elevated baseline DHEA-S. Our main finding is that adrenal androgen suppressibility to dexamethasone

Table 2. Logistic regression analysis of variables associated with DHEA-S suppression after 1-mg dexamethasone suppression test

Variables	B	SE	OR	*p*-Value	95% CI	
					Lower	Upper
Age, years	0.043	0.033	1.043	0.196	0.978	1.113
17-OHP, ng/mL	0.060	0.117	1.061	0.611	0.843	1.336
Total testosterone, ng/mL	-0.970	0.674	0.379	0.150	0.101	1.419
DHEA-S (baseline), μg/dL	0.004	0.003	1.004	0.257	0.997	1.010
% excess DHEA-S (baseline) above the age-specific ULN, %*	-0.055	0.013	0.947	<0.001‡	0.923	0.972
Baseline DHEA-S >700 μg/dL	-2.678	0.834	0.069	0.001‡	0.013	0.352

‡Statistically significant ($p < 0.05$). *% excess: [(baseline DHEA-S - age-specific ULN for DHEA-S)/(age-specific ULN for DHEA-S)] × 100. CI: Confidence interval; DHEA-S: dehydroepiandrosterone sulfate; OR: odds ratio; SE: standard error; ULN: upper limit of normal; 17-OHP: 17-hydroxyprogesterone

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is heterogeneous: while most participants (65.5%) showed adequate suppression (50% decrease and/or normalization), a substantial subgroup (34.5%) did not. Notably, this non-suppressed subgroup exhibited higher total testosterone and 17-OHP levels despite similar LH, suggesting a distinct biochemical phenotype with a possible functional adrenal contribution to hyperandrogenism.

The 1-mg DST is central to screening for CS by assessing cortisol suppressibility (5). Its role in evaluating adrenal androgen dynamics, however, is less established (5–9). Approximately 50% of women with PMOS demonstrate an exaggerated adrenal androgen response to exogenous ACTH administration, suggesting increased adrenal sensitivity to ACTH stimulation (10). The clinical presentation of CS varies widely due to differences in cortisol excess and glucocorticoid receptor sensitivity. Menstrual disturbances affect up to 80% of women with CS, nearly half of whom exhibit PMOS features (9). Historically, the 48-hour low-dose DST (LDDST) has been used to differentiate causes of androgen excess (6,8). In one study of 51 women with PMOS who underwent LDDST, 27.4% (14/51) had elevated baseline DHEA-S, yet both cortisol and DHEA-S were markedly suppressed to normal ranges after testing (6). Another study including 211 women with nontumorous hyperandrogenism and 17 patients with histologically confirmed androgen-secreting tumors reported elevated DHEA-S in 69% of tumor cases and 47% of nontumorous cases (8). Following LDDST, DHEA-S levels were adequately suppressed (≥40% reduction or normalization) in 92% of patients with nontumorous hyperandrogenism and two patients with ovarian tumors (8). LDDST thus achieved 100% sensitivity and 88% specificity in identifying androgen-secreting tumors (8), comparable to earlier data from longer dexamethasone protocols (3 mg/day for 5 days), in which DHEA-S suppression was complete in all patients with benign hyperandrogenism but absent in those with adrenal carcinoma (7).

A key methodological choice in our study was to use the 1-mg overnight DST rather than the multi-day LDDST. Unlike longer dexamethasone protocols, the 1-mg overnight DST is primarily used to assess cortisol suppressibility in selected patients with suspected hypercortisolism. In our clinical practice, when the 1-mg overnight DST was performed as part of the diagnostic evaluation in selected women with hirsutism, and post-DST DHEA-S levels were assessed in those with elevated baseline levels. This approach allowed us to evaluate DHEA-S changes after short-term dexamethasone exposure without requiring a multi-day protocol. However, adequate cortisol suppression does not necessarily validate the assessment of DHEA-S dynamics. Because DHEA-S has a longer half-life and distinct metabolic characteristics compared to cortisol, its response to short-term dexamethasone may be delayed or incomplete. Therefore, DHEA-S non-suppression after a single overnight DST should be interpreted with caution, as it may reflect incomplete suppression, interindividual variability in adrenal responsiveness, or a functional adrenal contribution, rather than definitive evidence of adrenal autonomy.

DHEA-S is produced almost exclusively by the adrenal glands and serves as a reliable marker of adrenal androgen production (2,4). In a series of 12 patients with adrenal carcinoma, only three had normal DHEA-S concentrations, while the remainder showed elevated levels (7). This observation supports that in some adrenal carcinomas lacking sulfotransferase activity, DHEA-S levels may remain normal. Thus, while normal DHEA-S values do not exclude adrenal disease, elevated levels are highly suggestive of adrenal-origin androgen excess (7,8). DHEA-S levels exceeding 700 μg/dL in premenopausal women should raise suspicion for an adrenal source, particularly adrenal neoplasms (1,5,11). This threshold aligns with existing recommendations to perform adrenal imaging in such patients to rule out adrenal tumors, which was performed in our cohort, revealing no adrenal masses. In the high-DHEA-S subgroup, six of 24 (25.0%) achieved normalization, and an additional three (12.5%) achieved ≥50% reduction after DST. The mean percent reduction following 1 mg DST was 43.39%. Therefore, while our findings suggest the 1 mg DST could help identify adrenal autonomy, more studies are needed to determine its utility in the differential diagnosis of adrenal carcinoma.

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The non-suppressed group represents a distinct biochemical phenotype. Unsurprisingly, these patients not only had higher baseline and post-DST DHEA-S levels, but also significantly higher total testosterone and 17-OHP levels. The observed association between DHEA-S non-suppression and elevated total testosterone is noteworthy. Elevations in both DHEA-S (adrenal) and 17-OHP (adrenal) alongside total testosterone (adrenal/ovarian), may suggest mixed-source hyperandrogenism underpinned by possible adrenal contribution. Our findings support the observation by Wu and cols. (12) that hirsutism frequently has a "mixed" etiology. The non-suppressed group, which exhibited both an adrenal component (elevated DHEA-S) and a probable ovarian component (indicated by both higher mean total testosterone levels and a greater frequency of patients with testosterone >ULN), likely represents this mixed cohort. Nonetheless, the % excess of baseline DHEA-S showed weak but statistically significant positive associations with total testosterone and 17-OHP levels. Although these findings may contribute to the biochemical characterization of this subgroup, they are not sufficient to precisely determine the source of hyperandrogenism or to establish a causal relationship. A notable finding is that the non-suppressed group demonstrated significantly higher total testosterone without a corresponding increase in LH, suggesting an LH-independent mechanism of hyperandrogenism. We propose that this may be driven by potential pathways consistent with the possible adrenal contribution in this group (11–15).

Elevated testosterone may partly reflect peripheral conversion of adrenal precursors (i.e., DHEA-S and 17-OHP), both significantly elevated in our cohort. This is a key finding, as it suggests that the adrenal contribution in our cohort may not be primarily driven by an exaggerated hypothalamic-pituitary-ovarian axis, but rather by an intrinsic adrenal mechanism, potentially a form of functional adrenal hyperandrogenism (FAH) commonly seen within the wide spectrum of PMOS (14,16). Previous studies have reported that women with PMOS and elevated DHEA-S (FAH phenotype) tend to be leaner and may exhibit a more favorable metabolic profile with lower insulin resistance compared to other PMOS phenotypes (15,17,18). Although we lacked body mass index (BMI) data and observed no difference in HOMA-IR between groups, this likely reflects the limitations of HOMA-IR as a surrogate marker rather than absence of metabolic alterations.

In our study, 79% of patients were diagnosed with PMOS and only two with LOCAH, indicating that DHEA-S non-suppression predominantly reflects a phenotype within the PMOS spectrum, often referred to as FAH rather than a distinct adrenal disorder. From a clinical standpoint, our ROC analysis showed that % excess of baseline DHEA-S was a strong predictor of DHEA-S suppression. In addition, the optimal cut-off of 32.22% above the age-specific ULN could help clinicians identify the degree of adrenal autonomy. Logistic regression further confirmed this dose-dependent association, showing that greater % excess of baseline DHEA-S was inversely associated with suppression. This finding was further supported by the analysis of the >700 µg/dL threshold. Patients with baseline DHEA-S levels above 700 µg/dL were 14.5 times more likely to exhibit non-suppression. Together, these results provide robust evidence that marked DHEA-S elevation predicts adrenal autonomy.

The strengths of this study include its large sample size, well-defined cohort of hirsute women with elevated DHEA-S, standardized laboratory protocols, and comprehensive hormonal assessments enabling exclusion of other endocrinopathies. However, several limitations must be acknowledged. First, as a retrospective study, detailed clinical characteristics such as hirsutism severity, acne, alopecia, and metabolic parameters (e.g., BMI and lipid profile) were unavailable for all patients, limiting a comprehensive clinical and metabolic characterization. Second, while we excluded CS and overt adrenal tumors, we did not employ gold standard procedures such as adrenal venous sampling to confirm the source of androgen excess, as this is an invasive procedure and therefore not feasible. Third, adrenal magnetic resonance imaging was only performed on a small subset of patients (n = 20) with the highest DHEA-S levels, so small, non-secreting micronodules in other patients cannot be definitively ruled out. Fourth, PMOS diagnoses were retrieved from patients' medical records; hence,

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ultrasonographic assessments were not standardized across a single radiologist. Finally, serum dexamethasone and ACTH levels were not measured post-DST; nevertheless, all patients in the final analysis demonstrated adequate cortisol suppression after 1-mg overnight DST test, supporting sufficient dexamethasone effect and appropriate test performance.

In conclusion, the 1-mg overnight DST may serve as a practical tool to assess DHEA-S suppressibility in hyperandrogenic women, complementing its established role in cortisol evaluation. Lack of DHEA-S suppression was associated with greater baseline DHEA-S elevation. In our cohort, both the % excess above the age-specific ULN (cut-off 32.22%) and an absolute DHEA-S level >700 μg/dL were useful in identifying a subgroup with the non-suppressed 1-mg DST phenotype. This phenotype was associated with higher total testosterone and 17-OHP levels despite similar LH, suggesting a possible functional adrenal contribution within the broader PMOS-related hyperandrogenism spectrum. Prospective studies are warranted to externally validate these cut-offs and clarify long-term metabolic and reproductive outcomes for this subgroup.

Ethical approval: this study was approved by the Clinical Ethics Committee and conducted in accordance with the Helsinki Declaration.

Disclosure: no potential conflict of interest relevant to this article was reported.

Data availability: datasets related to this article will be available upon request to the corresponding author.

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original

Quantitative magnetic resonance imaging (MRI) evaluation of visceral and hepatic fat in Cushing's syndrome: insights into cortisol-driven metabolic changes

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ABSTRACT

Objective: To quantify hepatic steatosis and visceral adiposity in active Cushing's syndrome (CS) using magnetic resonance imaging (MRI), evaluate associations between hypercortisolism and MRI-derived fat metrics, and characterize the relationship between hepatic and visceral fat compartments. **Subjects and methods:** This retrospective cross-sectional study assessed 36 patients with biochemically confirmed active CS. Hepatic proton density, fat fraction, and visceral adipose tissue area were quantified using chemical-shift encoded MRI with semi-automated segmentation. Biochemical assessments included serum cortisol, adrenocorticotropic hormone, liver enzymes, and lipid profiles. **Results:** MRI-defined hepatic steatosis (fat fraction ≥6.25%) was identified in 69.4% (25/36) of patients. Those with steatosis exhibited higher serum cortisol levels (832.9 ± 347.8 vs. 581.2 ± 331.9 nmol/L, $p = 0.049$) and greater visceral adiposity (35.0 ± 8.0 vs. 23.9 ± 6.7 cm^2, $p < 0.001$). Serum cortisol positively correlated with visceral fat area ($r = 0.512$, $p = 0.001$) and inversely correlated with liver signal intensity index ($r = -0.424$, $p = 0.01$). Visceral adiposity demonstrated a strong positive correlation with hepatic steatosis grade ($r = 0.601$, $p < 0.001$). **Conclusion:** Hepatic steatosis was highly prevalent in active CS and closely linked to hypercortisolism and visceral adiposity. These findings support a mechanistic coupling between central and ectopic fat accumulation. Integrated MRI-based fat quantification provides a non-invasive, radiation-free approach for refined cardiometabolic risk stratification in this high-risk population.

Keywords: Magnetic resonance imaging (MRI); Cushing's syndrome; hepatic steatosis; visceral fat; cardiometabolic

INTRODUCTION

Endogenous Cushing's syndrome (CS) is a state of chronic glucocorticoid excess that confers a significant metabolic burden, characterized by visceral adiposity, insulin resistance, diabetes mellitus, atherogenic dyslipidemia, and hypertension (1). Persistent elevations of cortisol promote hepatic gluconeogenesis and impair insulin sensitivity, while also altering both distribution and function of peripheral adipose tissue. Collectively, these effects create an environment conducive to both direct and indirect hepatic triglyceride deposition (2).

In parallel, the conceptual framework for fatty liver disease has evolved. The term non-alcoholic fatty liver disease has been replaced by metabolic dysfunction-associated steatotic liver disease (MASLD), defined by imaging- or histology-confirmed hepatic steatosis in the presence of at least one of five cardiometabolic risk factors, in the absence of significant alcohol consumption or other chronic liver disorders (3). Within this context, hepatic steatosis is regarded as the hepatic manifestation of the metabolic syndrome (4). Glucocorticoid

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excess contributes to MASLD pathogenesis through several convergent mechanisms: (a) expansion of visceral adipose tissue (VAT) with increased portal delivery of free fatty acids; (b) glucocorticoid receptor (GR)-mediated upregulation of hepatic lipogenic enzymes; (c) suppression of fatty acid oxidation; and (d) aggravation of systemic insulin resistance (5).

Although histological assessment of liver tissue remains the reference standard for diagnosing hepatic steatosis, liver biopsy is invasive, carries a non-negligible risk of bleeding and sampling variability, and is impractical for screening or large-scale epidemiological research (6). Consequently, non-invasive imaging has become central to clinical practice and research. Recent MRI techniques allow precise, non-invasive quantification of visceral and hepatic fat, improving diagnosis of metabolic syndrome and MASLD. These methods aim to deepen our understanding of the relationship between hepatic and visceral fat and obesity-related conditions such as metabolic syndrome, MASLD, and cardiovascular disease (7).

Chemical shift-encoded magnetic resonance imaging (MRI), using in-phase and opposed-phase acquisitions, enables accurate and reproducible quantification of hepatic fat by exploiting the resonance frequency differences between water and fat protons. Proton magnetic resonance spectroscopy offers even greater precision, although its use is primarily confined to specialized research settings due to technical complexity and limited accessibility (8). In contrast, most previous research on body fat distribution in CS employed computed tomography (CT) (9). Although CT provides excellent spatial resolution for adipose tissue mapping, it involves ionizing radiation, which is an essential concern in metabolically compromised populations, and is insufficiently sensitive to detect mild hepatic steatosis, particularly when fat content is below approximately 30% (10).

Various imaging modalities have been employed to assess body composition in CS, including whole-body MRI (11), dual-energy X-ray absorptiometry (12), magnetic resonance spectroscopy for marrow and intrahepatic lipids assessment (13), and CT (14).

This study introduces several methodological strengths and contemporary innovations. First, it uses MRI as a single, radiation-free modality to simultaneously obtain quantitative measurements of hepatic steatosis and visceral fat area in patients with active CS. Second, it incorporates a dedicated semi-automated segmentation platform (SliceOmatic V5.0) for precise visceral fat quantification, a technique not previously described in the CS literature. Third, it directly examines associations between circulating cortisol concentrations and high-resolution MRI-derived metabolic indices, offering granular links between biochemical hypercortisolism and organ-level fat phenotypes.

Accordingly, this study aimed to establish the prevalence and severity of hepatic steatosis (MASLD) in active CS using MRI-based quantification, examine associations between serum cortisol levels and MRI-derived markers of adiposity and steatosis, and characterize the relationship between visceral fat burden and hepatic steatosis grade.

SUBJECTS AND METHODS

Study design and population

This cross-sectional study retrospectively analyzed data from 36 consecutive patients with newly diagnosed, treatment-naïve active CS who were managed at Alexandria University Hospital between January 2023 and January 2025. The diagnosis of CS followed current Endocrine Society Clinical Practice Guidelines (15), requiring: (a) elevated midnight serum cortisol (>50 nmol/L) during sleep; (b) failure to suppress serum cortisol to <50 nmol/L at 48 h after administration of dexamethasone 0.5 mg every 6 h for 48 h; and (c) confirmatory dynamic testing consistent with either adrenocorticotropic hormone (ACTH)-dependent or ACTH-independent etiology.

Exclusion criteria

Patients were excluded if they met any of the following criteria: (a) known chronic liver disease of any cause, including viral hepatitis (hepatitis B surface antigen or hepatitis C antibody positive), autoimmune liver disease, drug-induced liver injury, cholestatic liver disease, alcohol-related liver disease (≥20 g/day for women, ≥30 g/day for men), or hereditary hemochromatosis; (b) previous treatment with any

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cortisol-lowering agents (ketoconazole, metyrapone, osilodrostat, or mitotane) for >4 weeks; (c) standard contraindications to MRI (ferromagnetic implants, claustrophobia, or pregnancy); or (d) age <18 years.

Clinical and anthropometric assessment

All patients underwent comprehensive medical history reviews and physical examinations. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured at the midpoint between the lower costal margin and the iliac crest.

Biochemical analyses

Fasting venous blood samples were collected from the antecubital vein between 8:00–9:00 am following an overnight fast of at least 10 h. All biochemical analyses were performed within 4 h of collection. Serum cortisol was measured using an electrochemiluminescence immunoassay (Cobas e601, Roche Diagnostics, Germany). The diurnal serum cortisol profile was calculated as the mean of five samples collected at 9:00 am, 12:00 pm, 5:00 pm, 9:00 pm, and 12:00 am. Plasma ACTH and serum dehydroepiandrosterone sulfate were measured using chemiluminescent immunometric assays (Immulite 2000, Siemens Healthcare Diagnostics, Germany). Additional laboratory assessments included alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, fasting plasma glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and serum uric acid. All biochemical assays were performed using standard enzymatic colorimetric methods on an automated analyzer (AU680, Beckman Coulter, USA).

Magnetic resonance imaging acquisition

All imaging was performed using a 1.5-Tesla scanner (MAGNETOM Aera, Siemens Healthineers, Germany) with an 18-channel body matrix coil and 32-channel spine matrix coils. Patients were scanned in the supine position with arms elevated above the head to minimize wraparound artifacts.

Hepatic fat quantification

A breath-hold three-dimensional T1-weighted dual-echo Dixon volumetric interpolated breath-hold examination sequence was acquired in the axial plane during a single 18-second breath-hold. Acquisition parameters were: repetition time of 7.0 ms; echo time 1 2.38 ms (opposed-phase); echo time 2 4.76 ms (in-phase); flip angle 10°; slice thickness 3.0 mm; intersection gap 0 mm; matrix 256 × 192; field of view 380 × 285 mm^2; voxel size 1.5 × 1.5 × 3.0 mm; and parallel imaging acceleration factor 2. Fat-only, water-only, in-phase, and opposed-phase images were automatically reconstructed by the scanner software.

Visceral fat quantification

Axial T1-weighted fast spin-echo sequences without fat saturation were acquired through the abdomen, centered at the L4-L5 intervertebral disc level. Acquisition parameters included: repetition time of 400 ms; TE 10 ms; echo train length 3; slice thickness 8.0 mm; intersection gap 2.0 mm; matrix 320 × 224; field of view 400 × 300 mm^2; number of signal averages 2; and total acquisition time of 2 minutes and 14 seconds.

Image analysis

All image analyses were performed by a single radiologist with 12 years of experience in abdominal imaging, who was blinded to all clinical and biochemical data. Intra-observer reproducibility was assessed by reanalyzing 10 randomly selected studies after a 4-week interval.

Hepatic steatosis quantification

Analysis was conducted using dedicated post-processing software (Syngo.via, Siemens Healthineers, Germany). Three circular regions of interest (ROIs) of 2.0 cm^2 each were placed in the right hepatic lobe (Couinaud segments V–VIII) on both opposed-phase and corresponding in-phase images. ROIs were carefully positioned to exclude hepatic vessels, bile ducts, the liver capsule, and artifacts. The mean signal intensity (SI) from the three ROIs was calculated. The hepatic fat fraction was then calculated using **Equation 1**:

$$\text{Fat fraction (\%)} = [(SI_{\text{in-phase}} - SI_{\text{opposed-phase}}) / (2 \times SI_{\text{in-phase}})] \times 100 \quad (1)$$

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Hepatic steatosis was defined as a hepatic fat fraction ≥6.25% based on the 95th percentile of hepatic triglyceride content in healthy, normal-weight populations, as determined by proton magnetic resonance spectroscopy (16).

Visceral fat quantification

Visceral fat area was quantified using semi-automated segmentation software (SliceOmatic V5.0, Tomovision, Canada). This study quantified VAT on a single axial slice at the L4-L5 level, a validated and widely adopted approach for total visceral fat estimation in large-scale studies due to its simplicity, reproducibility, and feasibility compared to multi-slice volumetric approaches. On the axial T1-weighted image at the L4-L5 intervertebral disc level, the visceral fat compartment was defined by manually tracing the inner aspect of the abdominal wall musculature (transversus abdominis, internal oblique, external oblique, and rectus abdominis) and the anterior border of the vertebral body. Pixels within the defined compartment falling within the fat intensity range (as determined by histogram analysis) were automatically segmented and quantified. Visceral fat area was expressed as total surface area in square centimeters (cm^2).

Statistical analysis

Statistical analyses were performed using SPSS v. 25.0 (IBM Corp., USA). The normality of continuous variables was assessed with the Shapiro-Wilk test and visual inspection of Q-Q plots. Continuous variables are presented as mean ± standard deviation for normally distributed data, or as median with interquartile range for non-normally distributed data. Categorical variables are described as absolute counts and percentages. For comparative analyses, patients were stratified into two groups according to hepatic steatosis status, defined by an MRI-derived fat fraction ≥6.25%. This a priori grouping enabled identification of clinical, biochemical, and anthropometric factors associated with hepatic steatosis in active CS. Comparisons between groups were carried out using the independent samples t-test for normally distributed continuous variables and the Mann-Whitney U test for non-normally distributed variables. Categorical variables were compared using Chi-square or Fisher's exact test, as applicable. Correlations were assessed by Pearson's correlation coefficient for normally distributed variables and Spearman's rank correlation coefficient otherwise. Multiple linear regression was originally planned to identify independent predictors of hepatic fat fraction; however, to avoid model overfitting due to small sample size, analyses were limited to bivariate correlations. A two-tailed p-value < 0.05 was regarded as statistically significant for all analyses.

RESULTS

Patient characteristics and group stratification

The study cohort comprised 36 patients with active, treatment-naïve CS. The mean age was 50.2 ± 13.8 years, most participants were female (29/36, 80.6%), the mean BMI was 28.0 ± 7.0 kg/m^2 and impaired glucose tolerance or type 2 diabetes mellitus was present in 15 (41.7%) patients (**Table 1**). Using the predefined MRI-derived hepatic fat fraction threshold of ≥6.25%, patients were stratified into two groups: those without hepatic steatosis (n = 11, 30.6%) and those with hepatic steatosis (n = 25, 69.4%). This stratification enabled identification of factors associated with hepatic steatosis in active CS and comparison of metabolic and imaging parameters between these clinically distinct phenotypes.

Demographic and anthropometric comparisons

Patients with hepatic steatosis were significantly older than those without steatosis (55.1 ± 11.9 vs. 44.0 ± 15.8 years, p = 0.03). BMI was also significantly higher in the steatosis group (29.6 ± 7.7 vs. 24.0 ± 5.0 kg/m^2, p = 0.03). No significant difference in sex distribution was observed between groups (p = 0.80) (**Table 1**).

Biochemical comparisons

As shown in **Table 1**, patients with hepatic steatosis had significantly higher serum cortisol concentrations than those without steatosis (832.9 ± 347.8 vs. 581.2 ± 331.9 nmol/L, p = 0.049). The lipid profile was significantly more atherogenic in the steatosis group, with higher total cholesterol (7.99 ± 2.71 vs. 6.22 ± 1.45 mmol/L, p = 0.01), triglycerides (2.00 ± 1.09 vs. 1.22 ± 0.65 mmol/L, p = 0.01), and LDL-C (5.42 ± 1.87 vs. 4.48 ± 1.18 mmol/L, p = 0.02). HDL-C was lower in the

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steatosis group, approaching but not reaching statistical significance (1.29 ± 0.24 vs. 1.46 ± 0.15 mmol/L, $p = 0.05$). Markers of hepatocellular injury were significantly elevated in patients with hepatic steatosis: ALT (52.0 ± 34.1 vs. 30.0 ± 14.9 U/L, $p = 0.049$), AST (27.0 ± 9.1 vs. 20.0 ± 10.2 U/L, $p = 0.048$), and GGT (41.2 ± 10.1 vs. 34.6 ± 8.2 U/L, $p = 0.045$). Total bilirubin was also significantly higher in the steatosis group (11.2 ± 6.4 vs. 7.0 ± 2.8 μmol/L, $p = 0.045$). The prevalence of hyperuricemia was higher in patients without hepatic steatosis (18.2% vs. 16%, $p = 0.76$), although this difference was not statistically significant. Impaired glucose tolerance or diabetes mellitus was more prevalent in the steatosis group (44.0% vs. 36.4%); however, this was not statistically significant ($p = 0.29$).

MRI-DERIVED QUANTITATIVE MEASUREMENTS

Hepatic steatosis

The mean hepatic fat fraction was significantly higher in patients with MRI-defined steatosis compared to those without (45.0 ± 10.1% vs. 5.40 ± 1.51%, $p < 0.001$). The hepatic fat fraction, defined as the signal-intensity ratio between opposed-phase and in-phase images and expressed as a percentage, was significantly lower in the steatosis group, reflecting greater intracellular lipid content (**Table 1** and **Figure 1**).

Visceral fat

Table 1 and **Figure 2** demonstrate that visceral fat area at the L4-L5 level was significantly greater in patients with hepatic steatosis (35.0 ± 8.0 vs. 23.9 ± 6.7 cm^2, $p < 0.001$).

Correlation analyses

Figure 3 demonstrated that serum cortisol showed a significant positive correlation with markers of hepatocellular injury, including AST ($r = 0.389$, $p = 0.02$), ALT ($r = 0.412$, $p = 0.01$), ALP ($r = 0.289$, $p = 0.01$), and GGT ($r = 0.378$, $p = 0.03$). Serum cortisol concentration also correlated positively with hepatic fat fraction ($r = 0.326$, $p = 0.001$) (**Figure 4A**), visceral fat area

Table 1. Demographic, biochemical, and MRI characteristics stratified by hepatic steatosis status

Parameter	Total (n = 36)	Steatosis		*p*-value
		Non-hepatic (n = 11)	Hepatic (n = 25)	
Demographics				
Age, years	50.2 ± 13.8	44.0 ± 15.8	55.1 ± 11.9	0.03
Female sex, n (%)	29 (80.6)	9 (81.8)	20 (80.0)	0.80
BMI, kg/m^2	28.0 ± 7.0	24.0 ± 5.0	29.6 ± 7.7	0.03
Biochemistry				
Serum cortisol, nmol/L	755.9 ± 364.2	581.2 ± 331.9	832.9 ± 347.8	0.049
Total cholesterol, mmol/L	7.45 ± 2.44	6.22 ± 1.45	7.99 ± 2.71	0.01
Triglycerides, mmol/L	1.76 ± 1.01	1.22 ± 0.65	2.00 ± 1.09	0.01
LDL-C, mmol/L	5.13 ± 1.72	4.48 ± 1.18	5.42 ± 1.87	0.02
HDL-C, mmol/L	1.34 ± 0.23	1.46 ± 0.15	1.29 ± 0.24	0.05
ALT, U/L	45.3 ± 30.6	30.0 ± 14.9	52.0 ± 34.1	0.049
AST, U/L	24.9 ± 10.0	20.0 ± 10.2	27.0 ± 9.1	0.048
GGT, U/L	39.2 ± 10.1	34.6 ± 8.2	41.2 ± 10.1	0.045
Total bilirubin, μmol/L	9.9 ± 5.7	7.0 ± 2.8	11.2 ± 6.4	0.045
Impaired glucose/diabetes (%)	15 (41.7)	4 (36.4)	11 (44.0)	0.29
Hyperuricemia, n (%)	6 (16.7)	2 (18.2)	4 (16.0)	0.76
MRI metrics				
Hepatic fat fraction, %	25.2 ± 5.8	5.40 ± 1.51	45.0 ± 10.1	<0.001
Visceral fat area, cm^2	31.6 ± 9.6	23.9 ± 6.7	35.0 ± 8.0	<0.001

Data presented as mean ± standard deviation.

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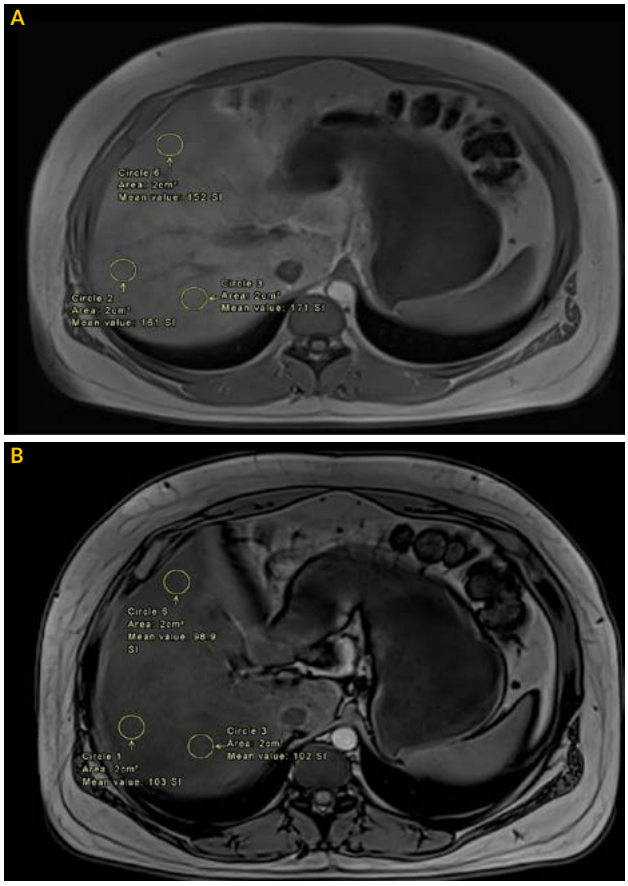


Figure 1. Liver MRI T1-weighted in-phase (**A**) and out-of-phase (**B**) images show diffuse hepatic steatosis, evidenced by a uniform signal drop on the out-of-phase sequence, consistent with diffuse fatty infiltration.

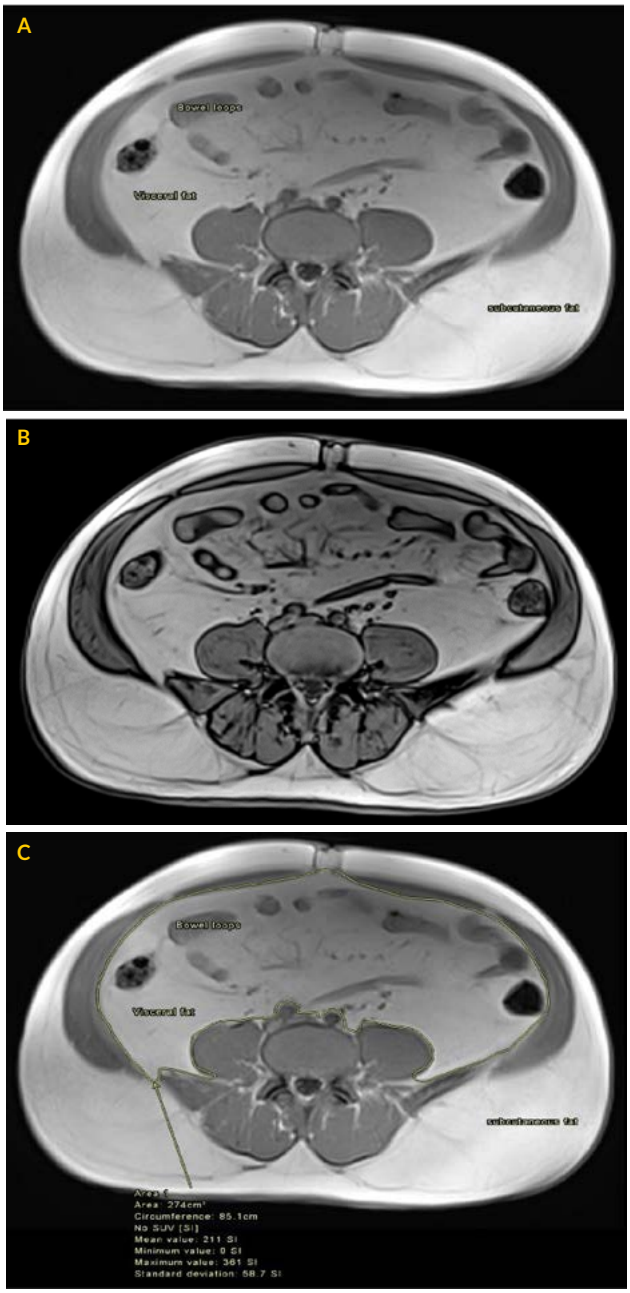


Figure 2. Axial abdominal MRI at the L4-L5 level, depicting visceral fat assessment. (**A**) In-phase T1-weighted image, (**B**) out-of-phase T1-weighted image, and (**C**) in-phase T1-weighted image with segmented visceral fat. The region of interest (ROI) was manually delineated along the abdominal wall (yellow line) to distinguish visceral fat from subcutaneous fat, and the visceral fat area within the ROI was quantified.

(r = 0.512, p = 0.001) (**Figure 4B**), and degree of hepatic steatosis (r = 0.263, p = 0.001) (**Figure 4C**). There was a significant negative correlation between the hepatic signal intensity index and cortisol levels (r = -0.424, p = 0.01), indicating that higher cortisol levels were associated with greater visceral adiposity and higher hepatic fat content. A strong, statistically significant positive correlation was observed between visceral fat area and the degree of hepatic steatosis (r = 0.601, p < 0.001). This finding represents one of the principal observations of this study, demonstrating a close quantitative relationship between these two glucocorticoid-sensitive fat depots.

DISCUSSION

In CS, fat tends to accumulate preferentially in visceral and periorgan compartments rather than subcutaneous tissue, leading to hypertrophic, inflamed VAT with dysregulated adipokines, enhanced lipolysis, and reduced storage capacity. This state is associated with ectopic fat deposition in the liver and other organs. Independent of BMI, active CS is marked by increased waist circumference and remodeling of inflammatory VAT macrophages, indicating qualitative changes in VAT beyond simple expansion (17).

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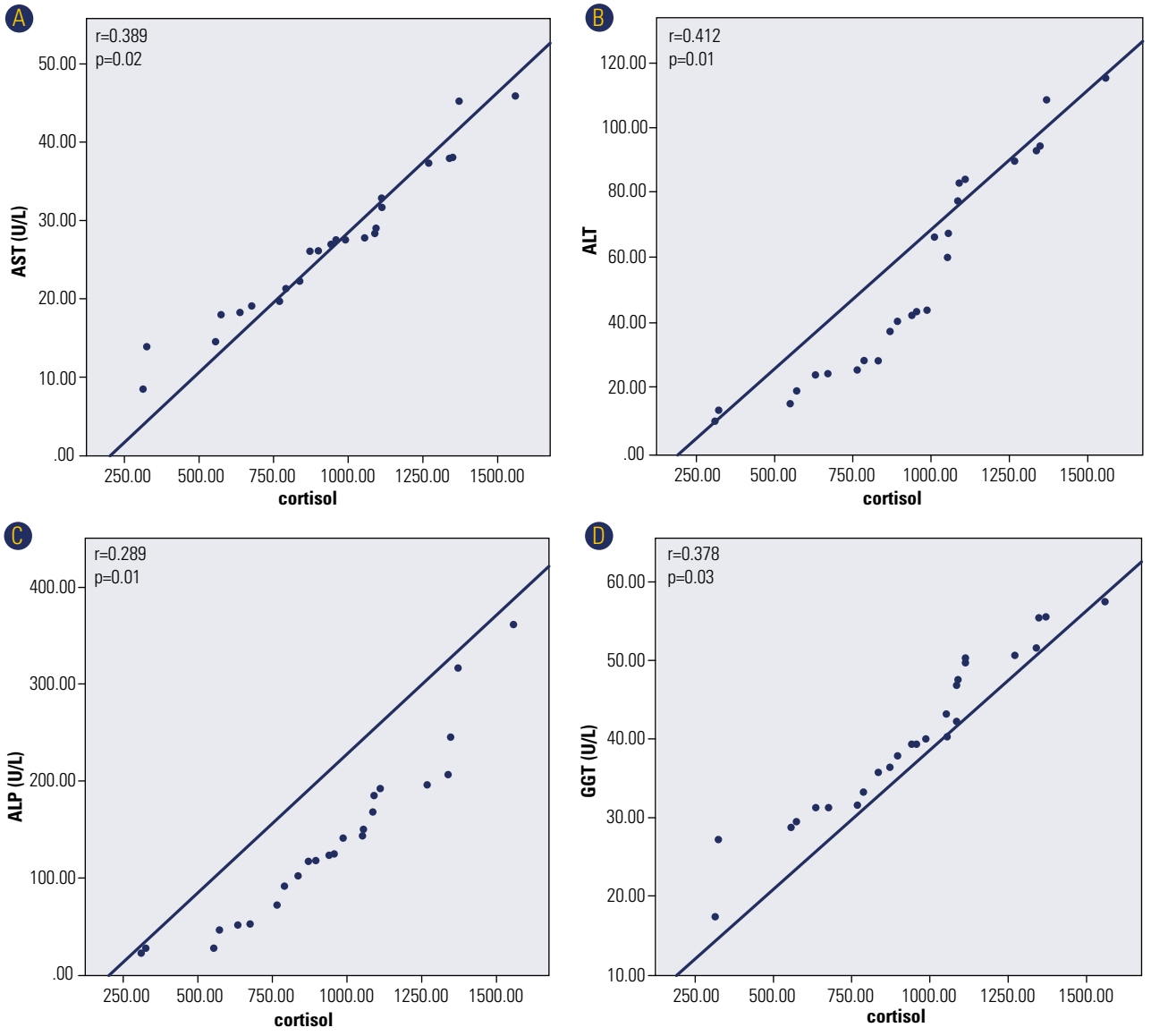


Figure 3. (**A**) Association between serum cortisol and AST. (**B**) Association between serum cortisol and ALT. (**C**) Association between serum cortisol and ALP. (**D**) Association between serum cortisol and GGT.

Conceptual models position hepatic steatosis as one of several ectopic fat depots closely linked to visceral adiposity, with intrahepatic triglyceride content correlating more strongly with visceral than subcutaneous fat, even among patients evaluated for adrenal disease. In CS, cortisol-induced visceral obesity, insulin resistance, and atherogenic dyslipidemia are associated with hepatic triglyceride accumulation due to increased de novo lipogenesis, higher free fatty acid flux from visceral fat, and reduced very low density lipoprotein export. Recent evidence indicates that hypercortisolism is linked to ectopic fat deposition in the pancreas, renal sinus, and skeletal muscle, further supporting CS as a clinical model of systemic ectopic lipid overload, with liver fat as a central feature of this phenotype (18).

Hepatic steatosis was highly prevalent in this cohort, with chemical shift-encoded MRI demonstrating MASLD in 69.4% of patients with active, treatment-naïve CS. This proportion is more than double that reported in the general Egyptian population (31.6%), and clearly exceeds global estimates for MASLD in unselected populations (25%–30%) (19,20), indicating that liver fat accumulation represents a core metabolic feature of active CS rather than an incidental co-morbidity. Notably, the 69.4% prevalence observed here

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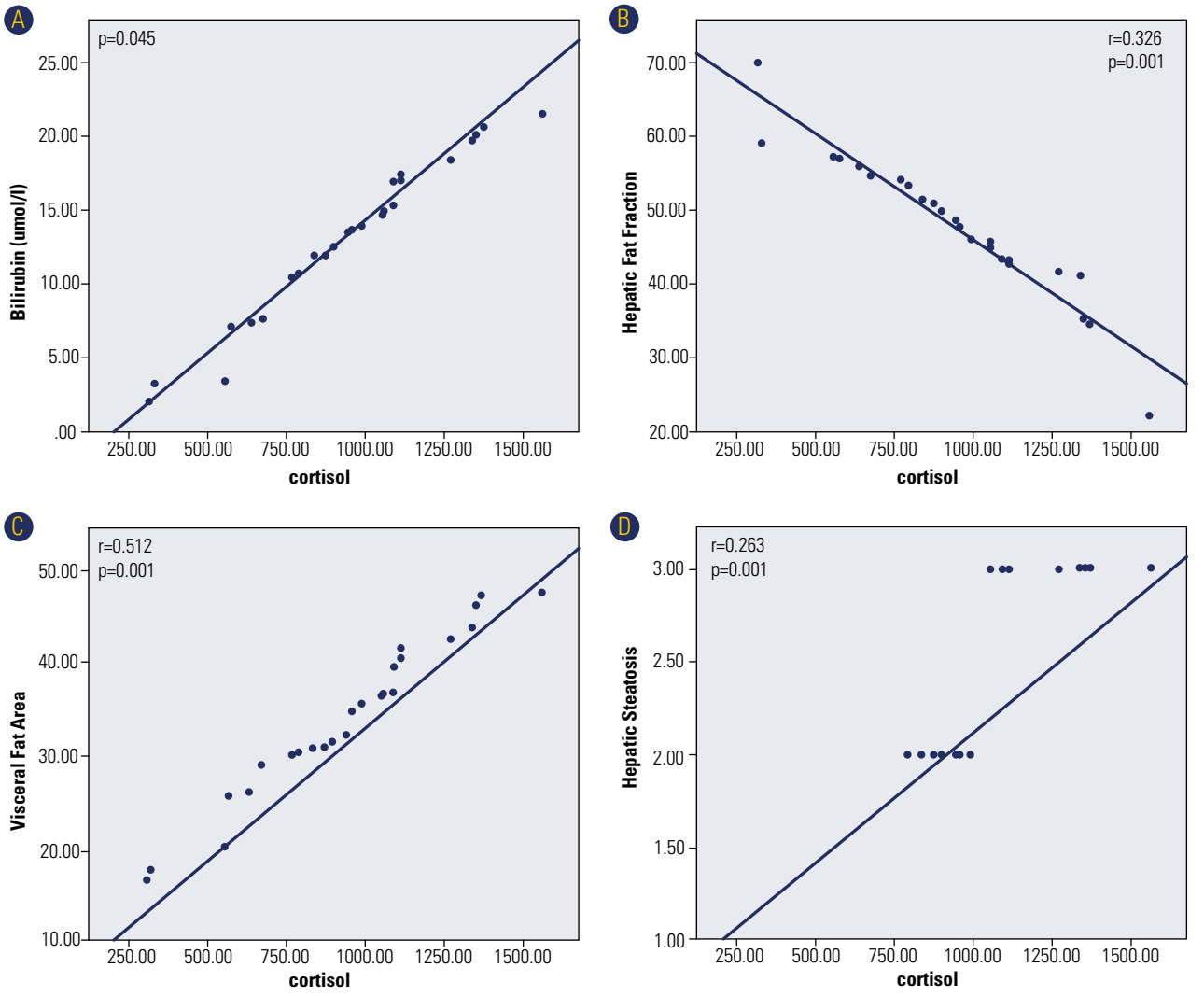


Figure 4. (**A**) Association between serum cortisol and bilirubin. (**B**) Association between serum cortisol and the degree of hepatic attenuation. (**C**) Association between serum cortisol and visceral fat distribution. (**D**) Association between serum cortisol and the degree of hepatic steatosis.

substantially surpasses the 20% reported by Rockall and cols. (9) among 50 CS patients, highlighting how advances in imaging and differences in study design can markedly affect the perceived disease burden.

Several methodological and demographic factors may account for these discrepancies. Foremost, earlier work relied on CT-based liver fat estimation, which infers steatosis from hepatic attenuation (Hounsfield units) and poorly detects mild-to-moderate fat accumulation (<30% histological steatosis), in addition to being susceptible to confounding by hepatic iron, edema, or glycogen (10). In contrast, chemical-shift-encoded MRI leverages the intrinsic resonance-frequency differences between water and fat protons to provide sensitive, quantitative fat-fraction measurements across the clinically relevant range, thereby capturing subclinical and early-stage steatosis that CT may miss. In addition, this study deliberately included only patients with active, untreated CS at the time of imaging, ensuring ongoing exposure to chronic hypercortisolism. Earlier studies may have inadvertently enrolled individuals with partially treated disease or intermittent cortisol excess, potentially underestimating the true prevalence of steatosis. It is also plausible that the Egyptian population has a higher baseline susceptibility to hepatic steatosis due to genetic background, dietary habits, or clustering of metabolic risk factors, as suggested by Tomah and cols. (19), further amplifying the effect of glucocorticoid excess on liver fat accumulation.

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Our findings are consistent with the recent work of Yu and cols. (21), who used proton magnetic resonance spectroscopy, the most sensitive noninvasive technique for hepatic fat quantification, to study 48 CS patients and 30 individuals with non-functioning adrenal incidentalomas. They reported a 66% prevalence of hepatic steatosis in the CS group, closely mirroring our 69.4%. The convergence of these results, despite differences in geography, patient selection, and MRI technique (spectroscopy versus chemical shift-encoded imaging), provides compelling evidence that hepatic steatosis is extremely common in active CS and likely under-recognized when less sensitive modalities are used.

In this current study, an external eucortisolemic control cohort matched for age, sex, BMI, or metabolic comorbidities was not included. These factors may substantially influence visceral adiposity and hepatic fat accumulation and therefore represent potential confounders. However, despite these limitations, significant associations remained among serum cortisol levels, visceral fat area, and hepatic steatosis within the active CS cohort, suggesting a biologically meaningful relationship between hypercortisolism and ectopic fat deposition.

Mechanistically, the relationship between hypercortisolism and hepatic steatosis is biologically plausible and appears to arise through direct and indirect pathways. Indirectly, chronic glucocorticoid excess preferentially expands and metabolically disrupts visceral adipose tissue, promoting increased lipolysis and reduced insulin sensitivity in these depots. As a result, the portal circulation is exposed to increased free fatty acid flux, thereby delivering more lipid substrate to hepatocytes and promoting intrahepatic triglyceride synthesis and storage. The strong positive correlation observed between visceral fat area and steatosis severity ($r = 0.601$, $p < 0.001$) is consistent with this portal "lipid spillover" model. Cortisol also acts directly on hepatocytes via GRs, upregulating key lipogenic enzymes such as fatty acid synthase and acetyl-CoA carboxylase, while simultaneously suppressing PPARα-driven fatty acid oxidation. Local regeneration of active cortisol within the liver by 11β-hydroxysteroid dehydrogenase type 1 further amplifies these effects by enhancing intrahepatic glucocorticoid signaling, independent of circulating cortisol concentrations (22). Collectively, these indirect (visceral adiposity-mediated) and direct (hepatocyte-intrinsic) mechanisms create a metabolic milieu that strongly favors hepatic fat accumulation, providing a coherent pathophysiological explanation for the high burden of MASLD observed in active CS.

The finding that roughly 70% of patients with active CS have MRI-detectable hepatic steatosis has clear clinical implications. This suggests that MASLD screening should be considered as part of the metabolic evaluation in newly diagnosed CS, utilizing the same chemical shift-encoded MRI sequences already obtained for adrenal imaging to allow concurrent liver fat quantification without additional scan time or radiation exposure. Nevertheless, the present cross-sectional design does not determine whether MRI-derived hepatic or visceral fat measurements independently predict clinical outcomes or improve risk stratification beyond standard metabolic markers.

The relatively high prevalence of hepatic steatosis reported in this cohort should be interpreted with caution. As this study was conducted at a single center in Egypt, where metabolic dysfunction and hepatic steatosis are known to be relatively high, population-specific genetic, environmental, dietary, and lifestyle factors may have contributed to the findings. Therefore, the reported prevalence estimates may not be directly generalizable to other ethnic or geographic populations, and confirmation in multicenter studies with more diverse cohorts is required.

Identification of steatosis is crucial, as MASLD is associated with increased cardiovascular mortality, progression to steatohepatitis, and, in some patients, fibrosis and cirrhosis. In this cohort, CS patients with hepatic steatosis exhibited higher cortisol levels, more atherogenic lipid profiles, elevated liver enzymes, and greater visceral adiposity, indicating a particularly high-risk cardiometabolic phenotype. These findings support the need for intensified management that normalizes cortisol excess and aggressively addresses cardiometabolic risk factors to reduce long-term hepatic and cardiovascular complications.

Although increased visceral and hepatic fat may contribute to adverse cardiometabolic outcomes in

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active CS, this cross-sectional study cannot determine whether these MRI-derived abnormalities independently predict clinical outcomes or improve risk stratification beyond established metabolic markers.

The main strength of this study is its precise quantification of adiposity. To our knowledge, this is the first CS cohort in which dedicated semi-automated software (SliceOmatic) was used to obtain reproducible, MRI-based measurements of visceral fat area, reducing operator dependence and providing continuous quantitative data that enhance statistical power and comparability.

Several limitations should be acknowledged. Adipose tissue distribution and hepatic steatosis in CS are likely influenced not only by cortisol excess, but also by disease duration, chronicity, severity of hypercortisolism, concomitant metabolic disorders, medication exposure, physical activity, and hormonal status, including menopause. Several of these variables were incompletely captured in this retrospective cohort, so residual confounding cannot be excluded. The absence of a matched eucortisolemic control group limited the ability to determine the independent contribution of cortisol excess relative to other determinants of visceral adiposity and hepatic steatosis. The relatively small cohort size, although comparable to prior MRI-based studies in rare endocrine disorders, may have limited statistical power and restricted the ability to perform comprehensive multivariable or subgroup comparisons. Despite the modest cohort size, several statistically significant associations were observed, including correlations between cortisol levels, visceral adiposity, and hepatic steatosis severity, suggesting that the detected relationships are biologically robust.

Although intra-observer reproducibility was assessed by repeat analysis of 10 examinations after a 4-week interval, quantitative reproducibility metrics such as the intraclass correlation coefficient and coefficient of variation were not reported; we acknowledge that inclusion of these metrics would further strengthen assessment of measurement reliability.

Larger multicenter, prospective studies are required to validate and extend these findings. The absence of a eucortisolemic control group with similar metabolic risk profiles limits the ability to attribute the high steatosis burden specifically to glucocorticoid excess. Interpretation of the observed prevalence of steatosis and group comparisons should be considered in light of these limitations. First, no age-, sex-, or BMI-matched eucortisolemic control group was included, limiting the ability to determine whether the high prevalence of hepatic steatosis observed is independently attributable to hypercortisolism rather than to metabolic risk factors. Second, patients with and without steatosis differed significantly in age and BMI, which are established determinants of hepatic fat and visceral adiposity. Therefore, these differences may have contributed to group differences in hepatic fat content. The cross-sectional design and limited sample size also necessitate that observed associations be interpreted with caution and not as evidence of a causal or independent effect of cortisol excess. Larger, controlled studies with multivariable adjustment are required to clarify the relative contributions of hypercortisolism and other metabolic risk factors to ectopic fat accumulation in CS. The absence of liver biopsy precludes assessment of steatohepatitis and fibrosis, which are key prognostic markers, and inter-observer variability in MRI fat measurements was not examined. Finally, the single-center design limits generalizability, underscoring the need for larger, multicenter studies in more diverse populations.

Although MRI offers a highly sensitive, quantitative assessment of hepatic fat content without exposure to ionizing radiation, CT remains an important and widely used modality in clinical practice. CT-based assessment of visceral adiposity is extensively validated, broadly available, relatively inexpensive, and can often be performed using scans obtained for other indications without additional imaging. Therefore, the choice between MRI and CT should be guided by clinical context, imaging resource availability, and specific research or diagnostic objectives. While MRI may offer greater sensitivity for detecting mild hepatic fat accumulation, CT remains a practical and clinically valuable tool for body composition assessment in many settings.

In conclusion, hepatic steatosis consistent with MASLD was detectable in approximately 70% of patients with active, treatment-naïve CS when evaluated

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using sensitive, quantitative MRI-based fat mapping. This burden clearly exceeds prior CT-derived estimates. The severity of liver fat accumulation showed a robust association with both circulating cortisol levels and MRI-derived visceral fat area. Because abdominal MRI is routinely performed for adrenal characterization in CS, leveraging the same examination to quantify hepatic and visceral fat provides a high-yield, radiation-free approach for integrated metabolic risk assessment. These findings support systematic incorporation of hepatic fat quantification into the baseline imaging workup for patients with newly diagnosed CS. Although our findings demonstrate significant associations among hypercortisolism, visceral adiposity, and hepatic steatosis in active CS, the small sample size and absence of a matched eucortisolemic control group limit the ability to determine the independent impact of cortisol excess. Larger, prospective, controlled studies are needed to validate these findings and clarify the relative contributions of hypercortisolism and other metabolic risk factors to ectopic fat accumulation.

Future research avenues

Prospective longitudinal studies with larger, multicenter cohorts are required to dete rmine the prognostic significance, therapeutic responsiveness, and clinical utility of MRI-derived quantification of hepatic and visceral fat in CS. These studies should evaluate whether effective surgical or medical control of cortisol excess results in meaningful regression of liver fat and visceral adipose tissue, and whether baseline hepatic steatosis identifies patients at risk for persistent metabolic dysfunction despite biochemical remission. Incorporation of advanced MRI techniques, such as MR elastography for noninvasive fibrosis staging and multiparametric protocols integrating fat, iron, and tissue stiffness mapping, would substantially enhance the depth of phenotyping in future research. Given the single-center design and population-specific characteristics of the current study, the reported prevalence should be interpreted with caution and not directly extrapolated to other populations. Further multicenter studies across different ethnic and geographic settings are needed to confirm the generalizability of these findings.

Ethical approval and consent to participate: this study was approved by the Ethics Committee of the Faculty of Medicine, Alexandria University (protocol code 0306946) and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

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brief communication

Obesity, diabetes, and other metabolic disorders among the Bororo Indigenous population

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ABSTRACT

Objective: Obesity and diabetes are widely recognized risk factors for cardiovascular disease, and their increase among Indigenous populations has been documented in the medical literature. Given the regional differences in the prevalence of metabolic disorders, this study aimed to evaluate the prevalence of obesity, diabetes, and other metabolic conditions in the Bororo population of the Central-West region of Brazil. **Subjects and methods:** In this cross-sectional study, 152 Bororo individuals from the Meruri Reservation in Mato Grosso, Brazil, underwent clinical, anthropometric, and laboratory assessments. **Results:** Women presented a worse metabolic profile than men, demonstrating significantly higher body mass index, waist circumference, total cholesterol, LDL-c, and 2-hour glucose levels, whereas men exhibited higher systolic blood pressure. Obesity was observed in 30.2% of the participants, with a higher prevalence among women (40.0% vs. 21.9%, p = 0.02). Prediabetes affected 51.3% of the participants, showing a higher prevalence in older men than in younger men (70.6% vs. 43.7%, p = 0.02). Diabetes was diagnosed in 9.2% of the participants, exclusively among women (20.0%), and rose to 38.7% in women aged ≥40 years. Hypertension was present in 23.0% of the participants and was positively associated with age. Central obesity was highly prevalent in this study cohort (72.3%). **Conclusion:** Our findings revealed an unfavorable metabolic profile among the Bororo Indigenous population, particularly in older women. This underscores the critical need for sex- and age-specific preventive measures and management strategies for metabolic diseases in this population.

Keywords: Indigenous population; weight excess; Brazil; metabolic disorders

INTRODUCTION

Indigenous Brazilian populations have experienced significant lifestyle changes driven by territorial and cultural losses. The depletion of natural resources and increasing urbanization have marginalized traditional practices, such as fishing and hunting, while concurrently increasing exposure to ultra-processed foods (1,2). This transition has profoundly altered dietary and physical activity habits, which act as key determinants of health (2,3).

While infectious diseases remain a significant cause of morbidity among Indigenous people (4), the high prevalence of metabolic disorders has exacerbated health inequities between Indigenous and non-Indigenous individuals (2,5). The Bororo is an indigenous Brazilian population inhabiting six demarcated territories within the state of Mato Grosso. These territories are currently 300 times smaller than their traditional lands and have been severely degraded by deforestation and mining activities (6). The Meruri Reservation, one of the largest Bororo territories, supports an estimated population of 811

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individuals, 47% of whom are under 17 years old (7). The Xavante, another Indigenous population inhabiting the same geographic region, differ in their ethnic identity and certain cultural practices. Previous studies have shown a high prevalence of diabetes (28.2%) and obesity (50.8%) within this population (8,9). Given this context, we aimed to investigate the prevalence of metabolic disorders, including prediabetes, diabetes, obesity, and hypertension, among the Bororo.

SUBJECTS AND METHODS

This cross-sectional study was conducted among the Bororo Indigenous population living in the Meruri Reservation. All individuals aged ≥17 years, excluding pregnant women, were invited to participate. Demographic data, medical history, and current medication use were recorded. Body weight was measured using a digital scale (Plenna), with participants wearing light clothing. Height was measured using a stadiometer (AlturExata). Body mass index (BMI) status was categorized according to World Health Organization standards (10). Waist circumference (WC) was considered elevated based on established cut-off points for South American populations: ≥90 cm for men and ≥80 cm for women (11).

Blood pressure (BP) was assessed using an automatic sphygmomanometer (OMRON HEM-742INTC®). Three sequential readings were taken, and the average of the last two was used for analysis. Hypertension was defined as a systolic BP of ≥140 mmHg, a diastolic BP of ≥90 mmHg, or the active use of antihypertensive medication (12). An oral glucose tolerance test (OGTT) was administered to participants with an initial fasting glucose (FG) of <200 mg/dL who were not taking antidiabetic medications. Capillary glucose levels were measured using a portable glucometer (HemoCue®). Diabetes was diagnosed based on the current use of antidiabetic drugs, a fasting or 2-hour post-glucose load capillary glucose level of ≥200 mg/dL, or an HbA1c level of ≥6.5%. Prediabetes was defined as having a 2-hour glucose level between 140 and 199 mg/dL or an HbA1c level of 5.7-6.4%. Cholesterol, HDL-c, and triglycerides were measured enzymatically. LDL-c was calculated using the Friedewald formula. HbA1c testing was performed via high-performance liquid chromatography (Tosoh G7, USA).

The normality of all continuous variables was assessed using the Shapiro-Wilk test (5% significance level). Data are presented as the mean ± standard deviation (SD) for continuous variables and as frequencies (categorical). Group comparisons were performed using Student's t-test, the Mann-Whitney U test, and Fisher's exact test, as appropriate. Statistical analyses were conducted using Stata/BE version 18.5 (StataCorp LLC, USA). The study protocol was approved by the Ethics Committee of the Escola Paulista de Medicina, Universidade Federal de São Paulo, and the Brazilian National Ethics Committee (approval no. 7.566.271, CAAE no. 79870324.3.0000.5505).

RESULTS

The study enrolled 152 participants (82 men and 70 women), with a mean age of 39.7 ± 14.6 years. **Table 1** summarizes their clinical characteristics by sex. Women exhibited significantly higher values for BMI, WC, total cholesterol, LDL-c, and 2-hour post-load glucose, whereas men demonstrated higher systolic BP. Age-stratified analyses (<40 years vs. ≥40 years) indicated that aging exerted more pronounced adverse metabolic effects on women than on men (**Table 2**). Older women had substantially higher rates of obesity, hypertension, and diabetes than their younger counterparts. Overall, diabetes was identified in 9.2% of the participants, exclusively in women, affecting 20% of them and 38.7% of women aged ≥40 years. Among individuals diagnosed with diabetes, the mean HbA1c was 9.8% ± 3.1% (range: 6.0%–14.8%). Five individuals (35.0%) had an HbA1c of ≤7.0%, two (14.0%) had levels between >7.0% and <8.0%, and seven (50.0%) exhibited levels ≥8.0%.

Prediabetes was identified in 51.3% of the participants, with no significant differences overall between the sexes. However, among men, prediabetes affected 54.9% and was notably more prevalent in those aged ≥40 years compared to younger men (70.6% vs. 43.7%, $p = 0.02$). Hypertension affected 23.0% of the participants, with equal overall distribution between the sexes, though its prevalence increased sharply with age, affecting 58.1% of older women and 35.2% of older men.

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Table 1. Demographic, anthropometric, and metabolic variables of the study population according to sex

Variable	Men n = 82 (53.9%)	Women n = 70 (46.1%)	Total n = 152	*p*
Age (years)	38.9 ± 15.4	40.5 ± 13.6	39.7 ± 14.6	0.38
BMI (kg/m²)	27.1 ± 4.4	29.2 ± 5.7	28.1 ± 5.1	0.03
WC (cm)	91.3 ± 11.7	97.5 ± 11.9	94.2 ± 12.1	<0.01
Basal glucose (mg/dL)	107.3 ± 13.7	120.2 ± 50.5	113.3 ± 36.1	0.68
2-h 75-g OGTT (mg/dL)	135.1 ± 22.2	152 ± 39.3	142.4 ± 31.8	0.01
Total cholesterol (mg/dL)	138.3 ± 30.1	154.3 ± 34.4	145.7 ± 33	<0.01
LDL-c (mg/dL)	70.2 ± 24.0	81.1 ± 29.3	75.2 ± 27	0.01
HDL-c (mg/dL)	47.9 ± 16.8	50.9 ± 16.5	49.3 ± 16.7	0.09
Triglycerides (mg/dL)	108.2 ± 60.4	123.1 ± 75.2	115.1 ± 67.8	0.12
Systolic BP (mmHg)	120.7 ± 17.3	112.5 ± 19.8	116.9 ± 18.9	<0.01
Diastolic BP (mmHg)	70.3 ± 12.5	68.8 ± 10.4	69.6 ± 11.6	0.56

Abbreviations: BMI: body mass index; WC: waist circumference; OGTT: oral glucose tolerance test; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; BP: blood pressure; systolic BP: systolic blood pressure; diastolic BP: diastolic blood pressure.

Table 2. Metabolic profile by sex and age groups in the study population

Variable	Women (n = 70)				Men (n = 82)				Total (n = 152)	
Age	< 40y (n = 39)	≥ 40y (n = 31)	*p	All women	< 40 y (n = 48)	≥ 40 y (n = 34)	*p	All men	Total	#p
Increased WC	36 (92.3%)	31 (100%)	0.24	67 (95.7%)	22 (45.8%)	21 (61.8%)	0.18	43 (52.4%)	110 (72.3%)	<0.01
Overweight	18 (46.1%)	7 (22.6%)	0.04	25 (35.7%)	20 (41.6%)	16 (47.1%)	0.65	36 (43.9%)	61 (40.1%)	0.32
Obesity	11 (28.2%)	17 (54.8%)	0.02	28 (40%)	09 (18.8%)	09 (26.5%)	0.42	18 (22%)	46 (30.2%)	0.02
Hypertension	4 (10.3%)	18 (58.1%)	<0.01	18 (25.7%)	05 (10.4%)	12 (35.2%)	0.01	17 (20.7%)	35 (23%)	0.56
Diabetes mellitus	02 (5.1%)	12 (38.7)	<0.01	14 (20%)	0	0	-	0	14 (9.2%)	<0.01
Prediabetes	16 (41%)	17 (54.8%)	0.33	43 (61.4%)	21 (43.7%)	24 (70.6%)	0.02	45 (54.9%)	78 (51.3%)	0.51

*p-values for comparison between age groups. #p-values for comparison between men and women. WC: waist circumference.

Excess weight was highly prevalent, affecting 70.3% of all participants. Obesity affected 30.2% of the cohort and was significantly more common in women than in men (40.0% vs. 21.9%, $p = 0.02$). Obesity rates were much higher in older women than in younger women (54.8% vs. 28.2%, $p = 0.02$). Overweight status was observed in 40.1% of the individuals. Only 29.6% had a normal BMI, and no participants were classified as underweight. Central obesity (increased WC) was broadly identified, affecting 72.3% of participants. The prevalence was overwhelmingly higher in women than in men (95.7% vs. 52.4%, $p < 0.001$), with 100% of women aged ≥40 years meeting the threshold criteria. Notably, even among women with normal weight (n=17), 88.0% (n = 15) presented with central obesity.

DISCUSSION

The Bororo exhibit higher rates of obesity (30.2%) and overweight (40.1%) compared to the general Brazilian adult population (25.9% and 34.4%, respectively) (13). Previous meta-analyses of Brazilian Indigenous adults from various ethnic groups reported lower overall rates of obesity (18%) and overweight (33%) (2). However, a systematic review of adult Indigenous populations in Brazil identified peak obesity prevalence among the Guarani, Kaiowá, Khisedje, and Terena groups (31%), closely aligning with our findings for the Bororo (14). In our previous study characterizing the Xavante population, obesity rates were even higher, affecting 52.4% of women and 47.6% of men (15). The Xavante and the Bororo inhabit the same geographic region and share similar patterns of interaction with the non-Indigenous population. The higher obesity rates observed in the Xavante may be attributable to their remarkably low genetic admixture (16) combined with a rapid nutritional acculturation toward a Westernized diet. While the Xavante have engaged in contact with non-Indigenous society primarily since

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the 1940s, formal contact for the Bororo dates back much further, to 1902 (17). This rapid nutritional acculturation may have driven the elevated obesity rates among the Xavante.

Sex disparities regarding obesity were prominent in our study; women demonstrated higher rates than men (40.0% vs. 21.9%), mirroring established patterns in both the general Brazilian population (30.2% vs. 22.8%) (13) and other Indigenous groups (15,18). Waist circumference, a well-validated marker of visceral fat, is independently associated with cardiovascular risk across various populations (19,20). Central obesity was notably high among Bororo women, with all women aged >40 years, showing increased waist circumference, indicating substantial cardiometabolic risk.

The overall prevalence of diabetes in the Bororo population (9.2%) was comparable to that of the general Brazilian adult population, which ranges from 6.6% to 9.4% depending on the diagnostic criteria employed (21). Among the Bororo, however, diabetes was diagnosed exclusively in women, particularly those aged ≥40 years. Pronounced sex discrepancies in diabetes rates among Indigenous populations are well documented, with a predictably higher prevalence in women across various Indigenous groups (22,23). However, on a global scale, the prevalence of diabetes is generally higher in men or remains comparable between sexes (22,24). The overwhelming prevalence of obesity, notably central adiposity, among Bororo women likely contributes substantially to these escalated diabetes rates. Factors such as relative differences in physical activity, early menarche, previous instances of gestational diabetes, high parity, unfavorable body composition changes, increased postmenopausal insulin resistance, and various psychosocial determinants, may conjointly explain the greater diabetes burden among Bororo women.

Prediabetes affected 51.3% of the total sample, a figure substantially higher than any rate normally reported for the general Brazilian population. For context, Malta and cols. (21) found a 16.9% prevalence using the HbA1c criterion, whereas Schmidt and cols. (25) reported a 20.3% prevalence utilizing the oral glucose tolerance test. Although prediabetes is a firmly established risk factor for both diabetes and cardiovascular disease (26), only Bororo women developed diabetes, despite similar prediabetes rates, suggesting sex-specific factors. Higher obesity rates alongside variations in physical activity, hormonal profiles, and diet may increase women's vulnerability to diabetes.

Hypertension was identified in 23.0% of participants, increasing with age. Among women, no cases were detected in those under 40 years of age, while 58.1% of those aged ≥40 years were hypertensive. Among men, the prevalence surged from 10.4% in the younger subset to 35.2% in those aged ≥40 years. In aggregate, these rates remain lower than the 30% rate observed in the Brazilian population (27), and are comparable to historical data derived from the Xavante population (9). Kramer and cols.'s review evaluating 46 studies of Indigenous Brazilian adults reported 11% overall hypertension prevalence, ranging from 1% in less urbanized regions to 30% in urban regions. This study noted that BP generally escalated alongside age specifically in urbanized Indigenous populations, although not in those maintaining traditional lifestyles (2). Furthermore, Oliveira and cols. (28) found a 29.5% prevalence of hypertension in an Indigenous community in Central Brazil. Ultimately, these collective findings highlight the volatile and highly varied prevalence of hypertension among Indigenous populations, differing by age, sex, region, and acculturation.

This study has several limitations. The characteristically irregular meal patterns typical of the Bororo people may have affected the accuracy of our fasting blood measurements. However, the 2-hour OGTT provides a more reliable assessment of glucose metabolism than FG alone, proving superior in predicting heart disease risk (29,30). Therefore, isolated impaired FG levels were not independently used to define instances of prediabetes. Triglyceride measurements were assumed to be in the fasting state, potentially introducing errors.

Our findings showed an unfavorable metabolic profile among the Bororo Indigenous population across numerous sex and age demographics. Women aged ≥ 40 years showed higher rates of obesity, especially central obesity, than men. Diabetes only

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occurred only in women, despite similar prediabetes rates between the sexes. These findings indicate the need for sex- and age-specific strategies for treating metabolic diseases in these populations.

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Authors' contributions: ALDF, JPBV, and LJF† contributed to the conception and design of this study. LFV, ALDF, LJF†, and RSM participated in data collection, analysis, interpretation of the results, and drafting of the manuscript. JPBV contributed to the conception, data collection, analysis, interpretation, and critical revision of this manuscript. All the authors have read and approved the final version of the manuscript. †In memory of Dr. Laércio Joel Franco, who passed away before the publication of this study. His dedication to research and the health of Indigenous populations is invaluable. He is greatly missed and fondly remembered by all.

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Data availability: the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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brief communication

Clinical and functional evidence supporting pathogenicity of a novel APOA5 variant in familial chylomicronemia syndrome

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ABSTRACT

Objective: This study aimed to evaluate the clinical and functional impact of a novel apolipoprotein A5 (ApoA5) variant by assessing lipoprotein lipase (LPL) activity. **Subjects and methods:** Demographic and clinical data, including blood lipid levels and body mass index, were retrospectively collected from an endocrinology clinic registry. Ten individuals with familial chylomicronemia syndrome (FCS) carrying a novel *APOA5* variant were included. Whole-exome sequencing was performed using the latest generation DNB-SEQ400 platform. LPL activity was measured in post-heparin plasma using a radiometric assay. Statistical analysis: Categorical variables were summarized as frequencies and percentages, and continuous variables as mean ± standard deviation or median (range), as appropriate. Group comparisons were performed using the Mann-Whitney U test or chi-square test. Analyses were conducted using the Statistical Package for the Social Sciences software (v. 25.0). A p-value < 0.05 was considered statistically significant. **Results:** We report ten cases of FCS with a homozygous missense variant of uncertain significance in *APOA5*: c.694T>C; p.(Ser232Pro), located in exon 3. In six patients assessed for LPL activity, levels remained consistently below 20% compared to normotriglyceridemic controls. The addition of exogenous serum containing *APOA5* restored LPL activity to above 20% in all cases, indicating functional rescue. **Conclusion:** Measurement of LPL activity demonstrated the functional impact of the APOA5 c.694T>C; p.(Ser232Pro) variant. These findings support reclassification of the variant as likely pathogenic and enable differentiation from multifactorial chylomicronemia syndrome.

Keywords: Familial chylomicronemia syndrome; hypertriglyceridemia; *APOA5*; pancreatitis; dyslipidemia

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INTRODUCTION

Apolipoprotein A5 (ApoA5) is a critical regulator of plasma triglyceride (TG) levels (1). It modulates hepatic synthesis of very low-density lipoprotein (2), enhances lipoprotein lipase–mediated triglyceride clearance (3), and promotes hepatic uptake of TG-rich lipoprotein remnants via the low-density lipoprotein (LDL) receptor family (4). ApoA5 enhances LPL activity through interactions with heparan

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sulfate proteoglycans (5) and glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (Gpihbp1) on endothelial cell surfaces (6). Biallelic loss-of-function (LOF) variants in *APOA5* underlie a rare condition with an estimated prevalence of 1–10 per million (7) and represent a known cause of familial chylomicronemia syndrome (FCS) (8,9). Even severely truncated *APOA5* variants are synthesized and secreted (10); however, premature truncation can disrupt key functional domains, leading to protein misfolding. In homozygous or compound heterozygous states, these alterations result in a significant reduction in *APOA5* mass and function (11,12). Only 38% of *APOA5* variants are classified as pathogenic or likely pathogenic (13), most of which are missense mutations. When genetic testing yields negative or inconclusive results, measurement of LPL activity can confirm the diagnosis of FCS, particularly when activity is reduced below 20–25% of the normalmedian (14,15). In this study, we describe the clinical and genetic characteristics of patients with severe hypertriglyceridemia carrying *APOA5* variants and assess their pathogenicity by measuring LPL activity in ten affected individuals.

SUBJECTS AND METHODS

Ten patients (3 men, 7 women; mean age: 42 ± 3.2 years) with strong clinical suspicion for FCS were included. Serum TG, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and apolipoprotein B were measured using commercial enzymatic assays on a Mindray BS360E autoanalyzer (Mindray, China). Body mass index and the Moulin score were calculated (16). Secondary causes of hypertriglyceridemia were excluded.

DNA preparation and sequencing

Peripheral blood samples were collected from all patients, and whole-exome sequencing was performed using the latest-generation DNB-SEQ400 platform. Genes associated with FCS (e.g., *LPL*, *APOC2*, *APOA5*, *GPIHBP1*, and *LMF1*) were analyzed. High-quality reads were aligned to the human reference genome (GRCh37/hg19). The analysis focused on variants in exonic and splice-site regions (minimum 20 bp), as well as small insertions and deletions (copy number variants). Variant classification followed the American College of Medical Genetics and Genomics (ACMG) guidelines (13), using the ClinGen framework (clinicalgenome.org). Databases consulted included Clinical Variation database, Human Gene Mutation Database, Leiden Open Variation Database, Database of Single Nucleotide Polymorphisms, Genome Aggregation Database, and in-house resources. The identified variant exhibited extremely low allelic frequency in the general population (0.000002502 in gnomAD v. 4.1), consistent with a pathogenic allele in a recessive inheritance model. *In silico* prediction tools (REVEL, BayesDel) classified the variant as deleterious.

LPL activity

To assess LPL activity, heparin was administered intravenously at a dose of 60 IU/kg body weight. Ten minutes after injection, blood samples were collected from the contralateral arm into ice-chilled tubes. Post-heparin plasma was obtained by centrifugation at 1500 × g for 10 minutes at 4 °C and stored at -70 °C until analysis. LPL activity was measured in post-heparin plasma from six patients. A standardized LPL activity assay was developed in our laboratory based on the Nilsson-Ehle method (17,18). This method has previously been applied in patients with FCS, in whom a median LPL activity of 33.3 mU/L (95% confidence interval, 28.5–41.3) was established for normotriglyceridemic (NTG) subjects (19). The intra-assay coefficient of variation was 5.1%, whereas the inter-assay coefficient of variation was 13.2%. Given the complexity of the assay, these values are considered acceptable. The calculated limit of detection was 0.144 mIU, and the limit of quantification was 1.83 mIU. A value of 6.6 mIU corresponds to 20% of normal activity. Therefore, LPL activity below this threshold is supportive of a diagnosis of FCS.

A functional assay was also performed. Two substrates were prepared, one of which included 10% v/v normolipemic human serum, heat-inactivated at 56 °C for 30 minutes and used as a source of exogenous cofactors, including ApoC-II and ApoA5. The *in vitro* assay was used to evaluate potential cofactor deficiency (18).

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In silico evaluation of APOA5-LPL: *GPIHBP1* interactions

Because no crystal structure is available for human *APOA5*, its structure was obtained from the AlphaFold Protein Structure Database (ID: Q6Q788) and used as a template. Structural modeling of the identified variant was performed using SWISS-MODEL, followed by energy minimization with SwissPDB Viewer (20). Energy-minimized models of both the native and mutant ApoA5 proteins, along with the LPL–Gpihbp1 complex (PDB ID: 6E7K), were uploaded to the HDOCK server, which calculates docking scores; lower scores indicate more favorable binding interactions (21).

Statistical analysis

Categorical variables were summarized using frequencies and percentages, while continuous variables were described using mean, standard deviation, median, and range (minimum–maximum). Results are presented as mean values with their corresponding standard errors. Comparisons between groups were performed using the Mann-Whitney U test or the Chi-square test, as appropriate. Statistical analyses were conducted using the Statistical Package for the Social Sciences software (v. 25.0). A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes the clinical and biochemical characteristics of the patients. All had a Moulin score ≥10 and a history of recurrent pancreatitis. The patients were unrelated and from different regions of Colombia. All carried the same homozygous variant in the *APOA5* gene: c.694T>C; p.(Ser232Pro). This missense variant, located in exon 3, results from a T-to-C substitution at nucleotide position 694, leading to a serine-to-proline substitution at amino acid position 232. To date, it has been classified as a variant of uncertain significance (9).

LPL activity and functional assay

LPL activity for six patients are shown in Figure 1, along with the results of the *in vitro* functional assay. In all cases, LPL activity was below 20% of that observed in NTG controls. The *in vitro* assay, which included the addition of normal serum as a source of key cofactors (i.e., ApoC-II and ApoA5), demonstrated restoration of LPL activity to normal levels in all cases. These findings suggest that LPL function was preserved in the presence of functional ApoA5 (Table 1). In four patients, samples could not be collected in time for shipment to Argentina for LPL activity analysis. No differences were observed between patients with and without LPL activity measurements in terms of diabetes status, history of pancreatitis, or lipid parameters, confirming the severity of the novel *APOA5* variant.

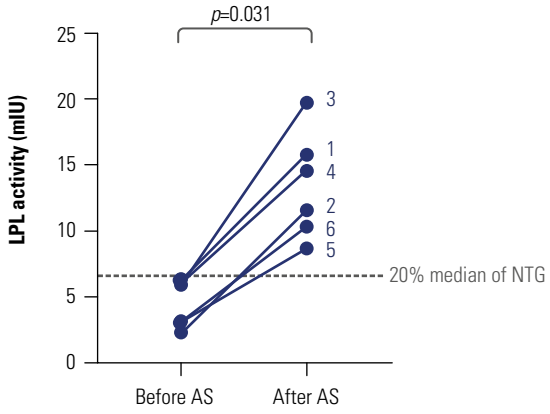
Table 1. Demographic, clinical, biochemical data, and LPL activity levels before and after addition of activator serum in the ten cases

Case	Sex	Age (years)	TG	HDL-c	LDL-c	ApoB	BMI (kg/m²)	MS	PPDM	AP	AP episodes and age at first episode	LPL (mIU)	Activity of median NTG (%)	LPL with AS (mIU)	Activity of median NTG with AS (%)
			mg/dL												
1	F	54	2748±345	19±3	31±8.3	75±7.9	24.6	12	No	Yes	1, 48 Y	6.35	19.05	15.80	47.40
2	M	68	5530±416	11±2.1	34±9.2	35.9±8.4	29.0	12	No	Yes	3, 27 Y	2.37	7.11	11.59	34.78
3	F	36	5128±398	18±3.1	U	U	24.3	12	No	Yes	5, 31 Y	5.97	17.91	19.75	59.26
4	M	64	5808±472	9.0±1.3	12±7.8	93±11.5	26.5	10	No	No		6.19	18.08	14.59	43.76
5	F	64	1664±178	13±4.2	40±9.1	98±12.4	20.1	13	No	Yes	1, 40 Y	3	9.0	8.67	26.00
6	F	65	1600±225	39±4.6	47±11.3	47±7.5	27.47	10	No	No		9.47	9.47	10.39	31.16
7	F	84	1105±248	30.4±4	89±12.4	47±9.3	30.4	10	U	No		NP	NP	NP	NP
8	F	52	4200±459	31±3.1	U	U	23.9	12	No	Yes	3, 30 Y	NP	NP	NP	NP
9	F	50	3638±298	19.3±2.4	100±14.6	U	27.1	10	U	Yes	1, 36 Y	NP	NP	NP	NP
10	F	54	1000±192	20.9±2.6	U	U	29.0	11	Yes	Yes	8, 45 Y	NP	NP	NP	NP

Gray rows correspond to patients who underwent lipoprotein lipase activity assessment.

AP: Acute pancreatitis; ApoB: apolipoprotein B; AS: activator serum; BMI: body mass index; F: female; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; LPL: lipoprotein lipase; M: male; MS: Moulin score; NP: not performed; NTG: normotriglyceridemic controls; PPDM: post-pancreatitis diabetes mellitus; TG: triglycerides (mg/dL); U: unknown; Y: years.

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AS: activator serum; LPL: lipoprotein lipase; NTG: normotriglyceridemic controls.

Figure 1. LPL activity levels before and after the addition of activator serum. The dotted line indicates 20% of the median LPL activity observed in NTG controls. Statistical comparisons were performed using the Wilcoxon signed-rank test.

DISCUSSION

This study describes ten patients carrying a novel homozygous *APOA5* variant, initially classified as a variant of uncertain significance. In patients with high clinical suspicion scores and markedly reduced LPL activity, these findings provide support for reclassifying this variant as likely pathogenic. ApoA5 contains several functional domains: a signal peptide, an N-terminal hydrophilic domain, a lipid droplet-binding domain, a positively charged Gpihbp1-interacting domain, and a C-terminal hydrophobic (lipid-binding domain) (11). Molecular analysis identified the homozygous variant NM_001371904.1: c.694T>C; p.(Ser232Pro), located in exon 3. This substitution impairs the interaction between ApoA5 and Gpihbp1. The introduction of a proline residue induces local conformational rigidity, altering the spatial orientation of residues critical for *GPIHBP1* recognition. In addition, substitution of a polar serine with proline may affect binding affinity and kinetics of the ApoA5-Gplhbp1 interaction, and its role in facilitating LPL-mediated TG lipolysis (22).

At least 118 definite or likely disease-causing variants in *APOA5* have been reported in the literature and databases, and are associated with FCS, hypertriglyceridemia, and atherosclerotic disease. Even minor alterations in the protein may impair its lipid-binding properties. The remaining three pathogenic LOF variants in *APOA5* could be associated with substantial functional impairment (22). The variant identified (*APOA5*: c.694T>C; p.Ser232Pro) likely disrupts Gpihbp1 interaction, similar to other pathogenic variants such as p.Ser232_Leu235del and p.Leu253Pro, resulting in impaired very low-density lipoprotein secretion and reduced LPL activity (12). No evidence of a dominant-negative effect has been reported for p.Ser232Pro. In previously reported cases (9), the variant showed a recessive pattern of inheritance, and heterozygous carriers exhibited normal or mildly elevated TG levels. These observations are consistent with a LOF mechanism, likely driven by impairment of the ApoA5-Gplhbp1 interaction.

Although the findings provide strong evidence for a deleterious effect of the *APOA5* c.694T>C (p.Ser232Pro) variant, the current classification as likely pathogenic is based on ACMG/AMP criteria that do not yet meet the threshold for pathogenicity. The study shows markedly reduced LPL activity (<20% of normal), with restoration upon addition of exogenous ApoA5, supporting a LOF mechanism. However, ACMG/AMP guidelines require either multiple independent functional studies or well-validated assays to support reclassification beyond this level (13).

The available genetic and functional evidence supports a LOF effect and disease association. Nonetheless, the data meet ACMG/AMP criteria for "likely pathogenic" classification. Additional independent functional studies, segregation analyses, and replication in other cohorts are necessary to support full classification as "pathogenic." Following ACMG/AMP guidelines for variant interpretation (13) (ClinGen framework updates), the variant met criteria PM3 (strong), PM2 (supporting), and PP3 (supporting), and was therefore classified as "likely pathogenic." Overall, p.Ser232Pro behaves similarly to other structurally disruptive *APOA5* variants, with pathogenicity consistent with a recessive LOF mechanism (21). The clinical and functional data support the pathogenicity of this variant.

LPL activity below 20% is strongly associated with a diagnosis of FCS and with the exclusion of multifactorial chylomicronemia syndrome (23,24), in combination with the Moulin score (16), and North American FCS scores (25). In our cohort, LPL activity was consistently below 20% of NTG controls, supporting the

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pathogenicity of this variant. Genetic testing fails to provide definitive results in a substantial proportion of FCS patients, even when the Moulin and North American FCS scores scores strongly suggest a high likelihood of disease (16,25). The APPROACH study reported that 21% of participants lacked identifiable genetic variants but were included based on their clinical presentation and markedly reduced LPL activity (14). LPL activity below 20% is strongly associated with FCS diagnosis and supports the exclusion of multifactorial chylomicronemia syndrome (23,24). In our cohort, LPL activity was consistently below 20% of NTG controls, supporting the pathogenicity of this variant. Although LPL activity testing is complex and not widely available, it is valuable in cases with inconclusive genetic results or unclear differentiation between FCS and multifactorial chylomicronemia syndrome. We recently reported a case of a pregnant patient with severe hypertriglyceridemia (6900 mg/dL) and a history of pancreatitis (24). Genetic testing identified a heterozygous *APOA5* variant (c.111delC; p.Asp37Glufs*20), classified as likely pathogenic. LPL activity was below 20% (9.6%), increasing to 31.2% after addition of activator serum, supporting a diagnosis of FCS and reclassification of the variant.

In conclusion, our findings showed that rare variants in *APOA5* play a critical role in the pathophysiology of FCS. In this study, we documented reduced LPL activity, supporting the diagnosis and demonstrating the functional impact of the *APOA5* variant. We propose a founder effect for this novel homozygous *APOA5* variant, which has also been reported in a prior case classified as a variant of uncertain significance. Novel *APOA5* variants are likely to be reclassified as pathogenic and LPL activity data continue to accumulate.

Limitations: although the cohort was well-characterized, the sample size was limited (n = 10), which was expected given the extremely low prevalence of FCS (approximately 1 in 1,000,000) and the description of a novel variant. In addition, no *in vitro* functional studies (e.g., Gpihbp1 binding assays) were performed to directly assess the mechanistic impact of the variant.

Ethical considerations: the study was approved by the Institutional Review Board of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires (RESCD-2021-842-E-UBA-DCT_FFYB). All investigations were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to DNA collection, blood sampling, and administration of heparin for post-heparin LPL activity analysis.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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cases report

Rare types of congenital adrenal hyperplasia: report of five children with 11β-hydroxylase deficiency including pathogenic and novel *CYP11B1* variants

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ABSTRACT

11β-hydroxylase deficiency (11β-OHD) is a rare form of congenital adrenal hyperplasia caused by biallelic pathogenic variants in the *CYP11B1* gene. It leads to impaired cortisol synthesis, resulting in increased adrenocorticotropic hormone stimulation and consequent accumulation of steroid precursors, which are diverted to androgen synthesis. In addition, the accumulation of 11-deoxycorticosterone, which is a potent mineralocorticoid, causes hyporeninemic hypokalemic hypertension. We report the clinical, hormonal, and genetic profiles of five children with 11β-OHD, emphasising phenotypic variability, a median 2-year diagnostic delay, the crucial role of hormonal profile in diagnosis, and management challenges, including post-treatment central precocious puberty. Two novel *CYP11B1* variants were identified in two unrelated patients. Hydrocortisone replacement resolved hypertension in only one of the three hypertensive patients; others required spironolactone. Early differentiation of 11β-OHD from 21-hydroxylase deficiency is critical to prevent hypertension-related morbidity.

Keywords: 11-beta hydroxylase deficiency; hormonal profile; hypertension; peripheral precocious puberty; genetic variations; India

INTRODUCTION

Congenital adrenal hyperplasia (CAH) refers to a group of autosomal recessive genetic endocrine disorders in children caused by variants in the genes encoding the various enzymes involved in adrenal steroidogenesis. Depending on the specific enzymatic defect, the synthesis of glucocorticoids, mineralocorticoids, and sex steroids may be impaired, resulting in varied manifestations ranging from genital ambiguity to potentially life-threatening adrenal insufficiency (1). The most common form of CAH is 21-hydroxylase deficiency (21-OHD), which is caused by variants in the *CYP21A2* gene, accounting for ~95% of cases. Other forms such as 11β-hydroxylase deficiency (11β-OHD) due to *CYP11B1* gene variants, 3β-hydroxysteroid dehydrogenase type 2 deficiency (*HSD3B2* gene variants), steroid 17α-hydroxylase/17,20 lyase (*CYP17A1* gene variants), P450 cholesterol side-chain cleavage enzyme (*CYP11A1* gene variants), or the steroidogenic acute regulatory protein (*STAR* gene variants), are rare and together account for ~5% cases (1). The prevalence of non-21-OHD forms of CAH varies across geographical regions. While 11β-OHD is generally considered the second most common form of CAH, this is not the case in certain countries. For example, 17-hydroxylase deficiency in Brazil and StAR protein deficiency in Japan, China, and India are the most common 21-OHD variants of CAH (1-5).

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In countries with well-established newborn screening (NBS) programs that employ 17-hydroxyprogesterone (17-OHP) measurements, the early detection of most classic 21-OHD cases and some rare variants has significantly improved long-term outcomes. However, in countries with limited or no nationwide NBS programs, such as India, timely diagnosis of CAH remains a challenge (6). Late diagnoses are common even for classic CAH; in a north Indian center, 75% were diagnosed only after they presented in adrenal crisis (7). Diagnosing rarer CAH variants is even more difficult due to variable increases in 17-OHP concentrations and significant clinical heterogeneity (8). Of all the non-21-OHD variants of CAH, 11β-OHD is often challenging to diagnose due to its distinct effects on adrenal hormone synthesis and consequent clinical manifestations (8). The enzyme 11β-OH mediates the conversion of 11-deoxycorticosterone (DOC) to corticosterone and 11-deoxycortisol to cortisol. 11β-OHD, therefore, results in the accumulation of 11-DOC, which is a potent mineralocorticoid and causes hyporeninemic hypokalemic hypertension. The diversion of steroid precursors to androgen synthesis produces features such as ambiguous genitalia in female infants, and virilization and precocious puberty during childhood mimicking simple virilizing CAH (9). During infancy, cortisol deficiency may present as a salt-wasting crisis similar to classic 21-OHD (1,8). Although hypertension is a distinguishing feature, it may develop late in the disease course (10). Due to such diagnostic challenges, children with 11β-OHD are often misclassified as 21-OHD initially (8).

Over the last few years, the detection and outcome of children with 11β-OHD have improved due to the wider availability of hormonal profiling by liquid chromatography tandem mass spectrometry (LC-MS/MS), as it simultaneously measures different adrenal steroids and their precursors (11). Additionally, the reduction in the costs of genetic analysis has enabled the confirmation of diagnosis in previously unresolved cases (12). The distribution of pathogenic variants also differs among different ethnicities. Of the more than 200 *CYP11B1* gene variants reported to date, most are missense or nonsense variants, occurring predominantly in exons 2, 6, 7, and 8. Worldwide, the most common variant identified is the p.R448H in exon 8; this is especially recurrent in Moroccan Jews. Other variants, such as p.R454C and p.R448P, are prevalent in China and Saudi Arabia, respectively, whereas p.Q356X is found in the Tunisian population and African Americans (13). At our tertiary care pediatric hospital in North India, we have been following a large cohort of children with CAH, most of whom are due to 21-OHD. This clinical brief describes our experience of 11β-OHD, focusing on varied clinical presentations, diagnostic delays, blood hormone profiling, genetic analysis, including two novel variants, and management strategies.

CASE SERIES

The Department Review Board (DRB-37-25) approved this retrospective study, and informed consent was obtained from the caregivers of all the patients for publication. Five patients [four boys, median age (IQR) 6.5 (3-6.8 years)] were diagnosed with 11β-OHD out of our 20-year cohort of approximately 60 patients with CAH. Boys presented with peripheral precocious puberty (PPP), skin hyperpigmentation, and hypertension, while the girl presented with clitoromegaly. One patient (Case 2) was born of third-degree consanguinity; his elder brother also had precocious puberty and short stature. There was no history of consanguinity in the other four cases. Clinical and laboratory work-up revealed tall stature and advanced bone age (BA) in all, metabolic alkalosis in four, hypertension, and hypokalaemia in three children (**Table 1**). Cases 1, 2, and 3 all had hypertension, metabolic alkalosis, and hypokalemia at presentation. Case 2, presented with hypertensive emergency, left ventricular hypertrophy (LVH), and testicular adrenal rests on ultrasound. Case 4 presented at 2 years of age with a history of ambiguous genitalia (clitoromegaly) noticed from 5 months of age. At her presentation, metabolic alkalosis was the only documented abnormality, without evidence of hypertension or hypokalemia. Four patients were evaluated using a hormonal profile by LC-MS/MS, which showed elevated levels of 11-deoxycortisol (median: 702 nmol/L), 11-DOC (median: 44.96 nmol/L), 17-OHP (median: 14.25 nmol/L), and androstenedione (median: 47.83 nmol/L) (**Table 1**).

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Table 1. Clinical, anthropometric, and biochemical parameters of patients

Variable	Case 1	Case 2	Case 3	Case 4	Case 5
At initial Presentation					
Age at presentation (years)/Sex	6.6/M	6.5/M	7/M	2/F	4.5/M
Height (SDS)	3.74	5.38	4.02	-0.31	3.44
Weight (SDS)	1.8	2.92	2.69	0.37	2.04
BMI (SDS)	0.1	0.64	1.51	0.77	0.01
Bone age (years)	13	12	14	4	8
Blood pressure	Stage 1 HTN	Stage 2 HTN	Stage 2 HTN	No HTN	No HTN
External genitalia findings	SPL 6 cm, TV 3 cc, PH 4	SPL 9.5 cm, TV 3 cc, PH 4	SPL 8 cm, TV 4 cc, PH 4	Clitoral length 2.5 cm	SPL 6.6 cm, TV 4 cc, PH 3
ACTH (pmol/L)/(pg/mL)	212.3 /965	92.8/422	440/2000	19.5/88.5	215.6/980
Cortisol (nmol/L)/(μg/dL)	92.9/3.37	48.56/1.76	61.9/2.24	94.5/3.43	69.36/2.51
Basal LH (mIU/mL)	0.41	0.27	0.30	0.28	0.10
Basal FSH (mIU/mL)	0.48	0.25	0.30	0.30	0.10
Serum potassium (mmol/L)	3	3	3.4	3.9	3.7
Metabolic alkalosis	Yes	Yes	Yes	Yes	No
17-OHP (nmol/L)/(ng/dL)	10.42/344	20.33/671	37.26/1230	31.51/1040	14.27/471
Testosterone (nmol/L) /(ng/dL)	7.30/210.56	13.15/379	7.40/213.36	2.77/80	4.70/471
Androstenedione (nmol/L) /(μg/L)	67.38/19.30	189/54.20	7.56/2.16	47.83/13.70	-
DHEAS (nmol/L)/(μg/dL)	5.69/210	6.31/233	2.62/97	5.01/185	2.34/86.7
11-Deoxycortisol (nmol/L) /(μg/L)	363.60/120	996.87/329	790.83/216	748.41/247	-
11-Deoxycorticosterone (nmol/L)/(μg/L)	21.63/7.14	45.75/15.10	44.23/14.6	136.35/45	-
CPP post-treatment (duration)	Yes (6 months)	Yes (3 months)	Yes (9 months)	No	Yes (6 months)
TV at diagnosis of CPP	5cc	5cc	6cc	-	6cc
LH (mIU/mL) at diagnosis of CPP	2.40	1.82	3.38	-	2.91
At the last follow-up					
Age at last follow-up (years)	7.6	7.5	9	3.5	18
Height (SDS)	3.10	2.97	1.95	-0.65	-3.03
BMI (SDS)	0.48	1.05	1.25	0.75	-1.09
Therapy at the last follow-up	Hydrocortisone, Leuprolide 11.25 mg	Hydrocortisone, Spironolactone, Leuprolide 11.25 mg	Hydrocortisone, Spironolactone, Leuprolide 11.25 mg	Hydrocortisone	Dexamethasone 0.5 mg

ACTH: Adrenocorticotropic hormone; SDS: Standard deviation scores; BMI: Body mass Index; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; DHEAS: Dehydroepiandrosterone sulfate; 11β-OHD: 11β-hydroxylase deficiency; 17-OHP: 17-hydroxyprogesterone; CPP: Central precocious puberty; GnRH: Gonadotropin-releasing hormone; SPL: Stretched penile length; TV: Testicular volume; PH: Pubic hair; HTN: Hypertension.

Note: Reference range for parameters in SI units: 17-OHP: 0.94–6.56 nmol/L (male), 0.30–2.42 nmol/L (female); Androstenedione: 0.27–1.74 nmol/L, 11-Deoxycortisol: 0.60–7.57 nmol/L, 11-Deoxycorticosterone: 0.06–1.03 nmol/L, ACTH: 1.58-13.93 pmol/L, Cortisol: 171-536 nmol/L, Testosterone: <0.34 nmol/L, DHEAS: 0.013–0.527 nmol/L (1-4yr), 0.076–2.314 nmol/L (5-9yr) **Reference range for parameters in conventional units:** 17-OHP: 31–217 ng/dL (male), 10–80 ng/dL (female), Androstenedione: 0.08–0.50 μg/L, 11-Deoxycortisol: 20–216 μg/L, 11-Deoxycorticosterone: 0.02–0.34 μg/L, ACTH: 7.2–63.2 pg/mL, Cortisol: 6.2–19.4 μg/dL, Testosterone: <9.8 ng/dL, DHEAS: 0.48–19.5 μg/dL (1-4 yr), 2.8–85.4 μg/dL (5-9 yr).

Molecular analysis of the *CYP11B1* gene by next-generation sequencing (NGS) using clinical exome sequencing identified five distinct variants in the five unrelated patients, including two novel variants (Table 2). All patients were homozygous for variants inherited in an autosomal recessive manner. Among these, one variant was classified as pathogenic, three as likely pathogenic, and one as a variant of uncertain significance (VUS) based on the standard guidelines of the American College of Medical Genetics and Genomics (ACMG) classification (14). The variants comprised two nonsense (p.Gln338Ter, p.Tyr266Ter), one missense variant (p.Cys450Tyr), one synonymous variant (p.Glu198=), and one homozygous contiguous gene deletion of 37.21 kb. Case 1 harbored a novel (ClinVar Accession number: SCV006555343) homozygous synonymous variant c.594A>G (p.Glu198=) in exon 3, classified as a VUS due to the

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lack of functional studies. Structural visualization using AlphaFold demonstrated that Glu198 localizes within a conserved region of the *CYP11B1* protein (**Figure 1A**). As the c.594A>G variant does not alter the amino-acid sequence, no conformational differences were observed between wild-type and variant proteins (**Figure 1B**). Case 5 also exhibited a novel (Accession number: SCV006555344) homozygous likely pathogenic missense variant c.1349G>A (p.Cys450Tyr) in exon 8. In silico parameters suggested damaging and deleterious effects. All variants were considered definitely homozygous based on the molecular tools used. However, segregation analysis could not be performed due to financial constraints. The ACMG classification of the variants is given in **Table 2**.

Table 2. Details of molecular analysis of the *CYP11B1* gene in the studied patients

Case	1	2	3	4	5
Location	Exon 3	Exon 6	Exon 4	Chr 8	Exon 8
Variant	c.594A>G	c.1012C>T	c.798C>G	g.(?*142875702*)(142912910_?) del	c.1349G>A
Amino Acid Change	p.Glu198=	p.Gln338Ter	p.Tyr266Ter	-	p.Cys450Tyr
Zygosity	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous
Inheritance	AR	AR	AR	AR	AR
Classification	VUS	Pathogenic	Likely Pathogenic	Pathogenic	Likely Pathogenic
Novel/Reported	Novel	Reported	Reported	Reported	Novel
ACMG Criteria	PM2, PP3	PVS1, PM2, PP5	PVS1, PM2, PP3	PVS, PM2, PP4	PM1, PM2, PP3, PP4
Type of Mutation	Synonymous	Nonsense	Nonsense	Homozygous deletion of 37.21KB	Missense

AR: autosomal recessive; ACMG: American College of Medical Genetics and Genomics; *CYP11B1:* Cytochrome P450 family 11 subfamily B member 1; VUS: variant of uncertain significance; 11β-OHD: 11β-hydroxylase deficiency PVS1: pathogenic very strong 1; PM2: pathogenic moderate 2; PP3: pathogenic supporting 3; PP4: pathogenic supporting 4; PP5: pathogenic supporting 5.

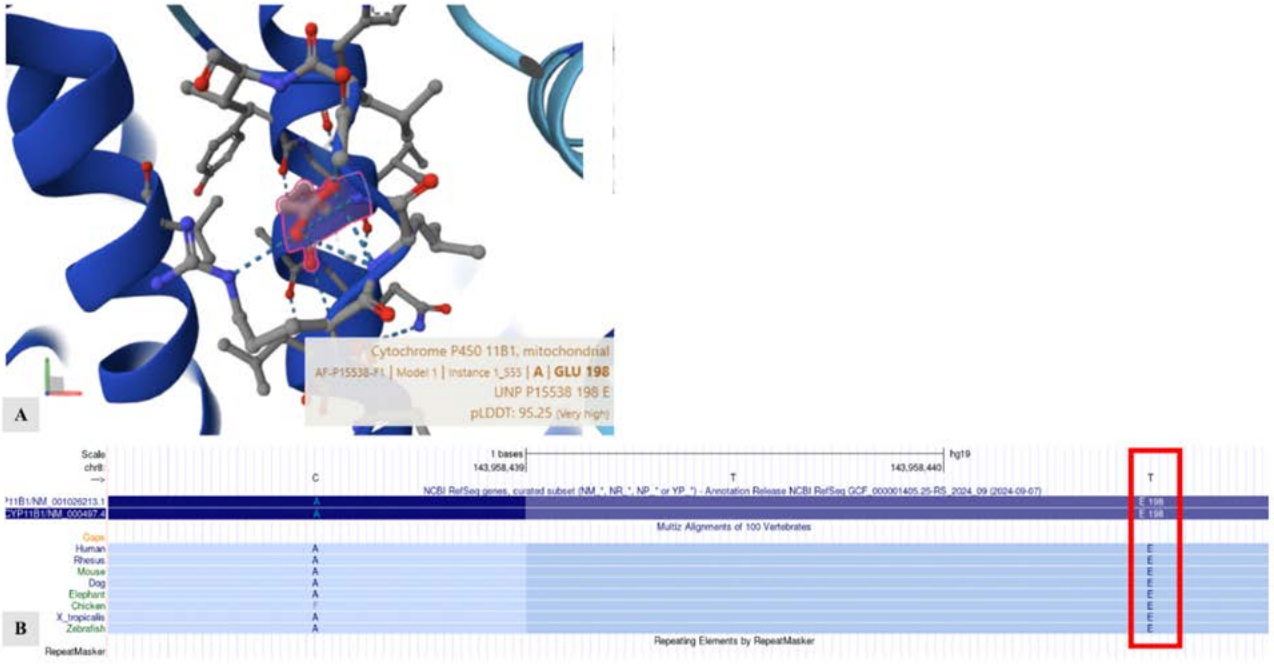


Figure 1. (A) AlphaFold-predicted structure of wild-type *CYP11B1* showing the spatial localization of residue Glu198. The three-dimensional structure of human *CYP11B1* (UniProt P15538) was obtained from the AlphaFold Protein Structure Database. Residue Glu198 (red sticks) is highlighted within the conserved cytochrome P450 fold. As the c.594A>G variant is synonymous (p.E198=), no alteration in protein conformation is observed, suggesting that pathogenicity is likely mediated through RNA-level regulatory mechanisms rather than structural changes. **(B)** Multiple sequence alignment of CYP11B1 protein sequences across vertebrate species.

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There was a median of 2 (IQR-0.5) years delay from the onset of symptoms to definitive diagnosis. Cases 1, 2, and 3 were diagnosed with 11β-OHD at initial presentation based on hypertension, hypokalemia, and metabolic alkalosis. The initial clinical diagnoses were simple virilizing CAH (21-OHD) in two patients (cases 4 and 5); hormonal profiling helped reach a definitive diagnosis of 11β-OHD in one of these (**Table 1**). In case 5, the definitive diagnosis was made only after molecular analysis at age 18 years.

All children received hydrocortisone (10–15 mg/m²/day); case 5 also received fludrocortisone until a confirmatory diagnosis was made and was shifted to dexamethasone at the age of 14 years. Two (cases 2 and 3) of the three children with hypertension at diagnosis required initial management with labetalol infusion. Only one child (case 1) showed resolution of hypertension with hydrocortisone replacement; the other two required the addition of spironolactone for control of hypertension (**Table 1**). All four boys developed central precocious puberty (CPP) within 3-9 months after initiation of glucocorticoid therapy. The diagnosis of CPP was based on an increase in testicular volume (>4 cc) and luteinizing hormone (LH) levels from baseline (**Table 1**). All four were started on leuprolide acetate 11.25 mg intramuscularly every 3 months. Cases 1, 2, and 3 are currently receiving leuprolide and have not shown further progression of puberty, while case 5 (current age 18 years) has completed puberty following leuprolide discontinuation at age 12 years. The median follow-up duration was 2.5 years (1.65-8.25 years).

DISCUSSION

11β-OHD impairs the conversion of 11-DOC to corticosterone and 11-deoxycortisol to cortisol. Cortisol deficiency leads to an increase in adrenocorticotropic hormone (ACTH), which causes the accumulation of steroid precursors such as 11-DOC and 17-OHP, which are diverted to androgen synthesis (1). Girls present with virilised genitalia at birth, but boys often present later with PPP and hypertension (1,8). Hypertension and hypokalemia occur due to chronic elevation of 11-DOC, which exerts potent mineralocorticoid effects (1). When present, hyporeninemic hypertension helps differentiate 11β-OHD from other CAH variants (10). However, not all patients present with hypertension. The onset and prevalence of hypertension are variable and usually attributed to the degree of increase in aldosterone precursors (10). However, hypertension in patients with 11β-OHD shows no direct correlation with 11-DOC levels or the degree of virilization (8). One proposed mechanism that warrants further exploration is genotype-dependent residual 11-β hydroxylase activity, as severe variants have been reported to be associated with worse hypertension (15). Two of our patients did not have hypertension at any time during their clinical course. When hypertension is a presenting feature, adrenal tumors and apparent mineralocorticoid excess also need consideration initially (16).

Based on the most commonly employed laboratory test for diagnosing CAH, i.e., 17-OHP, 11β-OHD is often misclassified as 21-OHD, leading to its underdiagnosis, even during NBS for CAH (17). In our patients, the 17-OHP elevations were also variable; case 5 was thus misdiagnosed as simple virilising 21-OHD for 14 years in the absence of hypertension or hypokalemia. Although a borderline elevation in 17-OHP warrants measurement of 11-deoxycortisol, the diagnostic cutoff for 11β-OHD is not defined (17). In countries where NBS for 21-OHD is performed, some false-positive results may still be 11β-OHD cases. A careful further evaluation of borderline 17-OHP values on NBS is therefore warranted. A previous study confirmed a diagnosis of 11β-OHD in 1% of their 133 patients with a previous diagnosis of 21-OHD (17). In such unsolved cases, hormonal profiling helps identify the enzymatic defect by estimating intermediate molecules in the steroidogenic pathway (11,17). In addition to 11-deoxycortisol, 11-DOC, and androstenedione, 21-deoxycortisol, a derivative of 17-OHP via 11-hydroxylase action, may serve as an alternative steroid marker for identifying 21-OHD subtypes, including heterozygotes, and may aid in distinguishing 11β-OHD (17). In our series, the 17-OHP levels of 31.47 nmol/L, without hypertension in case 4, pointed toward 21-OHD; however, the simultaneous elevations of 11-DOC and 11-deoxycortisol in the hormonal profile suggested 11β-OHD. In case 3, although 17-OHP levels were

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>30 nmol/L, the presentation with grade 2 hypertension provided an initial clue to the diagnosis. Thus, hormonal profiling further helps differentiate 11β-OHD from 21-OHD when initial clinical clues such as hypertension, hypokalemia, and metabolic alkalosis are absent. Molecular analysis subsequently confirms the enzymatic defect and is critical for genetic counseling and risk assessment in future pregnancies (17).

Our study highlights a heterogeneous variant spectrum in 11β-OHD, with two novel variants identified in five patients. A homozygous synonymous variant, c.594A>G (p.Glu198=) in exon 3, was classified as a VUS due to the absence of functional validation. Synonymous variants, however, can disrupt protein function by altering splicing, mRNA stability, translation efficiency, or transcription factor binding (18). Case 5 harbored a homozygous missense variant, c.1349G>A (p.Cys450Tyr) in exon 8, affecting a highly conserved residue in *CYP11B1* and predicted to impair protein structure and function. Prior studies indicate variant clustering in exons 2, 6, 7, and 8, consistent with known genetic hotspots. A nonsense variant, c.798C>G (p.Tyr266Ter) in exon 4, introduces a premature stop codon, producing a truncated, nonfunctional protein, as reported in ClinVar. The nonsense variant p.Gln338Ter in case 2, previously described in the Turkish population, results in complete enzyme inactivation (19). Case 4 exhibited a large 37.2 kb homozygous contiguous gene deletion encompassing *CYP11B1* and *CYP11B2*, consistent with earlier reports of locus loss or fusion leading to 11β-OHD (20). Our data provide further support for regional genetic heterogeneity in patients with 11β-OHD from India (21).

The prevalence of hypertension in patients with 11β-OHD varies between 30 and 66% and correlates significantly with older age at diagnosis (15,22,23). It often causes significant acute and chronic morbidity; case 2 presented with a hypertensive emergency, while his elder brother showed LVH due to chronic hypertension. If hypertension persists despite optimal glucocorticoid therapy, the addition of spironolactone, amiloride, a calcium channel blocker, or even bilateral adrenalectomy should be considered (15). Poorly controlled hypertension and glucocorticoid excess in 11β-OHD may cause cardiometabolic complications, including end-organ damage such as LVH, retinopathy, nephropathy, and cerebrovascular events (8,15).

In our series, four patients developed CPP after treatment initiation, unlike other forms of CAH, where CPP may be a presenting feature (24). This likely results from the withdrawal of prolonged gonadotropin-independent androgen exposure, which subsequently triggers activation of the hypothalamic-pituitary-gonadal axis (25). Intratesticular adrenal cell rests, as seen in case 2, may reflect disease severity and delayed diagnosis, and contribute to male infertility by compressing the seminiferous tubules and causing obstructive azoospermia (8). Advanced BA, seen in all our patients, also reflects chronic androgen exposure due to delayed diagnosis and carries a poor adult height prognosis (24,25).

CONCLUSIONS

11β-OHD is a rare form of CAH with high phenotypic variability, leading to diagnostic delays. Borderline 17-OHP elevations in suspected CAH cases should prompt evaluation for 11β-OHD. Hormonal profiling helps to differentiate this rarer form from 21-OHD. The two novel variants identified in our patients expand the known genetic spectrum of the *CYP11B1* in the Indian population.

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case report

Long-term follow-up of neonatal severe hyperparathyroidism: redefining calcium management

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ABSTRACT

Neonatal severe hyperparathyroidism (NSHPT) is a rare, life-threatening disorder caused by biallelic inactivation of the *CASR* gene, resulting in severe hypercalcemia and markedly elevated parathyroid hormone (PTH) levels in early life. Although total parathyroidectomy is often curative, long-term calcium balance and treatment requirements remain poorly understood. We describe the 25-year follow-up of a woman with NSHPT due to a homozygous *CASR p.Arg680His* variant who underwent total parathyroidectomy with autotransplantation at 32 days of age. Despite initial normalization of calcium levels, graft failure led to permanent hypoparathyroidism requiring long-term calcium and active vitamin D supplementation. Over time, calcium and calcitriol requirements progressively decreased despite persistently undetectable PTH, with recurrent episodes of hypercalcemia requiring careful dose adjustments. This case represents one of the longest documented follow-ups of genetically confirmed homozygous *CASR*-related NSHPT. The progressive decline in calcium requirements reflects impaired renal calcium excretion and an altered calcium–PTH set point characteristic of *CASR* inactivation. These physiological adaptations challenge the conventional supplementation strategies and suggest that standard hypoparathyroidism guidelines – largely derived from acquired or autoimmune forms – may require cautious individualization in patients with homozygous *CASR* variants, given their distinct renal calcium handling and the possibility of lower urinary calcium excretion. Our findings reinforce the complexity of long-term management in NSHPT patients and illustrates how calcium requirements may change over time. Long-term follow-up cases such as this may contribute to a better understanding of the physiological mechanisms and inform future guideline development for this rare condition.

Keywords: Hyperparathyroidism; receptors; calcium-sensing; parathyroidectomy; calcium homeostasis

INTRODUCTION

Neonatal severe hyperparathyroidism (NSHPT) is a rare, life-threatening condition caused by homozygous or compound heterozygous inactivating variants in the *CASR* gene, which encodes the calcium-sensing receptor (CaSR) (1). This receptor, expressed in parathyroid glands and renal tubules, regulates parathyroid hormone (PTH) secretion and renal calcium handling, thereby maintaining calcium homeostasis. Inactivating *CASR* variants raise the calcium–PTH set point, resulting in inappropriate PTH secretion and hypercalcemia despite elevated calcium levels (1–3).

We present the 25-year follow-up of a woman with a homozygous *p.Arg680His CASR* variant who underwent early parathyroidectomy for NSHPT. This case illustrates the unique long-term metabolic course and the evolving calcium requirements that challenge conventional supplementation strategies and current management guidelines.

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CASE PRESENTATION

A 25-year-old female is under long-term follow-up in our endocrinology department for permanent hypoparathyroidism after a total parathyroidectomy performed for NSHPT.

At 32 days of life, she presented with severe hypotonia, prostration, feeding refusal, and weight loss. Laboratory evaluation revealed marked hypercalcemia of 6.15 mmol/L (24.6 mg/dL; normal range [NR] 2.2-2.8 mmol/L or 8.5-10.5 mg/dL), hypophosphatemia of 0.73 mmol/L (2.25 mg/dL; NR 1.45-2.5 mmol/L or 4.49-7.74 mg/dL), and markedly elevated PTH levels of 89.26 pmol/L (842 ng/L; NR 1.6-6.9 pmol/L or 7-59 ng/L). She was initially treated with intravenous fluids, furosemide (3 mg/kg/day), and intramuscular calcitonin (8 mg/kg/day), with partial biochemical improvement.

Because of persistent hypercalcemia despite medical therapy, surgical intervention was pursued. Total parathyroidectomy with autotransplantation of one gland into the left deltoid was performed without complications. Histopathology revealed two parathyroid glands; however, the right superior parathyroid specimen consisted only of thymic tissue.

After a transient decline, serum calcium and PTH levels returned to elevated levels – 3.16 pmol/L (12.5 mg/dL) and 26.07 pmol/L (246 pg/mL), respectively – by postoperative day 17. A CT scan of the parathyroid region localized an additional parathyroid gland in the superior pole of the right thyroid lobe. Due to recurrent hypercalcemia, a second surgery (right hemithyroidectomy) was performed three months later, and histopathology confirmed the presence of parathyroid and thyroid tissue. This achieved biochemical remission of hyperparathyroidism.

Biochemical screening of both parents revealed features consistent with familial hypocalciuric hypercalcemia (**Table 1**), while screening of other family members was unremarkable. Genetic analysis identified a homozygous likely pathogenic variant in the *CASR* gene (c.2039 G>A; p.Arg680His) in the patient, with both parents confirmed as heterozygous carriers.

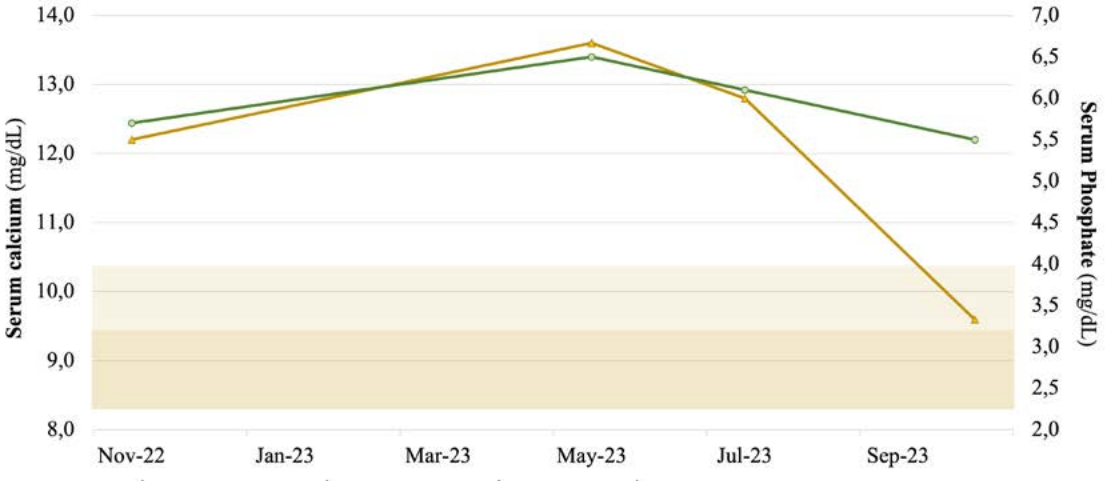
The patient was discharged on vitamin D supplementation. However, due to autograft failure, she developed permanent hypoparathyroidism, requiring long-term treatment with calcium and calcitriol. She has remained under continuous endocrinology follow-up since childhood, with regular biochemical monitoring and treatment adjustments, and has been followed at our tertiary center since 2016. Over the past decade, repeated dose adjustments were required, and progressive reduction of calcium and calcitriol became necessary in the context of recurrent hypercalcemia (**Figure 1**). During this period, despite hypercalcemia, the patient consistently exhibited low fractional excretion of calcium. Specifically, at one point during follow-up, despite a serum calcium level of 12.6 mg/dL, 24-hour urinary calcium excretion was 12.8 mEq (approximately 256 mg), corresponding to a fractional excretion of calcium of approximately 1.4%, which is inappropriately low for the degree of hypercalcemia and supports impaired renal calcium excretion related to CaSR inactivation. Following treatment dose adjustment, with calcium carbonate 1500 mg/day (corresponding to 600 mg of elemental calcium), her serum calcium stabilized at 2.4 mmol/L (9.6 mg/dL). Following guideline recommendations to maintain calcium in the lower half of the reference range, calcium supplementation was temporarily suspended, leaving only low-dose calcitriol. However, the patient developed paresthesias and was unable to tolerate complete withdrawal of calcium, leading to the reintroduction of calcium carbonate at 1500 mg/day.

Table 1. Blood and urine test results of the child and her parents

	Serum calcium (mg/dL)	Serum phosphate (mg/dL)	PTH (pg/mL)	FECa (%)	TRP (%)
Child (32 days)	24.64	2.25	842	0.9	10
Father (27 years)	10.4		46.8	0.4	
Mother (26 years)	10.0		33.7	0.5	

Normal range for serum calcium is 2.1-2.6 mmol/L (8.5-10.5 mg/dL). Normal range for serum phosphate is 1.45-2.5 mmol/L (4.49-7.74 mg/dL). Normal range for parathyroid hormone (PTH) is 1.6-6.9 pmol/L (7-59 pg/mL). Fractional excretion of calcium (FECa) (%) was calculated as: $\frac{U_{Ca} \times S_{Cr}}{S_{Ca} \times U_{Cr}} \times 100$. In CaSR inactivation, FECa is typically <1% despite hypercalcemia. Normal range for tubular reabsorption of phosphate (TRP) is 85–95%.

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Date	Serum calcium (mg/dL)	Serum Phosphate (mg/dL)	PTH (pg/mL)	Treatment
Nov 2022 → 24 years	12.2	4.5	1.2	Calcitriol 0.75 µg/day + Elemental calcium 1800 mg/day
May 2023 → 25 years	13.6	3.5		Calcitriol 0.75 µg/day + Elemental calcium 1800 mg/day
Jul 2023 → 25 years	12.8	4.1		Calcitriol 0.55 µg/day + Elemental calcium 1200 mg/day
Oct 2023 → 25 years	9.6	4.5	2.2	Calcitriol 0.55 µg/day + Elemental calcium 600 mg/day

Figure 1. Serum calcium, phosphate, and parathyroid hormone concentrations over recent years and corresponding treatment. The yellow line depicts serum calcium values, while the green line represents serum phosphate values. The yellow box represents the normal calcium range, with the darker shade indicating the therapeutic target recommended in hypoparathyroidism guidelines (i.e., the lower half of the normal reference range or just below it).

This case represents one of the longest documented follow-ups of genetically confirmed homozygous CASR-related NSHPT, highlighting the evolving calcium requirements over decades of care.

DISCUSSION

The CaSR is a G protein–coupled receptor expressed primarily in parathyroid glands and renal tubules. By sensing extracellular calcium, it regulates PTH secretion and renal calcium excretion, thereby maintaining calcium homeostasis (1,2,4). More than 230 variants in the *CASR* gene have been described, leading to either loss- or gain-of-function phenotypes (3). Inactivating variants raise the calcium–PTH set point, resulting in inappropriate PTH secretion and enhanced renal calcium reabsorption (4,5).

The clinical spectrum of *CASR* loss-of-function variants ranges from familial benign hypocalciuric hypercalcemia (FBHH), usually asymptomatic and caused by heterozygous variants, to NSHPT, a life-threatening disorder associated with homozygous or compound heterozygous variants (3,6,7). NSHPT typically presents in the neonatal period with severe hypercalcemia and markedly elevated PTH.

Initial management to control severe hypercalcemia includes intravenous fluid hydration and loop diuretics, with careful monitoring of volume status, as well as calcitonin, bisphosphonates, and calcimimetics (8–10). Although some reports describe neonates maintained on medical management (11–13), this approach is often complex and insufficient, and most patients ultimately require parathyroidectomy as definitive treatment (14,15). While subtotal parathyroidectomy was once preferred, most surgeons now favor total parathyroidectomy due to high recurrence rates (15). Autotransplantation of parathyroid tissue is commonly performed but carries a significant risk of graft-dependent hypercalcemia (≈33%) and a smaller risk of graft failure (≈6%) (16).

Postoperative permanent hypoparathyroidism is a frequent consequence following total parathyroidectomy without successful autotransplantation, and patients often require lifelong calcium and active vitamin D supplementation. Current guidelines for hypoparathyroidism recommend maintaining serum calcium in the lower half of the reference range or just below, to prevent hypercalciuria (17). However, our patient's long-term evolution illustrates that this

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recommendation may not fully apply to homozygous *CASR* variant carriers.

Over the years, she required progressively smaller doses of calcium and calcitriol to maintain normocalcemia, despite undetectable PTH. This clinically counterintuitive finding can be explained by the reduced renal calcium excretion associated with *CASR* inactivation. Because the CaSR normally enhances urinary calcium loss in response to hypercalcemia, its dysfunction limits calciuresis and allows calcium balance to be maintained with minimal supplementation. However, this same mechanism also increases the risk of hypercalcemia if treatment is excessive (2).

Published reports describe heterogeneous biochemical and clinical outcomes after parathyroidectomy in patients with NSHPT. While some individuals remain dependent on lifelong calcium and active vitamin D supplementation (10,18,19), others can maintain normocalcemia with calcitriol monotherapy (8,20,21). This variability likely reflects underlying genotype–phenotype differences. Marx and Sinaii analyzed *CASR* genotypes, biochemical profiles, and clinical courses of patients with NSHPT, distinguishing two broad phenotypic patterns: (1) homozygotes for clearly pathogenic variants, characterized by severe neonatal hypercalcemia and very high PTH concentrations; and (2) compound heterozygotes, who often present milder neonatal hypercalcemia and demonstrate a greater likelihood of partial receptor activity. Interestingly, the authors observed that homozygous individuals, although initially more severely affected, frequently required lower doses of calcium and calcitriol after total parathyroidectomy to maintain normocalcemia (22). These findings suggest that even minimal CaSR signaling in peripheral tissues — particularly in the kidney — may persist and influence calcium homeostasis independently of parathyroid function.

From a physiological standpoint, the CaSR modulates renal calcium reabsorption primarily in the thick ascending limb and distal convoluted tubule (2). Inactivating variants reduce receptor sensitivity to extracellular calcium, thereby blunting the renal excretory response to hypercalcemia. In the postoperative setting, this translates into reduced urinary calcium losses and a diminished requirement for exogenous supplementation. In contrast, excessive calcium or calcitriol replacement can easily precipitate hypercalcemia, given the kidney's impaired capacity to enhance calciuresis. This mechanism provides a plausible explanation for the wide interindividual variability in calcium needs and highlights the importance of cautious titration.

Furthermore, long-term tolerance to mild or moderate hypercalcemia in *CASR*-related hypoparathyroidism parallels that seen in FHH, in which heterozygous carriers remain asymptomatic despite chronic elevations of serum calcium (1,3). Similar to FHH, homozygous *CASR* variants carriers may exhibit lifelong protection against nephrocalcinosis and renal impairment due to persistently low urinary calcium excretion. Therefore, strict adherence to the conventional target of maintaining calcium in the lower half of the reference range may not be necessary (17) — and could even be counterproductive — in this specific population. Instead, maintaining biochemical stability and symptom control should take precedence over numerical targets.

In fact, current international guidelines for chronic hypoparathyroidism recommend targets that are derived from populations with acquired or autoimmune hypoparathyroidism and do not account for the unique renal physiology associated with *CASR* mutations.

Long-term calcium requirements in homozygous *CASR* mutation carriers remain unpredictable and may fluctuate over time. Some patients gradually reduce or discontinue supplementation as homeostatic adaptation occurs, whereas others relapse into symptomatic hypocalcemia after dose reductions. These divergent courses reinforce the need for personalized, physiology-based follow-up and careful long-term surveillance.

From a clinical perspective, this case illustrates that long-term calcium and active vitamin D requirements after parathyroidectomy in NSHPT may evolve over decades and differ substantially from those observed in other forms of hypoparathyroidism. Rather than rigid adherence to standard biochemical targets, clinicians should anticipate dynamic calcium requirements, closely monitor symptoms and laboratory

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parameters, and adjust supplementation cautiously to avoid both hypo- and hypercalcemia.

Given the extreme rarity of NSHPT, evidence is confined to small cohorts and isolated reports, precluding formal treatment algorithms. Nonetheless, accumulating long-term follow-up data — as exemplified by our patient — are critical to refine management goals and to inform future guidelines. A shift toward a personalized, physiology-based approach, acknowledging the unique renal and systemic consequences of *CASR* inactivation, will likely improve clinical outcomes and optimize quality of life in this rare population.

CONCLUSION

This case illustrates the complexity of long-term management in NSHPT due to homozygous *CASR* variants. Despite permanent hypoparathyroidism, our patient required progressively less supplementation, ultimately maintaining normocalcemia with minimal therapy. This clinically counterintuitive finding reflects impaired renal calcium excretion and suggests that, in selected patients with homozygous *CASR* variants, strict adherence to lower calcium targets may not always be necessary; however, any reduction in supplementation or acceptance of higher serum calcium levels should be individualized and accompanied by close biochemical monitoring.

Ongoing documentation of long-term outcomes in NSHPT cases will enhance treatment approaches and outcomes for this rare condition. Our case contributes to this limited body of evidence, emphasizing the need for individualized care and the development of guidelines adapted to CASR-related disorders.

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case report

Pregnancy in a patient with metastatic functioning midgut neuroendocrine tumor receiving lanreotide after peptide receptor radionuclide therapy

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ABSTRACT

Neuroendocrine tumors (NETs) are rare neoplasms that present significant challenges during pregnancy, particularly in the context of metastatic disease or carcinoid syndrome requiring systemic therapy. Data on the safety of somatostatin analogs during pregnancy remain limited and are primarily derived from studies involving patients with acromegaly. We report the maternal and fetal outcomes in a pregnant patient diagnosed with a metastatic NET of the ileocecal valve, complicated by carcinoid syndrome. The patient had previously undergone treatment with ^{177}Lu-DOTATATE, debulking surgery, and lanreotide therapy. Lanreotide administration was continued throughout the pregnancy under multidisciplinary supervision. Maternal monitoring revealed no complications, and postpartum evaluations confirmed disease stability. The newborn developed transient neonatal hyperbilirubinemia requiring phototherapy but demonstrated normal development at the 18-month follow-up. This case highlights important clinical considerations regarding the continuation of somatostatin analog therapy, biochemical monitoring, peri-delivery planning for a carcinoid crisis, and pregnancy after peptide receptor radionuclide therapy in patients with functioning metastatic NETs. It provides supportive observational evidence that the continuation of lanreotide during pregnancy may be feasible in carefully selected patients under close multidisciplinary supervision, although conclusions regarding safety remain limited by the rarity of available data.

Keywords: Neuroendocrine tumors; pregnancy; lanreotide; carcinoid syndrome; peptide receptor radionuclide therapy; somatostatin analogs

INTRODUCTION

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms that may arise in several organs, including the small intestine, pancreas, colon, and lungs (1,2). NETs may be benign or malignant, functional (hormone-secreting) or nonfunctional, as well as sporadic or associated with inherited syndromes (2).

Pregnancy in female patients undergoing systemic treatment for metastatic neuroendocrine neoplasms is rare, clinically challenging, and poorly documented, as these neoplasms are themselves rare. Consequently, clinical data on the maternal and fetal effects of treatment with somatostatin analogs (SSAs) in such patients are scarce (3). The limited available data on the safety of SSAs during pregnancy are primarily based on their use in acromegaly. Data regarding pregnancy after peptide receptor radionuclide therapy (PRRT) are even more limited.

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Here, we describe the maternal and fetal outcomes of a pregnant woman previously diagnosed with a metastatic NET of the ileocecal valve accompanied by carcinoid syndrome, who had undergone treatment with ^{177}Lu-DOTATATE and continued lanreotide therapy throughout her pregnancy under multidisciplinary supervision.

CASE PRESENTATION

We report the case of a 33-year-old woman initially diagnosed with a metastatic grade 1 (G1) NET of the ileocecal valve and carcinoid syndrome at the age of 29. At the time of diagnosis, she presented with multiple liver and lymph node metastases, experiencing symptoms of abdominal pain, flushing, nausea, vomiting, diarrhea, and unintentional weight loss. Her medical history included multiple consultations with various specialists for allergies, a diagnosis of irritable bowel syndrome, and recurrent urinary tract infections. Baseline staging with ^{68}Ga-DOTANOC PET/CT at the time of diagnosis demonstrated increased somatostatin receptor expression across multiple sites: two subcentimeter nodules in the left and right pericardial regions (interpreted as lymph node metastases), at least five hypermetabolic hepatic foci (notably, a 30-mm lesion at the segment IV/V junction), intense uptake in the body and tail of the pancreas, and focal uptake in the uncinate process. Additional uptake in the right lower quadrant suggested a small bowel NET with possible nodal involvement, and scattered mild-to-moderate uptake throughout the abdominopelvic cavity raised suspicion for peritoneal implants or synchronous disease.

Urinary 5-hydroxyindoleacetic acid (5-HIAA) and chromogranin A (CgA) levels were 61.08 mg/24 h (reference range [RR] 2.0–9.0) and 256.72 ng/mL (RR: < 98.1 ng/mL), respectively. A diagnosis of a G1 NET was confirmed through histopathological evaluation of a liver metastasis biopsy. The analysis demonstrated a well-differentiated NET with a Ki-67 index of 2% and a mitotic count of 2 per 2 mm^2, showing concordant findings between the primary tumor and the liver metastases. Carcinoid heart disease was ruled out based on normal N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and a transthoracic echocardiogram, which revealed normal valvular morphology and function without evidence of right-sided heart involvement or chamber dilation.

Long-acting subcutaneous lanreotide was initiated at a dose of 120 mg every 28 days. Follow-up magnetic resonance imaging and ^{68}Ga-DOTANOC PET/CT performed three months after starting lanreotide therapy demonstrated disease progression. There was increased radiotracer uptake in the ileocecal region (maximum standardized uptake value of 29.44), consistent with the known primary tumor, alongside new or more prominent uptake in previously identified sites: pericardial and epiphrenic lymph nodes, at least three hepatic metastases (with a maximum standardized uptake value of 72.51 in segment IV), pancreatic lesions, and abdominopelvic areas suggestive of further metastatic spread.

In light of this progression, ^{177}Lu-DOTATATE therapy was proposed, and a total of four cycles were completed. PRRT was administered as a standard-of-care treatment rather than within a clinical trial protocol. Despite disease stabilization, cytoreductive (debulking) surgery was undertaken; this procedure consisted of an ileocolectomy, a cholecystectomy, a hepatic metastasectomy, and the excision of uterine fibroids. A total of three liver metastases were resected, and residual metastatic disease remained stable on subsequent imaging. The surgical approach aimed to reduce the tumor burden and improve long-term disease control, aligning with current practices for selected young, motivated patients with stable, low-grade (G1), well-differentiated NETs. Histological analysis confirmed a diagnosis of NET G1 (pT4N1M1) according to the European Neuroendocrine Tumor Society classification (4,5). Lanreotide treatment (120 mg every 28 days) was continued over the subsequent two years. During the follow-up, the patient showed clinical improvement and disease stability, achieving radiologic tumor volume reduction and the biochemical normalization of urinary 5-HIAA (3.0 mg/24 h) and CgA (39 ng/mL) levels.

Unexpectedly, three years after the initial diagnosis, the patient became pregnant while receiving long-acting subcutaneous lanreotide treatment. By the time she became aware of her pregnancy at 5 weeks of

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gestation, she had already received one dose of lanreotide. She was promptly referred to a specialized center dedicated to high-risk obstetrics to ensure appropriate maternal and fetal care. A chronological summary of the patient's clinical course is presented in **Table 1**.

After evaluation by a multidisciplinary team, a shared decision was made to continue lanreotide therapy. This decision considered the functioning and metastatic nature of the NET, as well as the limited clinical reports describing favorable maternal and fetal outcomes with SSA treatments, including lanreotide. A transthoracic echocardiogram and NT-proBNP measurement at this time revealed no abnormalities. The patient received subcutaneous lanreotide (120 mg every 28 days) throughout the entire pregnancy, with no dosage adjustments.

A first-trimester combined risk assessment was conducted at 14 weeks' gestation, followed by a morphological ultrasound at 22 weeks and a fetal echocardiogram at 27 weeks. All evaluations showed normal fetal biometrics and no anomalies. A third-trimester ultrasound performed at 31 weeks revealed fetal growth at the 48th percentile and no fetal abnormalities.

Regarding maternal outcomes, standard screening protocols revealed no evidence of gestational hypertension, preeclampsia, or gestational diabetes. Biochemical analyses of CgA and urinary 5-HIAA levels remained within or near normal ranges throughout the pregnancy (**Figure 1**). A magnetic resonance imaging performed in the third trimester showed no evidence of metastatic disease progression. At 39 weeks' gestation, the patient was admitted for an elective induction of labor. Epidural analgesia was administered. To shorten the expulsion stage, vacuum extraction and additional maneuvers were necessary to resolve fetal shoulder dystocia. The delivery proceeded without further complications, and a preplanned octreotide infusion protocol, intended for the prevention of a carcinoid crisis, was ultimately not required.

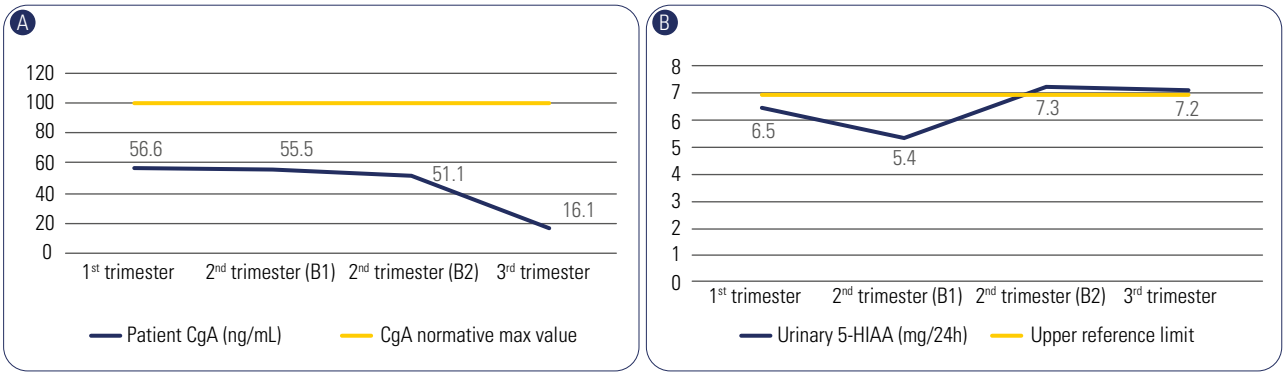
The male newborn presented with normal anatomical characteristics, a birth weight of 3,820 g, a length of 50.5 cm, and a head circumference of 37 cm — all parameters appropriate for gestational age. The Apgar scores at 1, 5, and 10 min were 8, 9, and 9, respectively, indicating good overall vitality. A pediatric evaluation within the first 24 h confirmed the absence

Table 1. Clinical timeline from diagnosis to postpartum follow-up

Age/timepoint	Clinical event
Age 29	Diagnosis of metastatic grade 1 (G1) ileocecal neuroendocrine tumor with carcinoid syndrome
At diagnosis	Elevated urinary 5-HIAA (61.08 mg/24h) and chromogranin A (256.72 ng/mL) ^{68}Ga-DOTANOC PET/CT demonstrated liver, lymph node, pancreatic, and probable peritoneal metastatic involvement
Immediately after diagnosis	Lanreotide 120 mg every 28 days initiated
3 months later	MRI and ^{68}Ga-DOTANOC PET/CT demonstrated disease progression
Age 29–30	Four cycles of 177Lu-DOTATATE administered
Following PRRT	Cytoreductive surgery performed (ileocolectomy, cholecystectomy, hepatic metastasectomy, and uterine fibroid excision)
Age 31 (following 2 years)	Stable disease under continuous lanreotide therapy with biochemical normalization
Age 33	Spontaneous pregnancy occurred while receiving lanreotide
5 weeks gestation	Pregnancy recognized after exposure to one dose of lanreotide
14 weeks gestation	First-trimester combined risk assessment performed
22 weeks gestation	Morphological fetal ultrasound showed no abnormalities
27 weeks gestation	Fetal echocardiography demonstrated normal findings
31 weeks gestation	Third-trimester ultrasound showed normal fetal growth (P48)
Third trimester	MRI demonstrated stable metastatic disease
39 weeks gestation	Elective induction of labor and vacuum-assisted vaginal delivery
Neonatal period	Transient neonatal hyperbilirubinemia requiring phototherapy
Age 34 (18 months postpartum)	Patient asymptomatic with stable disease; child with normal development

5-HIAA: 5-Hydroxyindoleacetic acid; G1: grade 1; MRI: magnetic resonance imaging; PET/CT: positron emission tomography/computed tomography; PRRT: peptide receptor radionuclide therapy.

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CgA: chromogranin A; 5-HIAA: 5-hydroxyindoleacetic acid; B1: first biochemical evaluation during the second trimester; B2: second biochemical evaluation during the second trimester.

Figure 1. (**A**) Changes in chromogranin A (CgA) levels (reference range: 0–100 ng/mL) and (**B**) urinary 5-hydroxyindoleacetic acid (5-HIAA) levels (reference range: 1.0–7.0 mg/24 h) during pregnancy.

of visible malformations or tremors and reported normal neonatal reflexes. Due to the lack of safety data regarding breastfeeding while on lanreotide, formula feeding was advised.

On his second day of life, jaundice was observed, and a transcutaneous bilirubin (TcB) level of 13.2 mg/dL (RR: 0.2–1.0 mg/dL) was recorded; this did not initially require phototherapy. However, by the fourth day, a clinical reassessment revealed a TcB level exceeding 20 mg/dL, requiring hospitalization for phototherapy. After 48 h of treatment, the newborn was discharged with a TcB level of 12.7 mg/dL. No congenital abnormalities or persistent developmental concerns were observed during the follow-up period. At two months of age, the infant exhibited normal psychomotor development. His somatometric parameters were as follows: a weight of 5510 g (50th percentile), a length of 56 cm (15th percentile), and a head circumference of 39 cm (50th percentile). Eighteen months after delivery, the patient was still asymptomatic, maintaining normal levels of urinary 5-HIAA and serum CgA, and with stable disease on morphologic and functional imaging.

DISCUSSION

Pregnancy in patients with metastatic midgut NETs and active carcinoid syndrome presents a complex therapeutic dilemma for which no formal guidance exists. We report a pregnancy with favorable maternal and fetal outcomes in a 33-year-old woman with a metastatic G1 ileocecal NET and carcinoid syndrome; conception occurred two years after the completion of PRRT, and the patient was managed with uninterrupted lanreotide (120 mg) throughout gestation. Despite the challenges of metastatic disease and the significant mechanical stress of a vacuum-assisted delivery complicated by shoulder dystocia, the patient maintained biochemical and radiological stability, resulting in the birth of a healthy, appropriate-for-gestational-age newborn. Although factors such as the psychological burden of the disease and the potential hormonal effects of systemic therapies may impair fertility in patients with NETs, pregnancy is increasingly encountered in clinical practice. This trend reflects both the prolonged survival achieved with modern SSAs and the rising age of first-time mothers, creating a need for clearer management strategies in this setting (6).

Safety of somatostatin analogs is the first-line systemic therapy for metastatic G1-G2 NETs and are a key component of carcinoid syndrome management (7,8). Although SSAs are known to cross the placental barrier via passive diffusion, *in vitro* studies suggest they have low-affinity binding to placental somatostatin receptors (9). Importantly, most previously reported cases of pregnancy in patients with metastatic NETs treated with lanreotide describe either the interruption of therapy during the first trimester or its reintroduction later in gestation (10–12). Published cases of pregnancy in patients with metastatic NETs receiving lanreotide are summarized in **Table 2**. In contrast, our patient received uninterrupted lanreotide therapy from early pregnancy until delivery. This decision was not based solely on theoretical safety, but on the clinical need to preserve hormonal stability in a patient

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Table 2. Published cases of pregnancy in patients with metastatic neuroendocrine tumors receiving lanreotide

Reference	Age at pregnancy (years)	Site of primary tumor	Grade	Treatment during pregnancy	Treatment before pregnancy	Pregnancy outcomes	NET status postpartum
Giulia Meoni et al. (10)	41	Ileal NET (breast, axillary lymph nodes, and liver metastases)	G2	Lanreotide depot (LAR) 120 mg every 28 days	LAR 120 mg every 28 days	Cesarean delivery due to hypertensive emergency (history of chronic hypertension before pregnancy). No other adverse maternal and fetal outcomes.	Stable disease
	37	Pancreatic NET (liver and bone metastases)	G2	LAR 120 mg every 28 days	Distal splenopancreatectomy and liver metastasis resection. LAR 120 mg every 28 days	Cesarean delivery. No adverse maternal and fetal outcomes.	Disease progression (1 year postpartum; everolimus added with disease stabilization)
Nynne Emilie Hummelshøj et al. (11)	32	Small intestinal NET (liver metastasis)	G1	1st trimester: lanreotide discontinuation 16 weeks: lanreotide resumed with dose titration to 120 mg every 28 days	LAR 120 mg every 28 days, debulking surgery	Cesarean delivery. No adverse maternal and fetal outcomes.	Stable disease
Ratnayake et al. (12)	35	Midgut NET (liver and pancreas metastases)	G2	Octreotide, LAR	Surgery, octreotide, LAR	Emergency cesarean delivery at 31 weeks (preterm pre labor rupture of membranes). Invasive ventilation at neonatal intensive care, nasogastric tube feeding, retinopathy of prematurity, ventriculomegaly, mild developmental delay in the baby	Stable disease
	31	Pancreatic (liver, abdominal node, pancreas metastases)	G2	Octreotide, LAR	Octreotide, LAR	Cesarean delivery. No adverse maternal and fetal outcomes.	Disease progression
	33	Midgut (liver, midgut, portocaval, pelvic and retroperitoneal metastases)	G2	Octreotide, LAR (not in 1st trimester)	Octreotide, LAR	Cesarean delivery. No adverse maternal and fetal outcomes.	Stable disease
	36	Midgut (liver, midgut, portocaval, pelvic and retroperitoneal metastases)	G2	Octreotide, LAR	Octreotide, LAR	Cesarean delivery. No adverse maternal and fetal outcomes.	Not available
	33	Pancreatic (liver metastases)	G2	LAR	LAR, PRRT within a year before pregnancy	Cesarean delivery. Gestational diabetes controlled by diet.	Stable disease

LAR: Long-acting formulation; NET: neuroendocrine tumor; PRRT: peptide receptor radionuclide therapy.

with active carcinoid syndrome and a significant metastatic burden. The stable urinary 5-HIAA and CgA levels observed throughout pregnancy, combined with the absence of radiological progression, suggest that maintaining antisecretory therapy may be feasible in selected patients with functioning metastatic NETs under close multidisciplinary supervision.

The safety profile observed in our patient aligns with data from populations with acromegaly, in which SSAs are not consistently associated with adverse

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maternal and fetal outcomes (13). However, the clinical context differs substantially. In acromegaly, SSAs are often discontinued unless significant tumor growth occurs, whereas in metastatic NETs, the risk of acute clinical deterioration due to uncontrolled carcinoid syndrome is considerably higher. Particularly in midgut NETs with hepatic metastases, serotonin-mediated complications and the increased hemodynamic workload of pregnancy necessitate a more cautious approach to treatment interruption (8,9,14). This case highlights the importance of individualized risk-benefit assessments when considering the continuation or interruption of SSA therapy during pregnancy.

An additional important aspect of this case was the interpretation of biochemical markers during pregnancy. A mild increase in urinary 5-HIAA was observed from the second trimester onward. Rather than definitively indicating tumor activity, this finding may partially reflect physiological, pregnancy-related changes, including increased placental serotonin production and plasma volume expansion (15). Recognizing this physiological interference is crucial to avoid misinterpretation and unnecessary therapeutic adjustments in patients with functioning NETs during pregnancy.

Our case also underscores the importance of anticipating the risk of a carcinoid crisis during delivery. Functioning NETs carry a well-recognized risk of hemodynamic instability during anesthesia or high-stress events due to massive amine release (16). Therefore, a preventive octreotide infusion protocol was prepared in advance. Notably, despite the need for vacuum extraction and additional maneuvers for shoulder dystocia, both of which represent significant physical stressors, no carcinoid crisis occurred. Continuous lanreotide therapy throughout the third trimester may have contributed to this biochemical stability, although this remains speculative (17). Such anticipatory peripartum planning represents a critical, yet rarely described, component of management in pregnant patients with carcinoid syndrome.

A particularly distinctive feature of this report is the successful pregnancy following prior PRRT. Data regarding pregnancy outcomes after 177Lu-DOTATATE therapy are extremely limited, although successful pregnancies following PRRT have been previously reported (18,19). Concerns also persist regarding potential adverse effects on the pituitary-gonadal axis due to somatostatin receptor expression (20). While some studies have reported transient changes in gonadotropin levels, recent data have not demonstrated a clear association with clinically significant hypogonadism or subfertility in premenopausal women (21–23). The ability of our patient to conceive spontaneously and maintain a stable G1-pT4N1M1 NET two years after PRRT adds meaningful, real-world reassurance in an area where evidence remains scarce.

Compared with previously published cases of pregnancy in patients with metastatic NETs receiving lanreotide, to our knowledge, this report uniquely combines prior PRRT, active carcinoid syndrome, uninterrupted SSA therapy throughout gestation, and structured peripartum planning for a carcinoid crisis (10–12). These elements provide practical information that extends beyond the demonstration of drug safety and contribute directly to clinical decision-making.

Despite the favorable outcome, this report has limitations, including its single-case nature and the absence of long-term pediatric follow-up. Nevertheless, in rare clinical scenarios where prospective studies are neither ethical nor feasible, well-documented, real-world cases play an essential role in guiding multidisciplinary management.

In conclusion, this case suggests that a pregnancy with favorable maternal and fetal outcomes is possible in selected patients with metastatic functioning NETs following PRRT and during continuous lanreotide therapy. Careful multidisciplinary planning, maintenance of hormonal stability, and cautious interpretation of biochemical markers are important considerations in selected patients managed during pregnancy.

Ethical approval: ethical approval was not required for this case report as the patient provided express written informed consent for the description and publication of the clinical data.

Consent to participate: written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editorial Board of this journal.

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Authors' contributions: Beatriz Tavares da Silva and Ariana Maia contributed to data collection, literature review, manuscript drafting, and manuscript revision. Maria Teresa Pereira, Sara Santos, Susana Garrido, Joana Vilaverde, Marta Sales Moreira, Clara Pinto, Ana Paula Santos, and Jorge Dores contributed to patient management, critical revision of the manuscript, and approval of the final version. Jorge Dores and Ana Paula Santos supervised the study. All authors contributed to the article and approved the submitted version.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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in memoriam

A tribute to Doris Rosenthal, "La grande Dame de la thyroidologie brésilienne" (1932-2026)

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Doris Rosenthal (**Figure 1**) passed away on June 5, 2026. With her passing, Brazil lost not only a distinguished scientist and educator, but also a pioneering woman whose life was inseparably intertwined with the advancement of medical education, endocrinology, and scientific research in the country. She also forged several leaders in the field.

Her presence at scientific conferences, where she would enter the lecture halls eager to follow the latest debates in the field, was always a source of admiration. At the age of 93, at the last International Thyroid Congress, she remained active and continued to inspire those around her (**Figures 2-4**).

Born in Düsseldorf, Germany, on August 23, 1932, Doris experienced the devastating consequences of Nazi persecution in her childhood. The daughter of a Jewish father, Frederick, and a Catholic mother, Maria, she arrived in Brazil with her family in late 1941, where they settled in Rio de Janeiro. Their journey was never easy, and they undoubtedly faced prejudice and hardship. Yet Doris and her family met those challenges with resilience, courage, and dignity, shaping a woman whose unwavering pursuit of knowledge and extraordinary determination became the hallmarks of her life. Her story proves that the struggle for a life of purpose, learning, and humanity was profoundly worthwhile.



Figure 1. Doris Rosenthal at the American Thyroid Association Annual Meeting, New York, 2007.



Figure 2. Doris Rosenthal and Rui Maciel at the International Thyroid Meeting in Rio de Janeiro, 2025.

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Figure 3. In front of the Meeting room, Doris Rosenthal at the International Thyroid Meeting in Rio de Janeiro, 2025.

Doris earned her medical degree from the State University of Rio de Janeiro (UERJ) in 1957. At the time, few women entered higher education, and even fewer pursued careers in medicine. Brazilian universities had been formally opened to women only in 1879, when Emperor Dom Pedro II issued a decree—seventy-one years after the founding of the Rio de Janeiro School of Medicine—allowing them to enroll.

Until then, women's education in Brazil had largely been confined to schools that emphasized social etiquette and proper manners. The pioneering women who entered medicine faced restrictions, prejudice, and countless obstacles from the outset of their careers, and only a small number succeeded in establishing themselves in this traditionally male domain. Doris was undoubtedly one of those pioneers. According to Brazil's 2023 medical demographic data, women were expected to become the majority of physicians in the country by 2024—a prediction that has since become a reality (1). Doris helped pave the way for that transformation.

She began her career as a research fellow in 1961. Between 1966 and 1968, she joined the pioneering group of professors at the University of Brasília's Faculty of Medical Sciences. From 1970 onward, she served as Professor of Endocrine Physiology at the Carlos Chagas Filho Institute of Biophysics at the *Universidade Federal do Rio de Janeiro* (UFRJ), which remained her academic home for more than five decades. Doris also devoted part of her professional life to the *Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione* (IEDE), where she played a key role in establishing translational thyroid research.

In 1975, she earned her doctoral degree in science from UFRJ's Postgraduate Program in Biophysics. In 1978, she completed a postdoctoral fellowship at INSERM in France.



Figure 4. Doris and former students from UFRJ during the International Thyroid Meeting, 2025.

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At the time, postgraduate education in Brazil was still taking shape, and Doris was among the faculty who played an active role in building what would become one of the country's great educational achievements. A central element of this success was the integration of undergraduate teaching, graduate education, and scientific research—a model that took root in Brazil in the 1970s. Throughout her career, she developed biochemical assays to measure thyroid metabolites and investigated defects in thyroid hormone synthesis by chromatographing iodinated amino acids. She also played a significant role in studies of iodine deficiency and endemic goiter in Brazil, participating in projects sponsored by the World Health Organization. In 1975, her work on the dynamics of radioactive iodine uptake in thyroid adenomas earned her the affectionate nickname "Madame Hot Nodules". The nickname remained with her throughout her career and became a symbol of her pioneering contribution to the study of thyroid disease.

As a professor and researcher at the Carlos Chagas Filho Institute of Biophysics at the Federal University of Rio de Janeiro (UFRJ), she led the Endocrine Physiology Laboratory, which now proudly bears her name. At the laboratory, Doris trained and inspired countless students and researchers. Nationally recognized for her exceptional career, she became only the second woman named an Honorary Member of the Brazilian National Academy of Medicine in 2015.

She supervised 27 master's and 13 doctoral students. She published seminal papers in thyroid research and authored 28 book chapters. Her legacy lives on in the generations of physicians, scientists, and educators she trained, inspired, and guided. The list of students she mentored is extraordinary and includes some of the most important leaders in Brazilian endocrinology, such as Egberto Moura, Carmen Pazos de Moura, Denise Carvalho, Belkiss Cardoso, Hans Graf, César Luiz Boguszewski, Gisah Amaral, Tânia Furlanetto, Yara Medeiros, Milena Braga, Marisa Breitenbach, Tamar Frankenfeld, Vânia Costa, Celly Nascimento, Débora Moreira, Michelle Marassi, Monique Monteaux, and Denise Boechat. Many of them were mentored by Doris as undergraduate, master's, and Ph.D. students. As a mentor, she treated every student with fairness, respect, and the utmost seriousness. She taught by example—not only through her scientific rigor but also through her integrity, generosity, and unwavering commitment to education.

A founding member of the Brazilian Society of Physiology, she also served as vice-president and president of the Latin American Thyroid Society. She was an active member of the Brazilian Society of Endocrinology and Metabolism (SBEM), particularly within its Basic Endocrinology and Thyroid Departments—both of which she helped establish and in which she served as vice-director, director, and board member.

Among the many legacies she leaves behind is the creation of the Brazilian Thyroid Meetings (*Encontros Brasileiros de Tireoide* — EBT) with João Romaldini and Rui Maciel. On March 22, 1984, as plans for the first edition of the event were underway, she wrote to Rui Maciel (**Figure 5**). Her words already revealed the special care and attention she devoted to young physicians, besides the integration between clinicians and basic scientists:

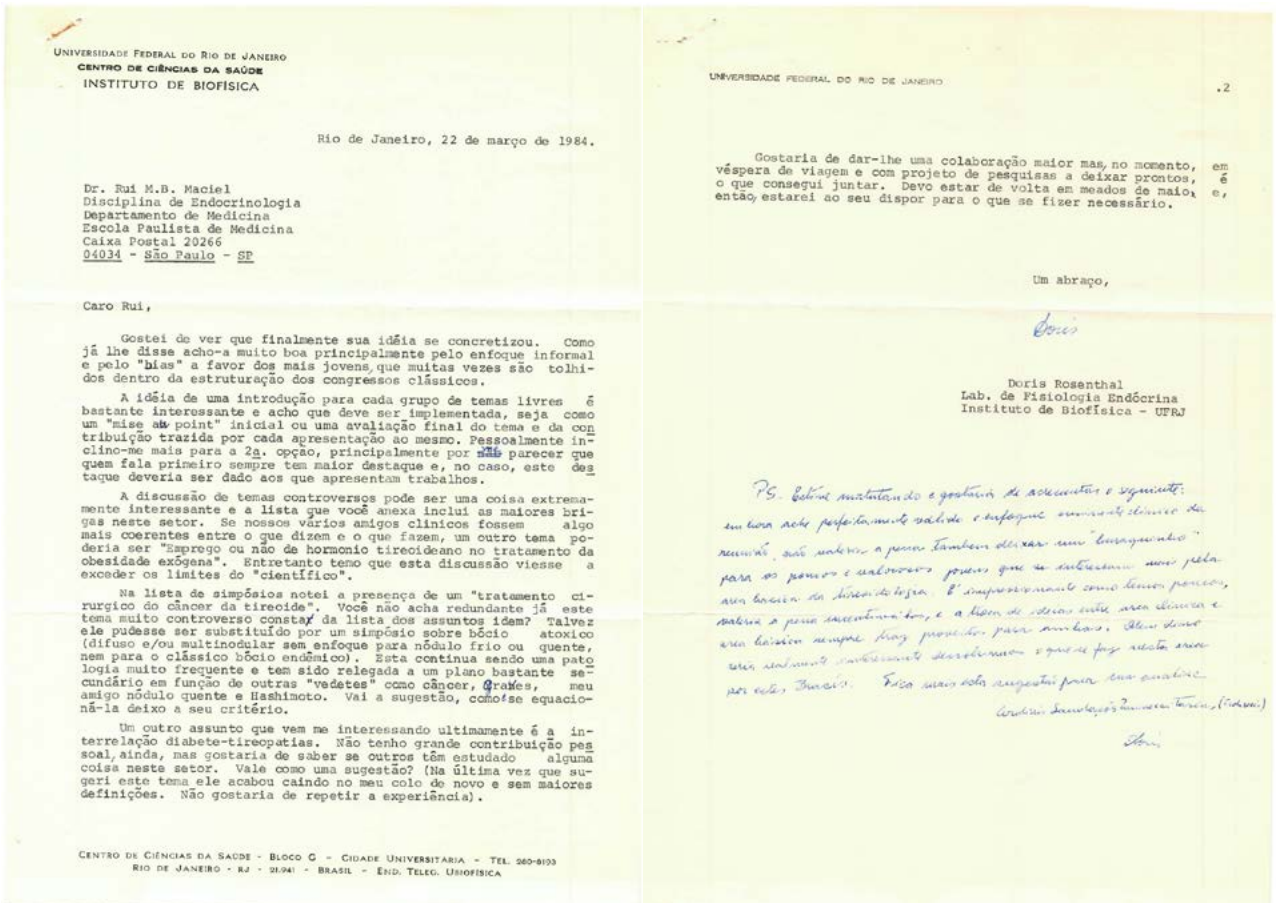
"Dear Rui,

I was delighted to see that your idea has finally become a reality. As I have told you before, I think it is an excellent idea, particularly because of its informal approach and its emphasis on younger participants, who are so often constrained by the structure of traditional conferences.

The idea of an introduction for each group of free-paper presentations is very interesting and, in my opinion, should be implemented—either as an opening overview or as a concluding assessment of the subject and the contributions made by each presentation. Personally, I am more inclined toward the second option, mainly because the person who speaks first always receives greater prominence, and in this case that prominence should belong to those presenting their work."

Her international recognition included membership in the American Thyroid Association and the European Thyroid Association. Throughout her career, she received numerous honors, including the Latin American Thyroid Award, the inaugural "Encontro Brasileiro de Tiroide" Lifetime Award, the José Schermann Award from IEDE, the SBEM-PR Award, and the *Prata da Casa* Award from IBCCF/UFRJ.

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UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
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Rio de Janeiro, 22 de março de 1984.

Dr. Rui M.B. Maciel
Disciplina de Endocrinologia
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Escola Paulista de Medicina
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04034 - São Paulo - SP

Caro Rui,

Gostei de ver que finalmente sua idéia se concretizou. Como já lhe disse acho-a muito boa principalmente pelo enfoque informal e pelo "bias" a favor dos mais jovens, que muitas vezes são tolhidos dentro da estruturação dos congressos clássicos.

A idéia de uma introdução para cada grupo de temas livres é bastante interessante e acho que deve ser implementada, seja como um "mise au point" inicial ou uma avaliação final do tema e da contribuição trazida por cada apresentação ao mesmo. Pessoalmente inclino-me mais para a 2a. opção, principalmente por não parecer que quem fala primeiro sempre tem maior destaque e, no caso, este destaque deveria ser dado aos que apresentam trabalhos.

A discussão de temas controversos pode ser uma coisa extremamente interessante e a lista que você anexa inclui as maiores brigas neste setor. Se nossos vários amigos clínicos fossem algo mais coerentes entre o que dizem e o que fazem, um outro tema poderia ser "Emprego ou não de hormonio tireoideano no tratamento da obesidade exógena". Entretanto temo que esta discussão viesse a exceder os limites do "científico".

Na lista de simpósios notei a presença de um "tratamento cirurgico do câncer da tireoide". Você não acha redundante já este tema muito controverso constar da lista dos assuntos idem? Talvez ele pudesse ser substituído por um simpósio sobre bócio atoxico (difuso e/ou multinodular sem enfoque para nódulo frio ou quente, nem para o clássico bócio endêmico). Esta continua sendo uma patologia muito frequente e tem sido relegada a um plano bastante secundário em função de outras "vedetes" como câncer, Graves, meu amigo nódulo quente e Hashimoto. Vai a sugestão, como se equacioná-la deixo a seu critério.

Um outro assunto que vem me interessando ultimamente é a interrelação diabete-tireopatias. Não tenho grande contribuição pessoal, ainda, mas gostaria de saber se outros têm estudado alguma coisa neste setor. Vale como uma sugestão? (Na última vez que sugeri este tema ele acabou caindo no meu colo de novo e sem maiores definições. Não gostaria de repetir a experiência).

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UNIVERSIDADE FEDERAL DO RIO DE JANEIRO .2

Gostaria de dar-lhe uma colaboração maior mas, no momento, em véspera de viagem e com projeto de pesquisas a deixar prontos, é o que consegui juntar. Devo estar de volta em meados de maio, e, então, estarei ao seu dispor para o que se fizer necessário.

Um abraço,

Doris

Doris Rosenthal
Lab. de Fisiologia Endócrina
Instituto de Biofísica - UFRJ

P.S. Estou mandando e gostaria de acrescentar o seguinte:

Figure 5. Reply to the invitation from Rui Maciel to create the Brazilian Thyroid Meeting, 1984.

In 2023, she received the UFRJ Minerva Medal for Academic Merit, following approval by the university's highest governing body—an honor that fittingly recognized a lifetime devoted to the institution and to Brazilian science.

Professor Doris Rosenthal was part of a generation of faculty who became pillars of higher education in Brazil. Her commitment to science, the creation of knowledge, and the training of young researchers helped establish the full-time academic career model, which proved essential to the country's scientific and technological development.

After her mandatory retirement in 2002, she continued working as a professor and researcher at the Endocrine Physiology Laboratory of the Carlos Chagas Filho Institute of Biophysics at UFRJ. More than a decade ago, the laboratory was named in her honor, following the Institute Council's unanimous approval.

Doris remained active as a collaborating professor and volunteer. Although the COVID-19 pandemic inevitably curtailed her activities, she continued to follow developments in her field and remained intellectually engaged until the very end of her life.

We now bid farewell to an exemplary Brazilian scientist and a remarkable human being. She trained a legion of professionals who continue to share knowledge and uphold the highest standards of excellence across Brazil and abroad. Through them and the institutions she helped shape, her influence will endure.

Doris Rosenthal leaves far more than an extraordinary academic record. She leaves a living legacy of courage, knowledge, perseverance, and devotion—a life dedicated to science and teaching in every sense.

Acknowledgements: the authors are very grateful to several friends who contributed to this work, especially Judy Botler and Cristina Dissat.

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letter to the editor

Methodological considerations in long-term diabetes mortality studies

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Dear Editor,

I read with great interest the article entitled "Death cause and age at death among individuals with diabetes: a 40-year retrospective Brazilian study" published by Lucas Casagrande Passoni Lopes and cols. (1). The temporal breadth of the analysis spanning four decades is noteworthy; furthermore, the use of Cox regression models provides valuable information regarding how causes of death among individuals with diabetes have evolved over time. However, I consider it pertinent to highlight some methodological considerations that may influence the interpretation of the results.

An important aspect to consider is the management of the temporal dimension. Although the study incorporated decade of death as a variable, it would be pertinent to consider the possible changes that occurred throughout the four decades evaluated in diabetes management, treatment availability, and modifications in death registration systems. Patients who lived longer had access to therapeutic advances and improved diagnostic systems, which could influence their prognosis. The omission of these changes could introduce calendar-time bias and distort estimates in longitudinal studies (2).

At a global level, challenges in death certification and registration still persist and may affect the accuracy of epidemiological data (3). In this context, the study relies on death certificates as the primary source for determining the etiological classification of mortality. Although this source is widely used, it may generate classification bias, particularly in chronic diseases such as diabetes mellitus.

It is particularly relevant that authors from the same research group previously reported a high rate of underreporting of diabetes mellitus as a cause of death, reaching up to 64.41%. This situation was observed mainly among patients with type 2 diabetes mellitus and in men (4). This finding is especially important because it reveals a systematic information bias in the death certificates used as a data source. Since these same subgroups showed significant associations in the present study, it is possible that the results were influenced by inaccuracies in the way the etiology of death was recorded. Additionally, the accuracy of a death certificate depends largely on the certifying physician's judgment and interpretation of the causal chain, which becomes particularly complex when patients present with multiple comorbidities (5).

Taken together, these points suggest that future studies could consider analyzing data according to time periods, exploring the impact of potential errors in the registration of causes of death, and incorporating clinical variables that may help improve the interpretation of the results.

Disclosure: no potential conflict of interest relevant to this article was reported.

Data availability statement: the authors are willing to share all data related to the study upon reasonable request.

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letter to the editor

Response to the letter to the editor: Methodological considerations in long-term diabetes mortality studies

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Dear Editor,

We would like to thank Chris Munayco Carhuamaca for her letter entitled "Methodological considerations in long-term diabetes mortality studies" (1) for her careful appreciation of our article "Death cause and age at death among individuals with diabetes: a 40-year retrospective Brazilian study" (2) and for their pertinent considerations presented. We believe that the observations highlighted contribute to deepening the scientific discussion on mortality studies in diabetes mellitus (DM) and offer a valuable opportunity to contextualize our findings.

We agree that analyses spanning for long decades are subject to the influence of substantial changes in the clinical management of DM, the availability of therapies, diagnostic criteria, and health information systems. Such transformations can introduce effects related to the observation period and should be considered in the interpretation of long-term longitudinal studies. As previously discussed, time-related biases represent a relevant methodological challenge in observational studies and can influence epidemiological estimates when important historical changes occur over the course of follow-up (3).

We also acknowledge the inherent limitations of using death certificates as a primary source for determining causes of death. Although they constitute one of the main tools for population-based mortality studies, their quality depends on multiple factors, including the correct identification of the causal chain of events leading to death and the proper completion of the death certificate by the certifying physician. International evidence demonstrates that errors and inconsistencies in the certification of causes of death remain frequent, and may impact the quality of mortality statistics (4). In this context, we agree that the underreporting of DM in death certificates represents a significant limitation. As cited, a previous study by our group identified a high frequency of omission of DM as a cause of death, particularly among individuals with type 2 DM and men (5). This aspect was discussed in the limitations section of the original article, as we recognize its potential to introduce classification bias and influence estimates related to mortality attributed to DM. On the other hand, we understand that these limitations reflect challenges widely recognized in the literature and inherent in the use of secondary mortality databases (5).

We agree that future investigations could benefit from analyses stratified by calendar period, sensitivity assessments for potential misclassification errors, and the incorporation of additional clinical information when available. We thank the author again for

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the constructive contribution and the opportunity to broaden the debate on the interpretation of mortality studies in DM.

Disclosure: no potential conflict of interest relevant to this article was reported.

Data availability: datasets related to this article will be available upon request to the corresponding author.

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letter to the editor

Sociodemographic considerations regarding the European Portuguese Validation of the Diabetes Distress Scale (DDS-17)

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Dear Editor,

The recent original article by Melo and cols. (1) successfully validated the Diabetes Distress Scale (DDS-17) for its implementation in the European Portuguese population. The introduction of this tool is of high clinical relevance, given that diabetes distress constitutes an affective dimension distinct from major depression and exerts a direct influence on therapeutic adherence and glycemic control. However, we consider that the sociodemographic characteristics of the evaluated cohort impose critical limitations that demand a cautious interpretation of the reported low levels of global distress.

In their study cohort, the mean age was 69.55 ± 12.55 years, and more than half of the participants (53.0%) had four years or less of education. On one hand, Almeida and cols. (2) demonstrated that perceived stress decreases in older adulthood due to reduced exposure to occupational stressors and lower reactivity to daily life conflicts. On the other hand, a low educational level is a well-known biasing factor in the use of self-report instruments. Data from the *All of Us* study (3) evidence that individuals with less than a high school education present an 11.6% rate of non-response or misunderstanding in stress scales, in contrast to the 5.3% observed in college graduates. Consequently, the reported low scores might not reflect a genuine absence of the condition, but rather marked differences in reading comprehension or cognitive fatigue within the sample.

Therefore, it would be pertinent to ask Melo and cols. whether a subgroup analysis regarding age and educational level was performed to clarify the variability of distress obtained in the study. In conclusion, we suggest that the clinical implementation of the DDS-17 in Portugal should rigorously account for these sociodemographic variables to mitigate the risk of false negatives. Furthermore, it would be advisable to complement questionnaire administration with strategies that ensure the correct interpretation of the items, such as assisted reading and a brief verification of question comprehension. Finally, we congratulate the authors for this valuable methodological contribution, which will undoubtedly optimize comprehensive disease management.

STATEMENT OF USE OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the process of preparation of this article, the author used Gemini (Google) for text structuring, stylistic refinement, and translation. After using this tool/service, the author reviewed and edited the content as necessary and assumes full responsibility for the content of the publication.

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Data availability: datasets related to this article will be available upon request to the corresponding author.

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letter to the editor

Response to the letter to the editor: Sociodemographic considerations regarding the European Portuguese Validation of the Diabetes Distress Scale (DDS-17)

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Dear Editor,

We would like to express our gratitude for the insightful and pertinent comments regarding our article "European Portuguese Validation of the Diabetes Distress Scale (DDS-17)"(1). The questions raised concerning the sociodemographic characteristics of our sample, specifically age and educational level, significantly enrich the discussion on the clinical applicability and robustness of this scale within the Portuguese population (2).

Regarding the impact of age on diabetes distress, we acknowledge that literature, such as the study by Almeida et al. cited by the commentators, suggests a reduction in overall perceived stress in older adulthood (3). However, it is crucial to distinguish general life stress from diabetes distress, which measures the distinct emotional burden inherently tied to managing a chronic condition. Furthermore, the mean age of our cohort (69.55 ± 12.55 years) is not an artifact of selection bias, but rather a faithful reflection of the epidemiological reality of Type 2 Diabetes (T2D) in Portugal, where the highest prevalence of the disease is concentrated precisely within the 60 to 79 age group (4). In these older patients, diabetes management often coexists with multiple comorbidities and age-related functional decline, which may, conversely, exacerbate disease-specific distress. Therefore, while the low global scores in our sample warrant cautious interpretation, they represent the actual clinical presentation of the target population.

In relation to educational attainment, the high prevalence of low formal education (53.0% with four years or less) also accurately reflects the historical and socio-educational context of this specific demographic in Portugal. For generations born until 1966, mandatory school attendance was legally limited to

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just four years. Consequently, any representative sample of older Portuguese T2D patients will naturally exhibit this characteristic. To actively mitigate the risks of non-response, reading comprehension difficulties, or cognitive fatigue highlighted by the commentators, the administration of the questionnaire in our study included dedicated assistance from healthcare professionals. They provided assisted reading and clarified individual items whenever a need was identified. This methodological safeguard minimized literacy barriers, ensuring that the scores reflected the patients' genuine emotional experiences rather than comprehension errors. The strong internal consistency and construct validity demonstrated in our paper further corroborate that the scale remained psychometrically robust within this population.

Finally, addressing the inquiry regarding subgroup analyses, this specific evaluation was not performed in the original article, as it fell outside the scope of this baseline psychometric validation study. However, we are pleased to share that this exact line of investigation is already underway. A follow-up study with a considerably larger and more diverse sample is currently being conducted, which will provide the statistical power necessary to thoroughly clarify the variability of distress across different age groups and educational levels, as well as to investigate the risk of false negatives.

We entirely endorse the commentators' recommendation that the clinical implementation of the DDS-17 in Portugal should be coupled with assisted reading strategies in populations with lower literacy, a practice we proactively adopted in our study design. We thank the commentators once again for their valuable contribution, which reinforces the importance of continuous debate to optimize comprehensive diabetes care.

Disclosure: no potential conflict of interest relevant to this article was reported.

Data availability: datasets related to this article will be available upon request to the corresponding author.

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erratum

In the article **"Dietary pattern and night work: metabolic syndrome in healthcare workers"**, published in Archives of Endocrinology and Metabolism, DOI: 10.20945/2359-4292-2026-0047:

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The authors reported that the names of Camila Pereira Baldin and Robson Martins Pereira were inadvertently omitted from the author list during the production process. This correction refers exclusively to the authorship information and does not affect the scientific content, results, analyses, or conclusions of the study.

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erratum

ERRATUM: Role of insulin-regulated aminopeptidase as potential biomarker in insulin resistant polycystic ovary syndrome patients

In the article: Köse O, Gök K, Köse E, Gözükara SI, Tüten A, Bostancı MS. **Role of insulin-regulated aminopeptidase as potential biomarker in insulin resistant polycystic ovary syndrome patients.** Arch Endocrinol Metab. 2026;70(2):e260011. doi: 10.20945/2359-4292-2026-0011.

Page 3, subtitle: Laboratory studies

Where it reads:

The blood samples taken with the second tube blood sample were centrifuged at 4,000 rpm for 10 minutes, then the serums were separated and put into a deep freezer to be stored at -80°C until the day of analysis. On the study day, all samples were thawed in the same month and Human ICE protease-activating factor (IRAP) levels were measured using ELISA kits (MyBioSource, San Diego, USA, Catalog no: MBS260922). The results were calculated using the Biotek ELX800 (USA) ELISA reader, while the intra-measurement CV was reported as < 8% and the inter-measurement coefficient of variation as < 12%.

Read:

The blood samples taken with the second tube blood sample were centrifuged at 4,000 rpm for 10 minutes, then the serums were separated and put into a deep freezer to be stored at -80°C until the day of analysis. On the study day, all samples were thawed simultaneously and Human Leucyl-cystinyl Aminopeptidase (LNPEP) levels were measured using ELISA kits (BT LAB, Zhejiang, China; Catalog no: E74293Hu). Absorbance values were measured at 450 nm using a Biotek ELX800 microplate reader (BioTek Instruments, USA), and concentrations were calculated from the standard curve according to the manufacturer's instructions. The intra-assay coefficient of variation was <8% and the inter-assay coefficient of variation was <10%, as reported by the manufacturer.

The correction refers exclusively to the description of the assay kit used in the Methods section and does not affect the results, analyses, or conclusions of the study.

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