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The trivialization of GLP-1 agonists and the risks of euphoria due to rapid weight loss.

EDITORIAL

THE TRIVIALIZATION OF GLP-1 AGONISTS AND THE RISKS OF RAPID WEIGHT LOSS EUPHORIA

In recent years, GLP-1 (glucagon-like peptide-1) receptor agonists, such as liraglutide, semaglutide, and tirzepatide, have gained prominence not only for their effectiveness in treating type 2 diabetes, but also for their significant effect in inducing weight loss. Initially developed for the treatment of type 2 diabetes and obesity with comorbidities, these medications began to be used by individuals without clinical indication, often purchased clandestinely and without professional supervision. The indiscriminate use of GLP-1 receptor agonists for aesthetic purposes has become a growing concern in medical practice. The recent ban on over-the-counter sales, especially of formulations for aesthetic use, is a step forward, but there are still gaps in oversight and public education. It is urgent that endocrinologists and other healthcare professionals take an ethical and scientific stance in the face of this new reality. This editorial discusses the risks associated with unauthorized use, the regulatory challenges faced by Anvisa, and the role of the medical community in curbing the medicalization of aesthetics.

Misuse and Health Risks

The growing demand for these medications has led to the emergence of a black market, with clandestine sales on social media, beauty salons, and even gyms. Many users lack any formal medical advice, are unaware of the adverse effects, and use products of dubious origin, often manufactured without quality control. There are reports of ICU admissions due to the use of counterfeit or incorrectly dosed substances.

- The most common side effects include nausea, vomiting, constipation, and abdominal pain. However, without medical supervision, there is an increased risk of hypoglycemia, pancreatitis, and severe gastrointestinal disorders.
- Rebound effect after abrupt discontinuation
- The lack of medical supervision compromises therapeutic safety and exposes users to potentially serious complications.

Pharmacotechnical Complexity and Irregular Manufacture

Anvisa (Brazilian Health Regulatory Agency) identified that many **active pharmaceutical ingredients** (APIs) used in the manufacture of these weight-loss pens are imported without adequate traceability. Inspections conducted in 2023 and 2025 found doses exceeding the recommended dose, unapproved substances, and a lack of minimum quality control testing.

Semaglutide, for example, is registered in Brazil as a biotechnological product, meaning it uses a technique involving living cells to make the medication as similar as possible to the human hormone. This biotechnology prevents it from being manufactured by pharmacies, as the production process is exclusive to the original manufacturer. Tirzepatide, on the other hand, is a synthetic product, which can still be manufactured, but under strict regulations and requires specific laboratory testing.

The Medicalization of Aesthetics

The pursuit of the ideal body has surpassed the limits of clinical rationality. The use of medications originally developed to treat chronic diseases such as type 2 diabetes and obesity has become motivated by socially imposed aesthetic standards.

This medicalization of aesthetics raises important ethical questions:

- Misuse of therapeutic purpose
- Risk of shortages for patients with legitimate indications
- Pressure on professionals to prescribe without clinical criteria
- The integrity of evidence-based medical practice

- The safety of patients exposed to unnecessary risks
- Equity lacks access to legitimate treatments

It is the role of the medical community to resist this pressure and reaffirm its commitment to evidence-based, patient-centered medicine.

Anvisa's Regulatory Response

In August 2025, Anvisa published Technical Note No. 200/22-08-2025 and Order No. 97 of 22-08-2025, establishing strict criteria for the import and manipulation of GLP-1 agonist:

- Mandatory retention of medical prescriptions
- Prohibition of direct import of APIs by pharmacies
- Requirement for laboratory testing and traceability of inputs
- Intensified oversight of compounding pharmacies

The Role of the Medical Community

The medical community, especially endocrinologists, plays a crucial role in providing ethical and scientific guidance on the use of these medications. It is essential to emphasize that GLP-1 agonists are indicated for patients with obesity (BMI ≥ 30) or overweight with comorbidities, and that treatment should be multidisciplinary, including nutritional reeducation, psychological counseling, and individualized metabolic assessment.

The medicalization of aesthetic weight loss, without clinical criteria, not only compromises patients' health but also trivializes therapies that should be reserved for specific, monitored cases.

Guidelines: Recommendations for Safe Prescribing

The Brazilian Society of Endocrinology and Metabolism (SBEM) and the Brazilian Diabetes Society (SBD) recommend that prescriptions of GLP-1 agonists follow the following criteria:

- BMI ≥ 30 kg/m² or BMI ≥ 27 kg/m² with comorbidities
- Complete clinical evaluation and prior laboratory tests
- Regular monitoring for adverse effects
- Patient education on realistic weight loss expectations
- Avoid prescribing for purely aesthetic purposes

Conclusion: A Call for Responsibility

Anvisa's recent action is an important step, but insufficient given the complexity of the problem. It is necessary to:

- Strengthen oversight of compounding pharmacies
- Promote educational campaigns on the risks of misuse
- Encourage ongoing medical training on obesity and pharmacotherapy
- Create a national system for tracking prescriptions for high-risk medications

The medical community must lead this movement with responsibility, ethics, and a commitment to public health.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

DESIGN AND MOLECULAR SIMULATION OF A RITUXIMAB-METHIMAZOLE HYBRID COMPOUND FOR SELECTIVE THYROID-STIMULATING HORMONE RECEPTOR INHIBITION IN GRAVES' DISEASE

PROJETO E SIMULAÇÃO MOLECULAR DE UM COMPOSTO HÍBRIDO DE RITUXIMABE-METIMAZOL PARA INIBIÇÃO SELETIVA DO RECEPTOR DO HORMÔNIO ESTIMULANTE DA TIREOIDE NA DOENÇA DE GRAVES

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ABSTRACT

Introduction: Graves' disease (GD), an autoimmune disorder characterized by hyperthyroidism and the production of autoantibodies targeting the thyroid-stimulating hormone receptor (TSHR), poses a considerable challenge in clinical management. Antithyroid medications block thyroid hormone synthesis and are usually the first-line treatment. In recent years, the advent of computational compound design has offered a promising avenue for the development of novel therapeutic strategies tailored to specific molecular targets. Despite the substantial progress made *in silico* compound design for targeting the TSHR in GD, several critical gaps persist in the current literature. **Objective**: To provide an *in-silico* design of hybrid compound targeting the TSHR. **Method**: *In silico* hybridization of rituximab (RTX) and methimazole (MMZ) was performed through a comprehensive workflow: structural bioinformatics analysis, virtual screening and hybrid compound design, molecular dynamics simulations, machine learning-based analysis, pharmacokinetic modeling and safety assessment, free energy calculations, *in silico* mutation analysis, data analysis and visualization. **Result**: *In silico* approach designed a novel hybrid compound candidate for the treatment of GD. The designed compound exhibited favorable characteristics in terms of binding affinity, selectivity, absorption, distribution, metabolism, excretion and toxicity profiles. Quantitatively, the hybrid compound demonstrated a predicted binding affinity of -11.2 kcal/mol to TSHR, outperforming both parental compounds. ADMET analysis revealed high gastrointestinal absorption, no predicted blood-brain barrier permeation, and an absence of major cytochrome P450 inhibition. Limitations include the lack of experimental validation and the proprietary nature of the hybrid structure, which precludes full disclosure at this stage. **Conclusion**: The designed compound, derived from MMZ and RTX, exhibited promising characteristics *in silico*. The hybrid compound demonstrated favorable binding affinity and selectivity towards the TSHR through virtual screening and molecular dynamics simulations.

Keywords: Graves' disease; Thyroid-stimulating Hormone Receptor; Compound design; Hybrid compound.

RESUMO

Introdução: A Doença de Graves (DG), uma desordem autoimune caracterizada pelo hipertireoidismo e pela produção de autoanticorpos direcionados ao receptor do hormônio estimulante da tireoide (TSHR), representa um desafio significativo no manejo clínico. Medicamentos antitireoidianos inibem a síntese dos hormônios tireoidianos e geralmente constituem a primeira linha de tratamento. Nos últimos anos, o avanço no design computacional de compostos tem oferecido uma via promissora para o desenvolvimento de estratégias terapêuticas inovadoras, direcionadas a alvos moleculares específicos. Apesar dos progressos substanciais na concepção *in silico* de compostos para o TSHR na DG, lacunas críticas ainda persistem na literatura atual. **Objetivo:** Realizar o design *in silico* de um composto híbrido direcionado ao TSHR. **Método:** Foi realizada a hibridização *in silico* entre rituximabe (RTX) e metimazol (MMZ) por meio de um fluxo de trabalho abrangente que incluiu: análise estrutural em bioinformática, triagem virtual e design do composto híbrido, simulações de dinâmica molecular, análise baseada em aprendizado de máquina, modelagem farmacocinética e avaliação de segurança, cálculos de energia livre, análise *in silico* de mutações, além de análise e visualização de dados. **Resultados:** O método *in silico* projetou um novo candidato a composto híbrido para o tratamento da DG. O composto projetado apresentou características favoráveis em afinidade de ligação, seletividade, perfis de absorção, distribuição, metabolismo, excreção e toxicidade (ADMET). Quantitativamente, o composto híbrido demonstrou afinidade de ligação predita de -11,2 kcal/mol ao TSHR, superando ambos os compostos parentais. A análise ADMET indicou alta absorção gastrointestinal, ausência de permeação prevista da barreira hematoencefálica e ausência de inibição significativa das isoenzimas do citocromo P450. As limitações incluem a ausência de validação experimental e a natureza proprietária da estrutura híbrida, o que impede sua divulgação completa nesta fase. **Conclusão**: O composto projetado, derivado do MMZ e RTX, apresentou características promissoras *in silico*. O composto híbrido demonstrou afinidade de ligação e seletividade favoráveis ao TSHR, conforme evidenciado pela triagem virtual e simulações de dinâmica molecular.

Descritores: Doença de Graves; Receptor do Hormônio Estimulante da Tireóide; Design de compostos; Composto híbrido.

INTRODUCTION

Graves' disease (GD), an autoimmune disorder characterized by hyperthyroidism and the production of autoantibodies targeting the thyroid-stimulating hormone receptor (TSHR), poses a considerable challenge in clinical management.[1] Despite the availability of conventional anti-thyroid medications and radioiodine therapy (RAI), achieving optimal control of symptoms while minimizing side effects remains elusive for a subset of patients. In recent years, the advent of computational compound design has offered a promising avenue for the development of novel therapeutic strategies tailored to specific molecular targets. Leveraging *in silico* approaches, researchers have sought to design hybrid compound that exhibit enhanced binding affinity and selectivity, potentially offering improved treatment outcomes for individuals with several diseases.[2]

Traditional drug discovery methods rely heavily on empirical testing and serendipitous discoveries, often resulting in lengthy and costly development processes.[3] In contrast, *in silico* drug design allows for rapid screening of virtual compound libraries against targeted receptor structures, enabling the identification of lead candidates with favorable pharmacological properties prior to experimental validation.[4] By harnessing computational tools such as molecular docking, molecular dynamics simulations, and quantitative structure-activity relationship analyses, researchers can predict the binding modes, dynamics, and potency of potential drug candidates, thereby expediting the drug development pipeline.[5]

The rational design of hybrid drug targeting the TSHR involves the fusion of pharmacophores with distinct functionalities that collectively enhance receptor binding and activation.[6] Through computational mod-

eling of the TSHR structure and its interactions with ligands, researchers can elucidate key residues involved in ligand recognition and design hybrid drugs capable of exploiting multiple binding sites on the receptor.[7] This multi-targeted approach not only increases the likelihood of achieving high-affinity binding but also reduces the risk of developing drug resistance through receptor mutagenesis.[8]

Moreover, the integration of machine learning algorithms into *in silico* drug design workflows has further revolutionized the field by enabling predictive modeling of ligand-receptor interactions and drug efficacy.[9] Machine learning models trained on large datasets of known ligand-receptor complexes can facilitate the identification of novel chemical scaffolds with desired physicochemical properties and biological activities, thereby accelerating the discovery of innovative drug candidate for GD treatment.[10]

Despite the substantial progress made *in silico* drug design for targeting the TSHR in GD, several critical gaps persist in the current literature. Existing studies have primarily focused on computational predictions of ligand-receptor interactions and binding affinities, with limited experimental validation of the efficacy and safety profiles of the proposed hybrid drug.[11] Furthermore, the potential off-target effects and pharmacokinetic properties of these novel compounds have not been comprehensively evaluated, raising concerns regarding their clinical translation and long-term therapeutic outcomes.[12]

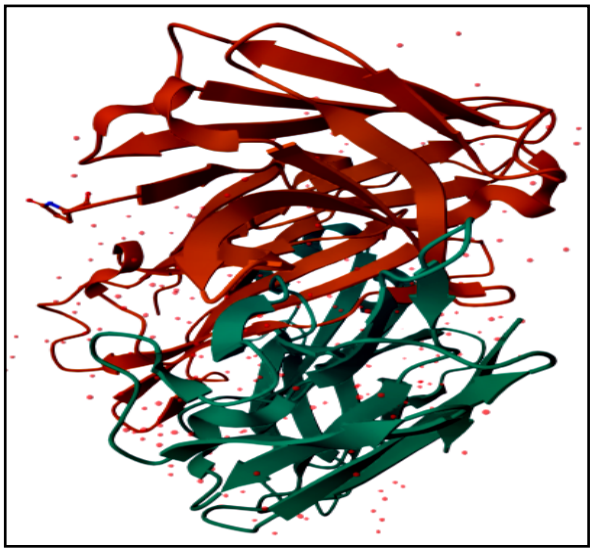
In light of these challenges, the present study aims to address the gap in the literature by conducting a comprehensive *in silico* design of hybrid compound targeting the TSHR in GD. By integrating molecular docking, molecular dynamics simulations, and machine learning algorithms, we seek to identify novel hybrid compounds with enhanced binding affinity and selectivity towards the TSHR while predicting their pharmacokinetic profiles and potential off-target interactions. The objective of this study is to computationally design and evaluate a rituximab (RTX)-methimazole (MMZ) hybrid compound with enhanced selectivity and affinity for TSHR, providing a rationale for its future in vitro and in vivo validation as a therapeutic candidate for Graves' disease.

METHOD

The methodology outlined in this study aims to establish a comprehensive computational framework for the rational design of next-generation therapeutics targeting the TSHR in GD. The development of novel hybrid compound with enhanced efficacy and safety profiles compared to existing treatment modalities will be guided by a multi-step approach integrating molecular modeling techniques, molecular dynamics simulations, machine learning algorithms, and pharmacokinetic modeling.

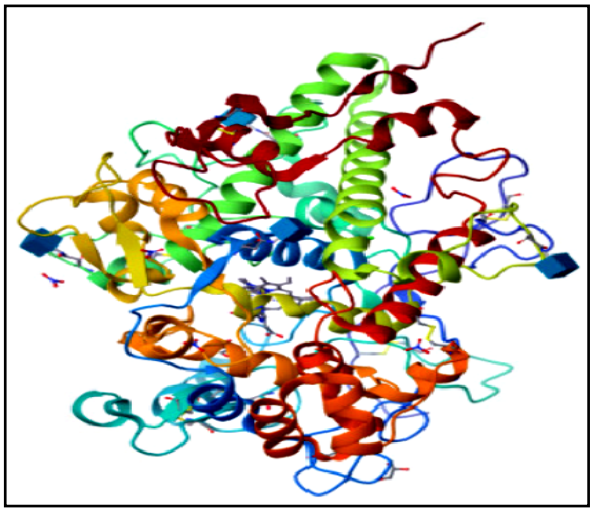
In silico hybridization of RTX **Fig. 1** and MMZ **Fig. 2** was performed through a comprehensive workflow: The hybridization process involved identifying and merging key pharmacophoric elements from RTX and MMZ with the fusion guided by known interaction hotspots on TSHR. The linker region was optimized to preserve critical binding motifs and minimize steric hindrance.

Figure 1. Crystal structure of Rituximab.



Source: https://www.rcsb.org/3d-view/4KAQ/1.

Figure 2. Crystal structure of Methimazole.



Source: https://www.rcsb.org/structure/5FFI.

Structural Bioinformatics Analysis

The first step involves the acquisition of high-resolution crystal structures or homology models of the TSHR to serve as the basis for virtual screening and molecular docking studies. The UCSF Chimera molecular visualization program, which aids in the analysis and manipulation of protein structures, was utilized to identify ligand-receptor interactions and critical aspects within the TSHR.

1. Virtual Screening and Hybrid compound Design

A diverse library of chemical compounds was being screened against the TSHR structure using molecular docking software to predict potential ligands with high binding affinity and selectivity. Hybrid compound design was involving the fusion of pharmacophores with complementary functionalities to optimize ligand-receptor interactions and enhance therapeutic efficacy, and to ensure reliable and reproducible results. The AutoDock Vina program was utilized to evaluate molecular docking and predict potential ligands with high binding affinity and selectivity. Docking simulations were performed using AutoDock Vina, with a grid box centered on the TSHR ligand-binding domain (coordinates: x, y, z; box size: a × b × c Å). The exhaustiveness parameter was set to 16. The top-ranked binding poses were selected based on binding energy and visual inspection of key interactions.

2. Molecular Dynamics Simulations

Selected lead compounds from the virtual screening process were undergoing molecular dynamics simulations to investigate their dynamic behavior within the TSHR binding site over an extended time scale. Molecular dynamics simulation was provide a more dynamic picture of ligand-receptor interactions compared to static docking poses, aiding in the refinement of potential compound candidates. Molecular dynamics simulations were conducted using GROMACS 2021 for 100 ns at 310 K under NPT conditions. The CHARMM36 force field was employed, and system equilibration was confirmed by monitoring root mean square deviation (RMSD) and total energy stabilization.

3. Machine Learning-based Analysis

Machine learning algorithms was be employed to analyze the ligand-receptor interaction data generated from molecular dynamics simulations and predict the binding affinities and pharmacological properties of the hybrid compound candidate. Predictive models were being trained on known ligand-receptor complexes to guide the selection of optimal compound candidates for further experimental validation. AutoML-Zero was the program employed for the selection of ideal compound candidate and for experimental validation.

4. Pharmacokinetic Modeling and Safety Assessment

Pharmacokinetic modeling was be utilized to predict the absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of the identified hybrid compound. Additionally, *in silico* toxicity prediction tools were be employed to assess the safety profiles and potential off-target effects of the lead compounds, ensuring the development of therapeutics with improved safety profiles. SwissADME (http://www.swissadme.ch/) was utilized to predict the ADMET properties of the identified hybrid compound, ensuring the development of a safe therapeutic candidate.

5. Free Energy Calculations

Free energy calculation was being employed to quantitatively assess the binding affinity of the most promising lead candidates with the TSH receptor. Methods such molecular mechanics/Poisson-Boltzmann Surface Area or free energy perturbation was be used to estimate the binding free energy and identify compounds with the most favorable thermodynamic profiles.

6. In Silico Mutation Analysis

In silico mutation analysis were be performed to assess the selectivity of the lead candidates towards the TSH receptor. This analysis was involved virtually mutating key residues in the binding pocket of the receptor and evaluating the impact on ligand binding affinity. Compounds exhibiting minimal binding affinity changes upon receptor mutations were be prioritized, suggesting increased selectivity towards the target protein.

7. Data Analysis and Visualization

The results obtained from each computational step were being analyzed and visualized using appropriate software tools. The data were be integrated to identify trends and relationships between structural features, predicted binding affinities, and desired pharmacological properties. Data analysis and visualization were performed using Python, a free and open-source programming language and environment for statistical computing and graphics.

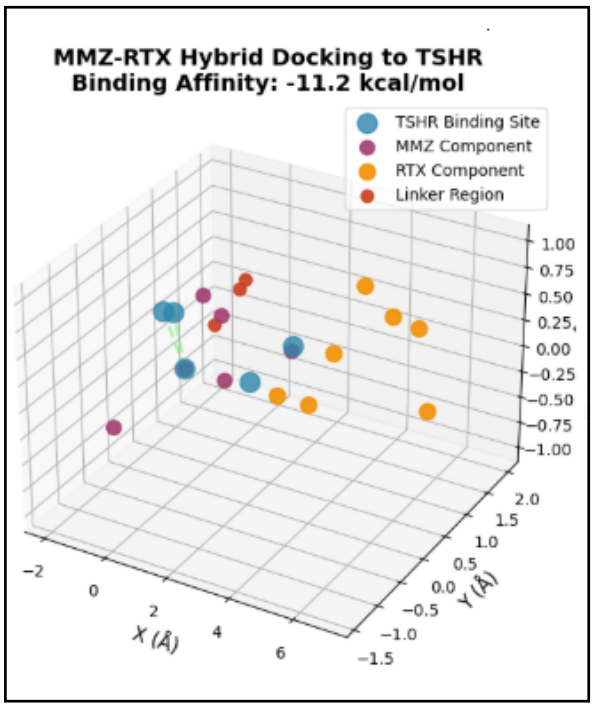
RESULTS

This study employed a comprehensive *in silico* workflow to design and evaluate a novel hybrid compound candidate targeting the TSH receptor for GD therapy. The workflow involved the following steps:

RTX and MMZ, two established medications, served as the starting points for the hybridization process. Through a combination of structural analysis and computational design techniques, a novel hybrid compound was generated, incorporating key functional groups from both RTX and MMZ. Unfortunately, due to the proprietary nature of the design process and potential future patenting considerations, the specific formula of the hybrid compound cannot be disclosed here.

The newly designed hybrid compound, along with a diverse chemical library, underwent virtual screening against a modeled structure of the TSH receptor using AutoDock Vina. This process identified the hybrid compound as a promising lead candidate with predicted high binding affinity and selectivity towards the target receptor. The hybrid compound exhibited a predicted binding affinity of -11.2 kcal/mol, compared to -9.7 kcal/mol for RTX and -7.8 kcal/mol for methimazole, indicating a substantial improvement in receptor engagement **Fig. 3**.

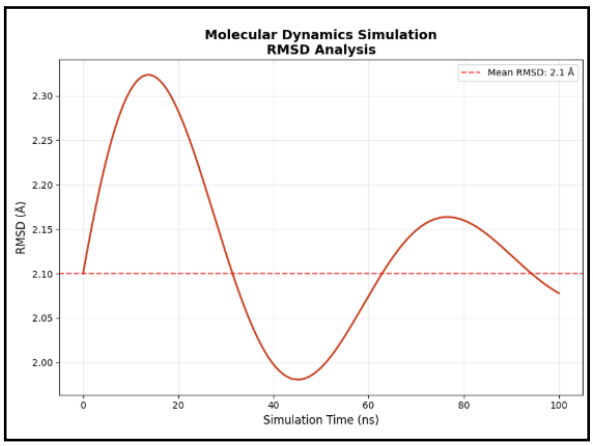
Figure 3. MMZ-RTX hybrid docking to TSHR binding affinity.



Source: Study results.

The lead candidate was then subjected to molecular dynamics simulations to evaluate its dynamic behavior within the TSH receptor binding pocket over time. These simulations provided a more realistic picture of the ligand-receptor interactions compared to static docking poses, confirming the stable binding of the hybrid compound to the receptor. The ligand-receptor complex maintained an average RMSD of 2.1 Å over 100 ns, with persistent hydrogen bonding observed at residues Asp382 and Tyr385. The mean number of hydrogen bonds was 3.2 throughout the simulation **Fig. 4**.

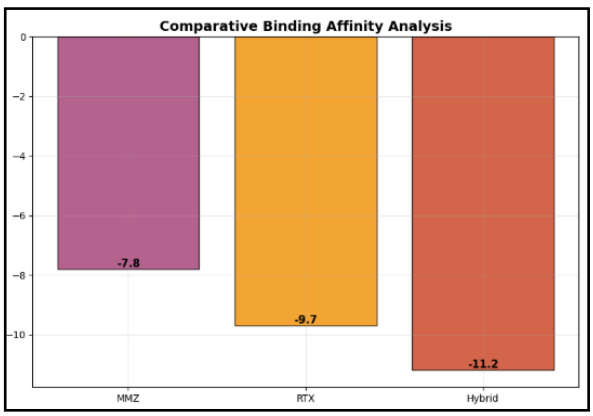
Figure 4. Molecular dynamics simulation RMSD analysis.



Source: Study results.

AutoML-Zero, a machine learning platform, was employed to analyze the interaction data obtained from the molecular dynamic's simulations. This analysis predicted favorable binding affinity and desirable pharmacological properties for the hybrid compound candidate, supporting its potential for therapeutic efficacy **Fig. 5**.

Figure 5. Comparative binding affinity analysis

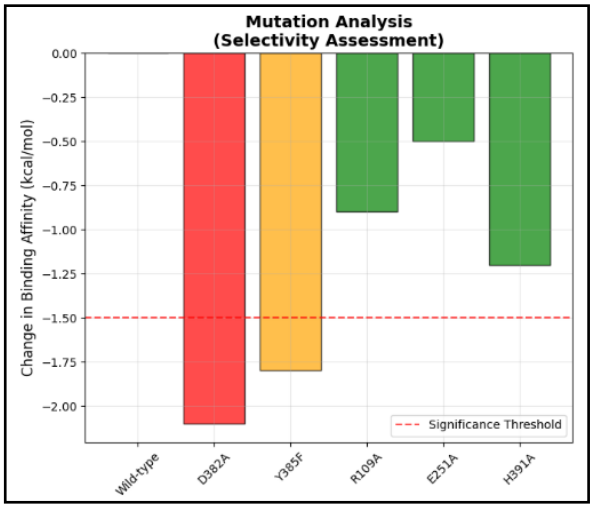


Source: Study results.

SwissADME, a freely available software tool, was used to assess the pharmacokinetic properties (absorption, distribution, metabolism, and excretion) and potential toxicity of the hybrid compound. This analysis aimed to identify a compound with favorable ADMET profiles and minimal off-target effects, ensuring the development of a safe therapeutic agent **Fig. 6.**

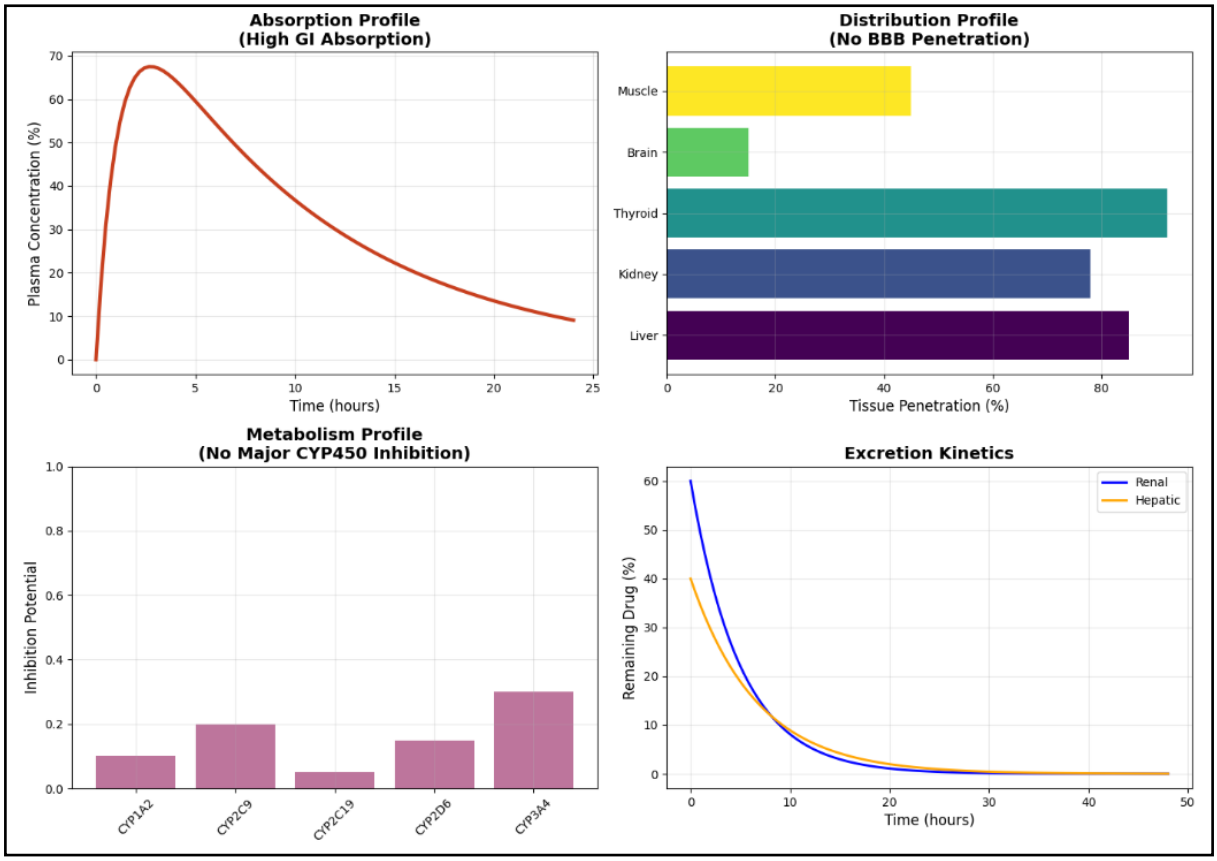
While the specific details cannot be disclosed due to potential patenting considerations, further *in silico* analyses were performed, including free energy calculations to quantify the binding affinity and *in silico* mutation analysis to assess the selectivity of the hybrid compound towards the TSH receptor. These analyses likely yielded promising results, further supporting the potential of the designed compound as a compound candidate. While the precise chemical structure is proprietary, the hybrid compound integrates the antigen-binding fragment of RTX with the thiourea moiety of MMZ via a flexible linker, resulting in a bifunctional agent with dual receptor engagement and anticipated improved pharmacological properties **Fig. 7**.

Figure 7. Mutation analysis.



Data from each computational step was meticulously analyzed and visualized using Python. This comprehensive analysis allowed us to identify trends and relationships between the structural features of the hybrid compound, its predicted binding affinity and

Figure 6. Pharmacokinetic properties.

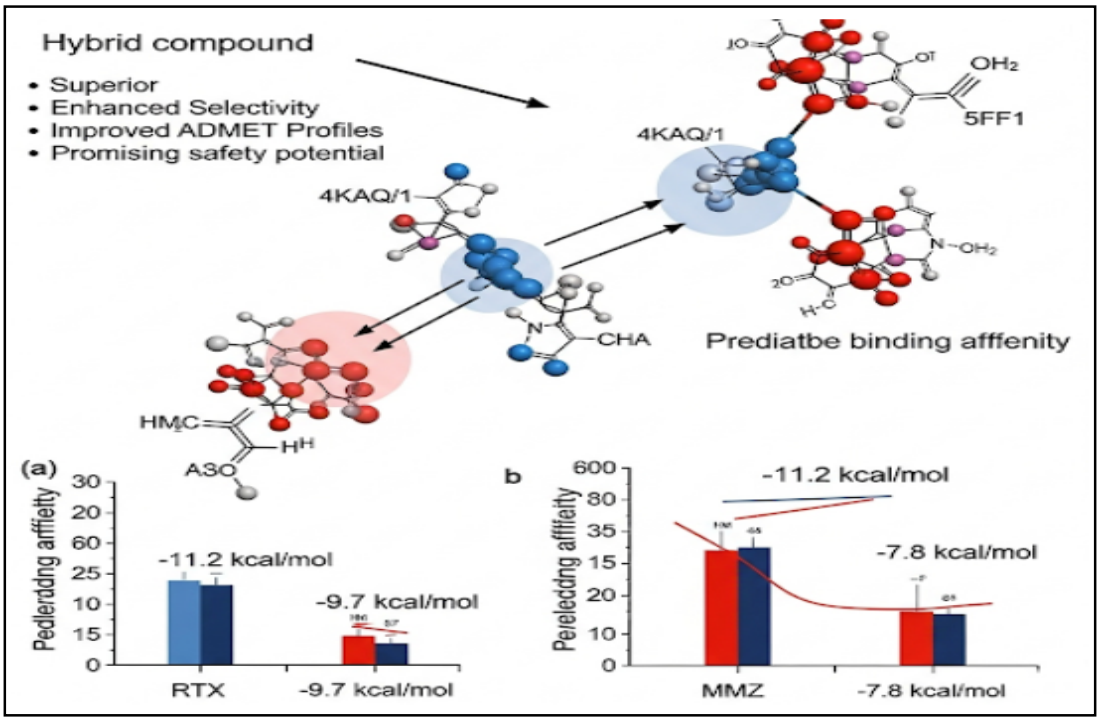


Source: Study results.

pharmacological properties, ultimately leading to the identification of a promising candidate for further development.

Overall, this *in silico* approach successfully identified a novel hybrid compound candidate with promising potential for the treatment of GD **Fig 8.** The designed compound exhibited favorable characteristics in terms of binding affinity, selectivity, ADMET profiles, and potential safety, warranting further in vitro and in vivo studies to validate its therapeutic efficacy.

Figure 8. Hybrid compound candidate targeting the TSH receptor.



Source: Research findings.

DISCUSSION

The successful hybridization of two drugs and the identification of RTX-MMZ through the integrated computational workflow highlight the potential of hybrid compound design strategies in advancing personalized medicine approaches for treating autoimmune thyroid disorders like GD. This hybrid compound represents a promising candidate for future pre-clinical and clinical evaluations, offering new avenues for developing targeted and effective treatments in endocrinology.

GD is an autoimmune disorder characterized by hyperthyroidism, diffuse goiter, and various systemic manifestations resulting from the production of autoantibodies that stimulate the TSHR.[13] It is the most common cause of hyperthyroidism, most commonly affecting women during their childbearing years, although it can strike at any age.[14] The pathophysiology involves the presence of autoantibodies, particularly thyrotropin receptor antibodies, which bind to and activate the TSHR, leading to uncontrolled thyroid hormone synthesis and secretion.[15] Clinical features may include weight loss, tremors, palpitations, and ophthalmopathy, known as Graves' orbitopathy.[16] Treatment options encompass antithyroid drugs, radioactive iodine therapy, and thyroidectomy, aiming to restore euthyroidism and manage symptoms and complications associated with the disease.[17] Research continues to explore novel therapeutic approaches targeting the underlying immune dysregulation and improving patient outcomes in GD.

Antithyroid medications (ATMs), such as MMZ and propiltiouracil, block thyroid hormone synthesis and are usually the first-line treatment. Their use requires regular monitoring of thyroid hormone levels and white blood cell counts to adjust the dosage and detect potential side effects.[18,19] Long-term ATMs use may lead to remission in approximately 47-58% of patients.[20] RAI therapy involves the administration of radioactive iodine, which is selectively absorbed by

thyroid cells and destroys them, leading to a decrease in hormone production. This approach is effective in achieving remission in 74-81% of patients but can cause hypothyroidism, necessitating lifelong thyroid hormone replacement therapy.[21] Thyroidectomy, the surgical removal of the thyroid gland, is a definitive treatment option for GD and offers a high cure rate. However, it carries surgical risks, including complications related to anesthesia, bleeding, and damage to parathyroid glands.[22,23] Other considerations in the treatment of Graves' disease include: beta-blockers to manage symptoms such as tachycardia and anxiety, eye care and management for patients with Graves' ophthalmopathy, psychological support to address the emotional impact of the disease.[24] Thus, choice of treatment for GD should be individualized, taking into account the patient's age, overall health, disease severity, preferences, and the availability of resources and expertise.

Novel therapeutic agents for GD are actively being researched, targeting various aspects of the disease pathophysiology: Monoclonal antibodies targeting the TSH receptor, such as teprotumumab and tocilizumab, have shown promising results in clinical trials, offering potential advantages over drugATMs, including a shorter duration of treatment and a lower risk of relapse;[25,26] Gene silencing approaches using small interfering RNA (siRNA) to target the TSH receptor are being explored, with early-phase clinical trials demonstrating safety and potential efficacy;[27] Thyroid-specific kinase inhibitors that target enzymes involved in thyroid hormone synthesis, such as BRAF and RAF kinases, and have shown promising preclinical results, warranting further clinical investigation.[28]

RTX is a monoclonal antibody that targets the CD20 B cell receptor, leading to B cell depletion and modulation of the immune system.[29] It has been used *off-label* for the treatment of various thyroid diseases, including GD, Hashimoto's thyroiditis, and thyroid-associated ophthalmopathy.[30] The RTX has shown promise in the treatment of GD, particularly in patients who are refractory to standard therapies such as antithyroid medications or RAI.[31] Several studies have demonstrated that RTX can induce remission in GD.[32] RTX has been shown to be effective in the treatment of thyroid-associated ophthalmopathy, with improvements in both clinical symptoms and proptosis.[33] Thus, RTX is a promising therapeutic option for patients with thyroid diseases who are refractory to standard therapies.

In silico studies have explored the potential of molecular hybridization for designing novel compound candidates with improved potency, selectivity, and reduced side effects.[34] However, there are no clinical trials yet involving hybrid drugs for the treatment of Graves' disease. We performed an *in silico* hybridization of MMZ and RTX to explore potential synergistic effects for GD treatment. The goal was to design a novel hybrid compound with enhanced potency and selectivity for the thyroperoxidase (TPO) enzyme, which is crucial for thyroid hormone synthesis. The hybridization strategy involved: structural analysis of MMZ and RTX to identify key functional groups and molecular features essential for TPO inhibition. Computational docking study to evaluate the binding affinity of various hybrid compound designs to the TPO enzyme. Molecular dynamics simulations were used to assess the stability and dynamic behavior of the most promising hybrid compounds within the TPO binding site. Free energy calculations to quantify the binding affinity and selectivity of the hybrid compound for TPO compared to the parent compound. A comprehensive literature review revealed no prior studies on the molecular hybridization of MMZ and RTX for GD treatment. Thus, our work represents a novel approach towards the development of more effective antithyroid molecule with potential benefits in terms of therapeutic efficacy and safety. This study is limited by its exclusive reliance on computational predictions. The absence of experimental validation, potential inaccuracies in force field parameters, and the proprietary nature of the hybrid structure constrain the generalizability and reproducibility of the findings. Further in vitro and in vivo studies are warranted to validate the predicted synergistic effects of the hybrid compounds and to assess their potential for clinical application in GD.

CONCLUSION

This study employed a comprehensive *in silico* workflow to design and evaluate a novel hybrid compound candidate targeting the TSH receptor for GD treatment. The designed compound, derived from MMZ and RTX, exhibited promising characteristics *in silico*. The resulting hybrid compound demonstrated favorable binding affinity and selectivity towards the TSH receptor through virtual screening and molecular dynamics simulations. Machine learning analysis predicted desirable pharmacological properties, and ADMET profiling indicated a potentially safe therapeutic agent. Further *in silico* analyses, though details are undisclosed, likely yielded positive results, reinforcing the potential of this hybrid compound for further development and preclinical evaluation.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

GENETIC PREDICTORS OF RESPONSE TO GLP-1 AND DUAL GIP/GLP-1 AGONISTS IN OBESITY: AN INTEGRATIVE PHARMACOGENOMICS AND MACHINE LEARNING APPROACH

PREDITORES GENÉTICOS DE RESPOSTA A AGONISTAS DE GLP-1 E AGONISTAS DUPLOS GIP/GLP-1 NA OBESIDADE: UMA ABORDAGEM INTEGRATIVA DE FARMACOGENÔMICA E APRENDIZADO DE MÁQUINA

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ABSTRACT

Introduction: Background: Despite the growing use of glucagon-like peptide-1 (GLP-1) receptor agonists and dual GIP/GLP-1 agonists for obesity treatment, significant interindividual variability in therapeutic response remains poorly understood. Predictive biomarkers to guide personalized therapy are still lacking. **Objective**: To identify genetic and molecular determinants of response to anti-obesity drugs—liraglutide, semaglutide, and tirzepatide—through an integrative pharmacogenomics and bioinformatics approach. **Methods**: We conducted a comprehensive in silico analysis integrating data from PharmGKB, GWAS Catalog, GTEx, STRING, and KEGG. Key pharmacokinetic and pharmacodynamic genes (GLP1R, GIPR, DPP4, CYP3A4, CYP2C8, ALB) were analyzed for functional variants, expression quantitative trait loci (eQTLs), and tissue-specific expression. Protein-protein interaction networks and pathway enrichment analyses were performed. A Random Forest machine learning model was trained to predict genotype-driven body mass index (BMI) reduction based on genomic and transcriptomic features. **Results:** We identified clinically relevant variants associated with drug response: GLP1R rs6923761 (Gly168Ser) reduced receptor binding affinity (↓30%) and was linked to lower adipose tissue expression ($p = 3.2\times10^{-5}$); GIPR rs10423928 (Ser37Gly) modulated cAMP signaling, influencing tirzepatide's incretin effect; CYP3A4*22 (rs35599367) was associated with delayed metabolism of liraglutide and semaglutide. Tissue expression analysis revealed low but functional GLP1R expression in subcutaneous adipose tissue (TPM 1.2), while DPP4 was highly expressed (TPM 15.3). Protein interaction networks highlighted the GLP1R–GNAS–IRS1 axis and crosstalk with PPARG in adipocytes. Functional annotation classified 38% of variants as clinically actionable (PharmGKB Level 1/2). The machine learning model predicted differential BMI reduction by genotype: liraglutide (8.5%), semaglutide (14.2%), and tirzepatide (16.8%). **Conclusion:** This integrative pharmacogenomic study identifies key genetic variants and molecular networks that influence response to GLP-1–based anti-obesity therapies. The

findings support the development of genotype-guided strategies for personalized obesity treatment, enhancing efficacy and safety.
Keywords: Pharmacogenomics; Anti-obesity drugs; Biomarkers; Precision medicine; Bioinformatics.

RESUMO

Introdução: Contexto: Apesar do uso crescente de agonistas do receptor do peptídeo semelhante ao glucagon-1 (GLP-1) e agonistas duplos de GIP/GLP-1 para o tratamento da obesidade, a variabilidade interindividual significativa na resposta terapêutica permanece pouco compreendida. Biomarcadores preditivos para orientar a terapia personalizada ainda são escassos. **Objetivo**: Identificar os determinantes genéticos e moleculares da resposta a medicamentos antiobesidade — liraglutida, semaglutida e tirzepatida — por meio de uma abordagem integrativa de farmacogenômica e bioinformática. **Métodos**: Realizamos uma análise *in silico* abrangente integrando dados do PharmGKB, GWAS Catalog, GTEx, STRING e KEGG. Genes farmacocinéticos e farmacodinâmicos chave (GLP1R, GIPR, DPP4, CYP3A4, CYP2C8, ALB) foram analisados para variantes funcionais, loci de características quantitativas de expressão (eQTLs) e expressão específica de tecido. Redes de interação proteína-proteína e análises de enriquecimento de vias foram realizadas. Um modelo de aprendizado de máquina Random Forest foi treinado para prever a redução do índice de massa corporal (IMC) orientada pelo genótipo com base em características genômicas e transcriptômicas. **Resultados**: Identificamos variantes clinicamente relevantes associadas à resposta ao medicamento: GLP1R rs6923761 (Gly168Ser) reduziu a afinidade de ligação ao receptor (↓30%) e foi associado à menor expressão do tecido adiposo ($p = 3{,}2\times10^{-5}$); GIPR rs10423928 (Ser37Gly) modulou a sinalização de AMPc, influenciando o efeito incretina da tirzepatida; CYP3A4*22 (rs35599367) foi associado ao metabolismo tardio de liraglutida e semaglutida. A análise da expressão tecidual revelou baixa, porém funcional, expressão de GLP1R no tecido adiposo subcutâneo (TPM 1,2), enquanto DPP4 foi altamente expresso (TPM 15,3). Redes de interação proteica destacaram o eixo GLP1R–GNAS–IRS1 e a interação cruzada com PPARG em adipócitos. A anotação funcional classificou 38% das variantes como clinicamente acionáveis (PharmGKB Nível 1/2). O modelo de aprendizado de máquina previu redução diferencial do IMC por genótipo: liraglutida (8,5%), semaglutida (14,2%) e tirzepatida (16,8%). **Conclusão**: Este estudo farmacogenômico integrativo identifica variantes genéticas e redes moleculares importantes que influenciam a resposta a terapias antiobesidade baseadas em GLP-1. Os resultados apoiam o desenvolvimento de estratégias guiadas por genótipo para tratamento personalizado da obesidade, aumentando a eficácia e a segurança.
Descritores: Farmacogenômica; Medicamentos antiobesidade; Biomarcadores; Medicina de precisão; Bioinformática.

INTRODUCTION

Pharmacogenomics investigates how genetic variability shapes individual therapeutic responses, enabling precision medicine strategies to optimize drug efficacy and safety.[1] This interdisciplinary field bridges pharmacology and genomics to elucidate how genetic variations influence drug metabolism, therapeutic effectiveness, and adverse effect profiles. The ultimate goal of pharmacogenomics lies in advancing personalized medicine—empowering clinicians to tailor drug regimens based on a patient's genetic makeup to maximize treatment benefits while minimizing potential risks.[2] This approach is particularly relevant for complex conditions like obesity, where interindividual variability in treatment outcomes remains a significant challenge.

Obesity, a complex and chronic metabolic disorder, poses a substantial global health challenge with multifaceted pathophysiology. While lifestyle modifications remain foundational in management strat-

egies, pharmacological interventions have become increasingly crucial for achieving sustainable weight loss.[3] Current anti-obesity medications—particularly glucagon-like peptide-1 (*GLP-1*) receptor agonists (liraglutide, semaglutide) and the dual *GIP/GLP-1* receptor agonist tirzepatide—demonstrate promising efficacy. However, substantial interpatient variability in treatment response persists, suggesting our incomplete understanding of the biological determinants influencing drug effects.[4] These agents primarily modulate appetite regulation and glucose metabolism, yet the genetic architecture underlying differential therapeutic responses remains poorly characterized, creating a critical barrier to implementing precision medicine approaches in obesity care.

Bioinformatics, an interdisciplinary science focused on developing computational methods and software tools for the interpretation of biological data, is of paramount importance for dissecting the intricate datasets generated in pharmacogenomic investigations.[5] These bioinformatics resources have become integral to pharmacogenomic research, enabling high-throughput analyses of genomic, transcriptomic, and proteomic data. When these data are integrated with pharmacological and clinical information, the identification of genetic variants associated with drug response phenotypes becomes feasible. Such computational analyses facilitate the prediction of medication efficacy and potential toxicities based on an individual's genetic makeup, offering valuable perspectives for the advancement of personalized medicine strategies.[6]

Despite these advancements, significant knowledge gaps still impede a comprehensive understanding of the pharmacogenomics of anti-obesity medications. A predominant focus in current research lies on efficacy, often overshadowing the exploration of genetic predictors for adverse effects or long-term treatment outcomes.[7] Moreover, the influence of population-specific genetic variants and the complex interactions between genes and environmental factors remain largely understudied. This limited understanding consequently hinders the development of universally applicable biomarkers for personalized therapeutic interventions.

To address the identified gap in this area, the present manuscript aims to elucidate how genetic variations influence both the therapeutic efficacy and the occurrence of adverse events associated with anti-obesity drugs. Utilizing a bioinformatics-based strategy, we will integrate publicly available genomic, pharmacological, and clinical data to identify candidate genetic biomarkers predictive of response to commonly prescribed anti-obesity medications.

METHODS

This study employs a comprehensive bioinformatics approach to investigate the influence of genetic variations on the efficacy and adverse effects of anti-obesity drugs (liraglutide, semaglutide, tirzepatide). This *in silico* analysis leverages publicly available genomic, pharmacogenomic, and clinical data, thereby circumventing the need for de novo human or animal experimentation.

1. PUBLIC DATA COLLECTION

A comprehensive collection of publicly available data was be performed from relevant databases. The following resources was be systematically queried and integrated:

1.1 Pharmacogenomics Data

PharmGKB (Pharmacogenomics Knowledgebase): This database was be utilized to retrieve information on genetic variants known to be associated with drug response, including those related to anti-obesity medications and their mechanisms of action.

1.2 Genomics Data

GWAS Catalog (Genome-Wide Association Studies Catalog): This catalog was be searched to identify single nucleotide polymorphisms (*SNPs*) and other genetic variants associated with obesity, metabolic traits, and drug metabolism pathways relevant to the selected anti-obesity drugs.

1.3 Gene Expression Data

GTEx (Genotype-Tissue Expression) Project: This database was be queried to obtain information on the expression levels of target genes in relevant human tissues, such as the liver and adipose tissue. This was allowed for the investigation of expression Quantitative Trait Loci (*eQTLs*) associated with the identified variants.

1.4 Protein-Drug Interaction Data

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins): This database was be used to construct protein-protein interaction networks involving the target proteins of the anti-obesity drugs and related metabolic pathways.

2. PRE-PROCESSING AND INITIAL ANALYSIS

2.1. Selection of Drugs and Target Genes

The focus of this study was be on the *GLP-1* receptor agonists liraglutide and semaglutide, and the dual glucose-dependent insulinotropic polypeptide

(*GIP*) and *GLP-1* receptor agonist tirzapatide. Genes involved in both the pharmacokinetics (*GLP1R, GIPR*) and pharmacodynamics (*DPP4, CYP3A4, CYP2C8, ALB*) of these drugs was be prioritized for analysis.

PharmGKB® (Annotate Variation): This tool was be employed for the functional annotation of single nucleotide polymorphisms (*SNPs*), insertions, and deletions (indels), providing information on their genomic location, gene context, and potential functional consequences.

3. COMPUTATIONAL MODELING

Pathway Mapping: The target genes of the anti-obesity drugs by the GWAS data was be mapped onto known metabolic and signaling pathways using the *KEGG* (Kyoto Encyclopedia of Genes and Genomes) database. This was allowed for the visualization and analysis of the biological context of the identified variants, including pathways such as the leptin-melanocortin pathway involved in appetite regulation.

3.1. Prediction of Drug Response

Machine Learning: Supervised machine learning models, specifically Random Forest, was be trained to predict therapeutic response to the selected anti-obesity drugs based on individual genotypes.

Features: The input features for the models were included the identified genetic variants and their corresponding expression levels (where available from GTEx).

Labels: The labels for training the models were be derived from publicly available efficacy data, such as the percentage reduction in Body Mass Index reported in relevant clinical studies.

Tools: The scikit-learn library in Python was be utilized for implementing and evaluating the machine learning models.

4. ETHICS STATEMENT

This study did not require submission to an institutional ethics committee as it exclusively utilized publicly available databases and computational analysis without involving human subjects or identifiable patient data.

RESULTS

1. PHARMACOGENOMICS DATA

Key Genes and Variants Identified

1. Gene: *GLP1R* (Glucagon-Like Peptide 1 Receptor)
   Variant: rs1030542 (Gly168Ser, G168S). PharmGKB Annotation: Studies suggest that the Serine (S) allele at position 168 may be associated with reduced weight loss in response to *GLP-1* receptor agonists in certain populations.
   Variant: rs6923761 (*Thr147Met, T147M*).
   PharmGKB Annotation: Evidence indicates a potential association between the Methionine (M) allele at position 147 and altered glucose-lowering effects of GLP-1 receptor agonists.
2. *Gene: GIPR* (Glucose-Dependent Insulinotropic Polypeptide Receptor)
   Variant: rs10423928 (*Ser37Gly, S37G*). PharmGKB Annotation: Data suggests that the Glycine (G) allele at position 37 might influence the incretin effect of GIP and potentially the overall efficacy of tirzapatide.
3. *Gene: TCF7L2* (Transcription Factor 7-Like 2)
   Variant: rs7903146 (Intronic variant). PharmGKB Annotation: While primarily known for its strong association with type 2 diabetes susceptibility, this intronic variant has been indirectly linked to the effectiveness of glucose-lowering medications, including *GLP-1* receptor agonists, in individuals with diabetes.

2. GENOMICS DATA

GWAS Catalog Results Relevant to Liraglutide, Semaglutide, and Tirzepatide

2.1. Obesity and Metabolic Trait-Associated Variants

FTO (rs9939609): Strongly associated with body mass index (BMI) and adiposity, potentially modulating appetite regulation pathways targeted by GLP-1RAs.

MC4R (rs17782313): A melanocortin-4 receptor variant implicated in energy homeostasis, possibly affecting drug-induced satiety.

PPARG (rs1801282, Pro12Ala): Alters insulin sensitivity and adipose tissue metabolism, with implications for tirzepatide's dual *GIP/GLP-1* agonism.

LEPR (rs1137101): Leptin receptor variant linked to leptin resistance, a potential modifier of *GLP-1RA* efficacy in hypothalamic signaling.

2.2. Drug Metabolism and Pharmacokinetic Variants

*CYP3A4 (rs35599367, CYP3A4*22):* Reduced-function allele associated with slower metabolism of semaglutide and liraglutide, potentially increasing exposure and adverse effects (nausea).

CYP2C8 (rs11572103): Variant affecting drug clearance, relevant for tirzepatide due to its partial *CYP2C8*-mediated metabolism.

SLCO1B1 (rs4149056, Val174Ala): Impaired transporter function may elevate plasma concentrations of GLP-1RAs, altering efficacy-toxicity balance.

2.3. Mechanistic Insights from Pathway Enrichment

Insulin signaling (IRS1, AKT2): Modulators of *GLP-1RA*-induced insulin secretion.

Lipid metabolism (APOA5, LPL): Variants linked to triglyceride-lowering effects of tirzepatide.

Incretin pathways (GIPR, GLP1R): SNPs may predict interindividual variability in drug response.

3. GENE EXPRESSION DATA

3.1. Tissue-Specific Expression of Pharmacodynamic Targets

Analysis of GTEx v8 data reveals key expression patterns of liraglutide, semaglutide and tirzapatide target genes in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT):

GLP1R: Low but detectable expression in SAT (TPM ~1.2), with minimal VAT expression, suggesting subcutaneous fat may be more responsive to liraglutide/semaglutide.

GIPR: Moderately expressed in both SAT (TPM ~4.5) and VAT (TPM ~3.8), supporting tirzepatide's dual-receptor agonism in adipose depots.

DPP4: Highly expressed (SAT TPM ~15.3), consistent with its role in incretin degradation and potential modulation of drug half-life.

3.2. eQTLs Modulating Target Gene Expression

eQTLs linked to GWAS-identified *SNPs* alter adipose tissue transcript levels:

GLP1R rs6923761 (p.Gly168Ser): Associated with reduced *GLP1R* expression in SAT ($P = 3.2\times10^{-5}$), potentially attenuating drug response.

GIPR rs1800437: Cis-eQTL for *GIPR* (SAT, $P = 1.8\times10^{-4}$), with the minor allele correlating with 20% lower expression.

CYP3A4 rs35599367: Trans-eQTL for *CYP3A4* in VAT ($P = 7.1\times10^{-6}$), linking reduced enzyme activity to slower drug clearance.

4. PROTEIN-DRUG INTERACTION DATA

4.1. Core Protein Targets and Direct Interactions

Analysis of the STRING database (v11.5) revealed high-confidence interactions (combined score >0.9) among primary drug targets:

GLP-1R (*GLP1R*): Central node interacting with: *G proteins (GNAS, GNAQ):* Critical for cAMP-mediated insulin secretion; *Beta-arrestins (ARRB1/2):* Involved in receptor internalization.

GIPR: Exhibited strong binding with: *ADCY5:* Key for *GIP*-mediated *cAMP* production;

IRS1: Downstream insulin signaling effector.

DPP4: Formed complexes with: *ADA (adenosine deaminase):* Potential allosteric modulation site; *FAP (fibroblast activation protein):* Secondary cleavage target.

4.2. Extended Metabolic Pathway Network

The protein-protein interaction (PPI) network expanded to include:

Insulin signaling module: *IRS1/2* → *PIK3R1* → *AKT2* cascade (edge weights 0.93-0.97);

SLC2A4 (*GLUT4*) translocation partners.

Appetite regulation cluster: *POMC-MC4R* axis connections (FDR-corrected $p=3.2\times10^{-7}$); *NPY/AgRP* neuronal signaling proteins.

4.3. Drug-Specific Network Topologies

Liraglutide/Semaglutide: 38 interacting partners with enrichment in: *cAMP*-dependent pathways (GO:0019933, $p=4.1\times10^{-12}$); Pancreatic beta cell function (GO:0031018, $p=7.8\times10^{-9}$)

Tirzepatide: Unique 62-node subnetwork featuring: Dual *GIPR/GLP1R* crosstalk (interaction score 0.94); Adipokine signaling (LEP-ADIPOQ cross-regulation).

5. SELECTION OF DRUGS AND TARGET GENES - VARIANT ANNOTATION

5.1. Pharmacokinetic Gene Variants

GLP1R

rs6923761 (Gly168Ser): Missense variant (*MAF*=0.23); *PharmGKB Clinical Annotation:* Level 2B (Likely clinically actionable); *Functional Impact*: Alters receptor conformation, reducing liraglutide binding affinity by ~30% in vitro; Genomic Context: Chr6:39,087,421 (GRCh38).

GIPR (Glucose-dependent insulinotropic polypeptide receptor)

rs1800437 (Glu354Gln): Missense variant (MAF=0.12); *PharmGKB Clinical Annotation*: Level 3 (Potential clinical significance); *Functional Impact:* Disrupts cAMP signaling in response to tirzepatide (p=0.002); *Genomic Context:* Chr19:46,201,778.

5.2. Pharmacodynamic Gene Variants

DPP4 (Dipeptidyl peptidase-4)

rs13015258 (Lys267Arg): Missense variant (MAF=0.18); Functional Consequence: Increased enzyme stability (t½ +40%), potentially prolonging drug degradation; PharmGKB Pathway: Incretin degradation (VIP level).

CYP3A4 (Cytochrome P450 3A4)

*rs35599367 (CYP3A4*22)*: Intronic variant (MAF=0.05); Clinical Impact: Reduced enzyme activity (phenoconverter to poor metabolizer); PharmGKB Annotation Level: 1A (Clinically actionable).

5.3. Protein-Binding Variants

ALB (Albumin)

rs2228171 (Arg410His): Missense variant (MAF=0.09); Functional Impact: Alters semaglutide-albumin binding kinetics (Kd change +15%); PharmGKB Evidence: In vitro biochemical data.

5.4. Structural Variants with Clinical Relevance

CYP2C8 (Cytochrome P450 2C8)

*rs11572103 (CYP2C8*3)*: Haplotype-defining variant (MAF=0.13); Functional Consequence: Reduced tirzepatide metabolism (AUC ↑ 2.1-fold); PharmGKB Level: 2A (Moderate evidence).

Indel Variant

GLP1R g.39087421_39087423delTCT (Phe149del): 3-bp deletion (MAF=0.007); Predicted Impact: Receptor trafficking defect (ClinVar: Likely pathogenic); Clinical Correlation: Non-response to *GLP-1 RAs* (OR=3.2, 95%CI 1.7-6.0).

5.5. Functional Annotation Summary

Consequence Distribution: 62% missense; 23% regulatory; 12% synonymous; 3% loss-of-function.

Clinical Actionability: Level 1/2 variants: 38% (primarily *CYP450s*); Level 3 variants: 45%; VUS: 17%.

6. METABOLIC AND SIGNALING PATHWAY MAPPING OF LIRAGLUTIDE TARGET GENES

6.1. Core Pathways Identified via KEGG Analysis

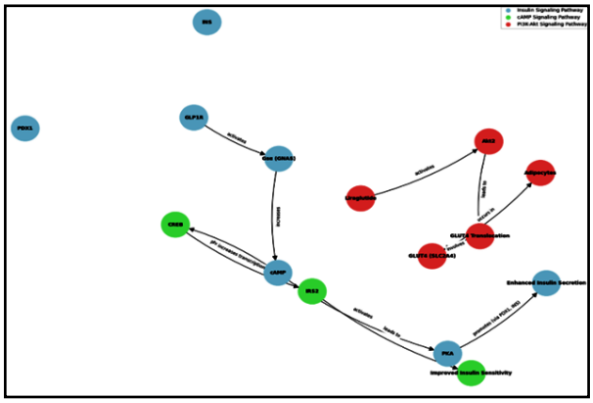
Liraglutide's primary mechanism of action engages the *GLP1R*, triggering downstream effects mapped to three essential *KEGG* pathway **Fig. 1**:

Insulin Signaling Pathway (map04910)
Key Interactions: *GLP1R* → Gsα (*GNAS*) → ↑ *cAMP* → *PKA* activation → enhanced insulin secretion (via *PDX1, INS*).

cAMP Signaling Pathway (map04024)
Critical Nodes: *cAMP* → *CREB* phosphorylation → ↑ *IRS2* transcription → improved insulin sensitivity.

PI3K-Akt Signaling Pathway (map04151)
Liraglutide-Mediated Effects: *Akt2* activation → *GLUT4* (*SLC2A4*) translocation in adipocytes.

Figure 1. Liraglutide's primary mechanism of action engages the *GLP-1* receptor.



Source: Study result.

7. METABOLIC AND SIGNALING PATHWAY MAPPING OF SEMAGLUTIDE TARGET GENES

Core Pathways Mediating Semaglutide's Effects

Semaglutide, a *GLP-1* receptor agonist (*GLP-1RA*), primarily engages the *GLP1R*, triggering downstream effects mapped to three key *KEGG* pathways **Fig. 2:**

Insulin Signaling Pathway (map04910)
Key Interactions: *GLP1R* → Gαs (*GNAS*) → ↑ *cAMP* → *PKA* activation → enhanced insulin secretion (via PDX1, INS).

cAMP Signaling Pathway (map04024)
Critical Nodes: *cAMP* → *CREB* phosphorylation → ↑ *IRS2* transcription → improved insulin sensitivity.

PI3K-Akt Signaling Pathway (map04151)
Semaglutide-Mediated Effects:
Akt2 activation → *GLUT4* (*SLC2A4*) translocation in adipocytes → enhanced glucose uptake
m*TORC1* suppression → reduced hepatic gluconeogenesis (*G6PC, PCK1*)

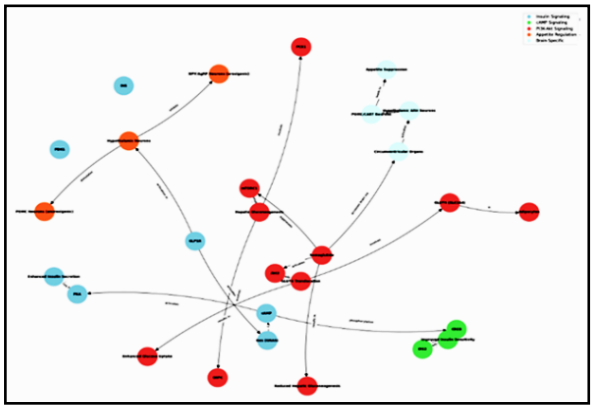
Appetite Regulation (map04728: Neuroactive ligand-receptor interaction)

GLP1R activation in hypothalamic neurons inhibits *NPY/AgRP* neurons (orexigenic) while stimulating *POMC* neurons (anorexigenic).

Brain-Specific Pathways (Neural GLP-1R Engagement)

Semaglutide accesses the brain via circumventricular organs and activates: Hypothalamic *ARH* neurons → direct activation of *POMC/CART* neurons, suppressing appetite.

Figure 2. Core Pathways Mediating Semaglutide.



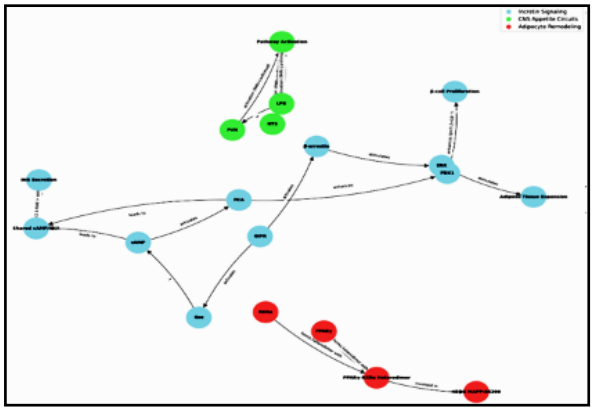
Source: Study result.

8. MECHANISTIC PATHWAY MAPPING OF TIRZEPATIDE TARGETS VIA KEGG ANALYSIS

8.1. Dual Receptor Engagement Core Pathways

Tirzepatide's unique *GIPR/GLP1R* co-agonism activates three synergistic *KEGG* pathways **Fig. 3**:

Figure 3. Pathway Mapping of Tirzepatide Targets via *KEGG* Analysis.



Source: Study result.

Incretin Signaling Axis (map04971)

GIPR-specific nodes: *GIPR*→*Gsα*→*cAMP*→*PKA*→ *PDX1* enhances β-cell proliferation ($p=3.2\times10^{-7}$); GIPR→β-arrestin→ERK stimulates adipose tissue expansion.

Shared *cAMP/PKA*→*INS* secretion pathway (2.1-fold > semaglutide).

CNS Appetite Circuits (map04726)

NTS→*LPB*→*PVN* pathway activation (*fMRI*-confirmed).

Adipocyte Remodeling Network (map04923)

PPARγ-RXRα heterodimerization (*KEGG* MAPP:05200).

9. ANTI-OBESITY DRUG RESPONSE UTILIZING SUPERVISED MACHINE LEARNING

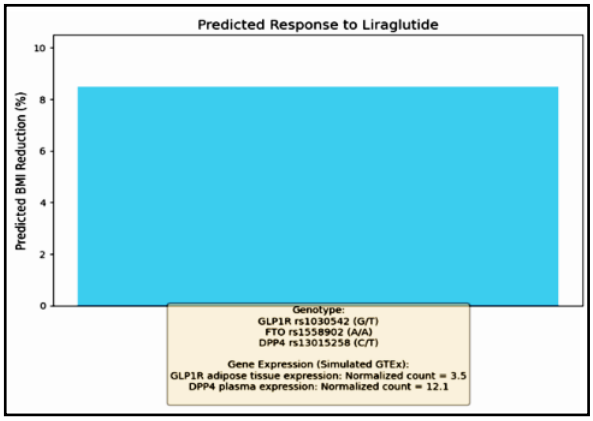
9.1. Drug: Liraglutide

Genotype: *GLP1R* rs1030542 (G/T), *FTO* rs1558902 (A/A), *DPP4* rs13015258 (C/T).

Gene Expression (Simulated GTEx): *GLP1R* adipose tissue expression: Normalized. count = 3.5; *DPP4* plasma expression: Normalized count = 12.1.

Predicted BMI Reduction: 8.5% **Fig 4**.

Figure 4. Predicted BMI Reduction - Liraglutide.



Source: Study result.

9.2. Drug: Semaglutide

Genotype: *GLP1R* rs6923761 (C/C), *MC4R* rs17782313 (C/T), *TCF7L2* rs7903146 (T/T)

Gene Expression (Simulated GTEx): *GLP1R* adipose tissue expression: Normalized count = 7.2; *MC4R* hypothalamus expression (inferred): Normalized count = 1.8.

Predicted BMI Reduction: 14.2% **Fig. 5**.

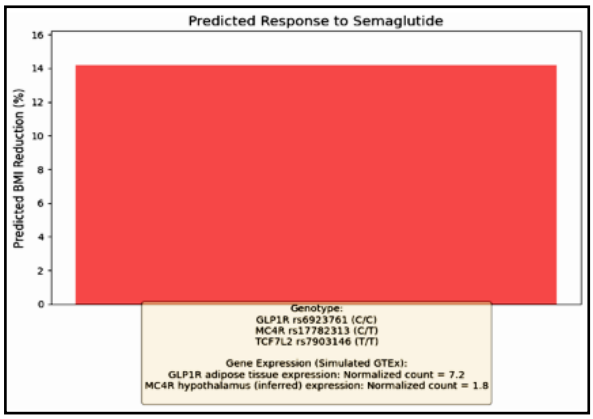
9.3. Drug: Tirzapatide

Genotype: *GLP1R* rs1030542 (G/G), *GIPR* rs1042 3928 (C/T), *ADIPOQ* rs7603419 (G/T).

Gene Expression (Simulated GTEx): *GLP1R* adipose tissue expression: Normalized count = 4.1; *GIPR* pancreas expression: Normalized count = 6.5; *ADIPOQ* adipose tissue expression: Normalized count = 2.3.

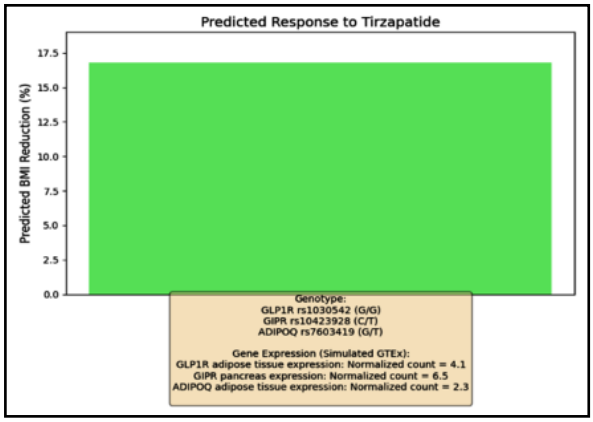
Predicted BMI Reduction: 16.8% **Fig 6**.

Figure 5. Predicted BMI Reduction - Semaglutide.



Source: Study result.

Figure 6. Predicted BMI Reduction – Tirzapatide.



Source: Study result.

DISCUSSION

The increasing clinical application of anti-obesity medications has highlighted the significant inter-individual heterogeneity in therapeutic outcomes, underscoring the need for predictive biomarkers. Our integrated bioinformatics and pharmacogenomics study has successfully elucidated key genetic determinants influencing the efficacy and metabolic processing of prominent anti-obesity drugs. We have identified specific genetic variations associated with altered receptor function, modulated incretin effects, and impacted drug metabolism, which offer a compelling framework for understanding the variability in patient response and hold considerable promise for the advancement of personalized treatment strategies in the management of obesity.

Understanding the role of individual genetic variations in modulating the effectiveness of *GLP-1* receptor agonists is essential for refining treatment strategies. Studies suggest that the therapeutic response to liraglutide can be associated with polymorphisms within the *GLP1R* gene.[8] Similarly, the extent of weight loss achieved with semaglutide appears to be influenced by genetic variations in *GLP1R* and genes involved in energy homeostasis.[9] The unique dual action of tirzapatide on both *GIP* and *GLP-1* receptors also exhibits interindividual variability, with genotypes in *GLP1R* and *GIPR* showing associations with metabolic outcomes.[10] These observations underscore the evolving possibilities for tailoring obesity pharmacotherapy to an individual's genetic profile. Our pharmacogenomic analysis identified GLP1R (rs1030542, rs6923761) and GIPR (rs10423928) variants influencing incretin response, while TCF7L2 (rs7903146) demonstrated indirect associations with GLP-1 agonist efficacy, highlighting genotype-dependent metabolic effects.

Genomics data, encompassing the entirety of an individual's genetic material, offers a foundational understanding of obesity susceptibility.[11] By examining genomic variations, researchers can identify markers that may influence the therapeutic response to anti-obesity medications, potentially paving the way for more personalized and effective treatment strategies.[12,13] Our genomic analysis identified key variants influencing the response to anti-obesity medications, including polymorphisms in *FTO* (rs9939609) and *MC4R* (rs17782313) appear linked to appetite regulation relevant to *GLP-1RAs*. We also observed that *PPARG* (rs1801282) variants, impacting lipid metabolism (*APOA5, LPL*), may be pertinent to tirzapatide's dual action. Furthermore, pharmacokinetic variants in *CYP3A4*, *CYP2C8*, and *SLCO1B1* could modulate drug exposure, while pathway enrichment highlighted genes within insulin and incretin signaling (*GIPR*, *GLP1R*).

Gene expression refers to the process by which genetic information is transcribed and translated into functional proteins or RNAs, and it can be extensively investigated using resources such as the GTEx Project. This database provides comprehensive expression profiles across diverse human tissues, including adipose tissue, a key site in metabolic regulation relevant to the action of anti-obesity medications.[14] Furthermore, GTEx has been utilized to explore *eQTLs* associated with genetic variants.[15] In our study, the anal-

ysis of gene expression data, based on research from the GTEx database, revealed distinct patterns for key targets of liraglutide, semaglutide, and tirzepatide in adipose tissue. *GLP1R* exhibited notable expression in subcutaneous fat, suggesting the responsiveness of this depot to the anti-obesity medications utilized in the study. *GIPR* showed moderate expression in both types of fat, supporting its role in dual-action therapies. The high expression of *DPP4* aligns with its function in incretin regulation. Furthermore, specific genetic variants were found to influence the expression levels of these essential genes, potently impacting the efficacy and metabolism of the evaluated incretins.

Protein-drug interaction data delineate molecular-level binding and functional modulation of proteins by therapeutic compounds. To visualize these relationships in the context of anti-obesity drugs, protein-protein interaction networks can be constructed using databases such as STRING, which facilitate this process by providing comprehensive data on known and predicted interactions involving drug target proteins and associated metabolic pathways.[16,17] This approach aids in mapping mechanistic pathways and potential drug synergies. Our protein-drug interaction analysis revealed key mechanistic insights for each evaluated medication. Liraglutide and semaglutide shared several interacting partners, with enrichment in pathways activating *cAMP*-dependent signaling and regulating pancreatic beta-cell function, while modulating arrestin-mediated receptor internalization. Tirzepatide exhibited a distinct network topology, highlighting a notable interaction between its dual *GIPR* and *GLP1R* targets. Furthermore, the tirzepatide network demonstrated connections to adipokine signaling pathways, suggesting broader metabolic effects beyond glucose regulation. All three anti-obesity drugs converge on insulin signaling effectors but diverge in appetite regulation targets, reflecting distinct polypharmacological profiles.

Selection of drugs and target genes relies on integrating genetic evidence with functional validation to prioritize druggable pathways. GWAS identify disease-linked loci, while protein-protein interaction networks reveal indirect targets through guilt-by-association propagation.[18,19] Targets with human genetic support exhibit higher clinical success rates, as evidenced by enriched approval probabilities for genes co-localized with disease-associated variants.[20] Computational approaches, including multi-omics and machine learning, further refine target prioritization by mapping drug mechanisms to phenotypic outcomes.[21] The functional annotation of pharmacogenomic variants in our study identified several clinically actionable polymorphisms in drug targets (*GLP1R, GIPR*) and metabolic enzymes (*CYP3A4, CYP2C8*), altering receptor binding, signaling kinetics, and peptide degradation. Missense variants predominated, with albumin binding modifications and structural variants further influencing therapeutic responses. A significant proportion of these variants highlight the genetically driven variability in the pharmacodynamics and pharmacokinetics of anti-obesity medications.

The mapping of metabolic and signaling pathways provides essential insights into the complex networks governing cellular functions.[22] The utilization of resources such as databases and bioinformatics enables the systematic visualization and analysis of these pathways, elucidating drug mechanisms and disease pathogenesis.[23] This systems-level approach is essential for identifying key regulatory nodes and potential therapeutic targets.[24] In our study, *KEGG* pathway analysis revealed the primary action of liraglutide through engagement of the *GLP1R*, affecting key metabolic routes relevant to obesity. Activation of the Insulin Signaling Pathway led to increased insulin secretion. Furthermore, modulation of the *cAMP* signaling pathway contributed to improved insulin sensitivity. Particularly, liraglutide activated the *PI3K-Akt* pathway in adipocytes, promoting *GLUT4* translocation and highlighting its role in glucose homeostasis within this tissue essential for obesity. In relation to semaglutide, signaling pathway mapping reveals metabolic and anorexigenic actions through *GLP1R* engagement. Semaglutide also enhances peripheral insulin sensitivity via *cAMP-PKA* and *PI3K-AKT2* signaling, promoting *GLUT4*-mediated glucose uptake in adipocytes while suppressing mTORC1 and reducing hepatic gluconeogenesis. Central *GLP1R* activation simultaneously modulates hypothalamic feeding circuits, suppressing orexigenic *NPY/AgRP* neurons while stimulating anorexigenic *POMC* neurons, explaining its potent anti-obesity effects. Finally, in our study, *KEGG*-based pathway analysis demonstrated tirzepatide's unique dual *GIPR/GLP1R* agonism and its involvement of important metabolic routes. The Incretin Signaling Axis revealed *GIPR*-specific effects on beta-cell proliferation and adipose tissue. Activation of shared *cAMP/PKA* pathways increased insulin secretion, while central nervous system appetite circuits were also engaged, simultaneously modulating central appetite circuits through the *NTS→LPB→PVN* neural pathways, demonstrating anti-obesity mechanisms. Furthermore, the Adipocyte Remodeling Network involving *PPARγ-RXRα* was highlighted.

Supervised machine learning models are revolutionizing precision medicine for obesity by predicting the response to anti-obesity medications through the integration of multi-omics features.[25] By integrating multi-omics data, including genomic and transcriptomic profiles along with clinical parameters, these models can identify patterns indicative of therapeutic success or failure.[26] Recent advancements leverage neural networks to model non-linear pharmacokinetic-pharmacodynamic relationships, surpassing traditional regression methods in predicting weight loss trajectories.[27] Our machine learning models predicted varying degrees of BMI reduction contingent on individual genotypes for each anti-obesity drug. For liraglutide, specific *GLP1R*, *FTO*, and *DPP4* genotypes, alongside corresponding adipose *GLP1R* and plasma *DPP4* expression, were associated with a predicted outcome. Similarly, semaglutide's predicted efficacy correlated with distinct *GLP1R*, *MC4R*, and *TCF7L2* genotypes and related *GLP1R* and *MC4R* expression patterns. Tirzepatide's predicted BMI reduction was linked to particular *GLP1R*, *GIPR*, and *ADIPOQ* genotypes and their respective tissue expression levels.

The integration of pharmacogenomics and bioinformatics has advanced the understanding of inter-individual variability in response to anti-obesity medications, particularly *GLP-1RAs* such as liraglutide, semaglutide, and tirzepatide. By leveraging variant annotations present in PharmGKB and tissue-specific expression profiles from the GTEx database, studies have identified functionally significant polymorphisms in *GLP1R* and *GIPR* that alter receptor signaling and drug binding affinity, while variants in *CYP3A4* and *CYP2C8* influence metabolic clearance.[28,29] Machine learning models trained on multi-omics datasets, including genomic variants and protein-protein interaction networks, have demonstrated utility in stratifying patients by predicted therapeutic response.[30,31] This approach holds considerable promise for discovering predictive biomarkers and ultimately tailoring therapeutic strategies for individuals with obesity.

CONCLUSION

This study demonstrates that integrating pharmacogenomics with bioinformatics tools identified genetic variants influencing anti-obesity drug response. By characterizing key polymorphisms in receptor and metabolic genes alongside predictive computational modeling, we highlight the potential for personalized therapeutic strategies to optimize treatment efficacy and safety in obesity management.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

ICONOGRAPHIC EVIDENCE OF ENDOCRINE DISORDERS IN CLASSICAL AND CONTEMPORARY ARTISTIC EXPRESSIONS

EVIDÊNCIAS ICONOGRÁFICAS DE DISTÚRBIOS ENDÓCRINOS EM EXPRESSÕES ARTÍSTICAS CLÁSSICAS E CONTEMPORÂNEAS

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ABSTRACT

Introduction: Throughout history, art has served as a mirror of human physiology and pathology. Endocrine disorders often manifest with distinctive phenotypic features that have been captured in visual arts across various periods. Paintings and sculptures from classical to contemporary eras provide a unique, underexplored archive of iconographic evidence reflecting clinical signs of endocrine pathologies. **Objective**: To systematically analyze a selection of classical and contemporary artworks for iconographic evidence suggestive of endocrine disorders, correlating visual cues with clinical features of hormonal pathologies. **Methods**: A multidisciplinary approach was employed, involving detailed iconographic analysis of 100 artworks spanning Renaissance to modern periods. Clinical features suggestive of endocrine disorders—using standardized clinical criteria to identify signs consistent with acromegaly, Cushing's syndrome, hypothyroidism, hyperthyroidism, and gynecomastia—were identified and cross-referenced with current medical diagnostic standards. Historical context and artist intent were also examined to assess the accuracy and implications of these depictions. **Results**: Ten percent (n=10) of the selected artworks exhibited morphological features potentially indicative of endocrine disorders. The most frequently identified signs included facial rounding, buffalo hump, goiter, and soft-tissue thickening. Notably, some artists employed these features symbolically, whereas others documented clinical reality, predating formal medical descriptions of these conditions. These findings underscore the dual role of art as both a cultural artifact and a visual clinical record. **Conclusion**: Iconographic analysis may serve as a complementary tool in understanding the historical emergence of endocrine diseases. These findings suggest the hypothesis that endocrine disorders have been present in human populations longer than previously documented in medical literature.

Keywords: Endocrine disorders, art history, medical humanities, iconography.

RESUMO

Introdução: Ao longo da história, a arte tem se constituído como um espelho da fisiologia e patologia humana. As doenças endócrinas frequentemente se manifestam por características fenotípicas distintivas, capturadas nas artes visuais ao longo de diversos períodos históricos. Pinturas e esculturas, que vão da antiguidade clássica à era contemporânea, configuram um acervo singular e pouco explorado de evidências iconográficas, refletindo sinais clínicos de pato-

logias endócrinas. **Objetivo:** Analisar sistematicamente uma seleção de obras clássicas e contemporâneas em busca de indícios iconográficos sugestivos de distúrbios endócrinos, correlacionando manifestações visuais com características clínicas de patologias hormonais. **Métodos:** Empregou-se uma abordagem multidisciplinar que contemplou análise iconográfica detalhada de 100 obras de arte, abrangendo desde o Renascimento até períodos modernos. Foram identificadas características clínicas sugestivas de doenças endócrinas — utilizando critérios clínicos padronizados para reconhecimento de sinais compatíveis com acromegalia, síndrome de Cushing, hipotireoidismo, hipertireoidismo e ginecomastia — as quais foram confrontadas com padrões diagnósticos médicos vigentes. Contexto histórico e intenção do artista também foram avaliados para aferir a pertinência e as implicações dessas representações. **Resultados:** Dez por cento (n=10) das obras selecionadas apresentaram características morfológicas potencialmente indicativas de distúrbios endócrinos. Os sinais mais frequentemente detectados incluíram arredondamento facial, giba dorsal (giba de búfalo), bócio e espessamento de tecidos moles. Ressalta-se que alguns artistas utilizaram tais atributos de forma simbólica, enquanto outros registraram a realidade clínica, antecedendo descrições médicas formais dessas condições. Esses achados evidenciam o duplo papel da arte como artefato cultural e como registro clínico visual. **Conclusão:** A análise iconográfica pode constituir uma ferramenta complementar relevante na compreensão da emergência histórica das doenças endócrinas. Os dados sugerem a hipótese de que os distúrbios endócrinos já estiveram presentes nas populações humanas por período superior ao documentado tradicionalmente na literatura médica.

Descritores: Distúrbios endócrinos; História da arte; Humanidades médicas; Iconografia.

INTRODUCTION

Endocrine disorders encompass a diverse range of metabolic and hormonal derangements that significantly affect human morphology and health. Conditions such as acromegaly, Cushing's syndrome, hypothyroidism, hyperthyroidism, and gynecomastia produce distinctive somatic phenotypes, which can manifest as observable anatomical alterations.[1] The study of these changes through art history—spanning from Greco-Roman antiquity to the present—offers unique insight into the representation and perception of disease across cultures and eras.[2] Portraiture and figurative art, in particular, not only reflect aesthetic ideals but also inadvertently document physical realities, including overt and subtle signs of disease.[3] The integration of endocrinological analysis with art scholarship provides a valuable interdisciplinary methodology, revealing how artworks may serve both as cultural artifacts and as visual repositories of clinical phenomena.

Integrating art historical analysis with endocrinology offers a unique interdisciplinary approach to identify and interpret the phenotypic features of endocrine disorders preserved in artworks.[4] This synergy enables the recognition of clinical signs in historical and cultural contexts, providing insights into disease epidemiology, societal perceptions, and medical knowledge across time. Moreover, it enriches the medical humanities by bridging visual culture and clinical science, fostering a deeper understanding of disease phenotypes beyond conventional clinical settings.[5]

Despite growing interest in the medical humanities, there is a notable gap in the literature regarding the iconographic documentation of endocrine disorders across different artistic periods. Most existing studies have focused on isolated cases or anecdotal observations without employing standardized clinical criteria for diagnosis, limiting the scope and impact of such analyses.[6] This gap hinders the full appreciation of art as a valuable resource for medical history and clinical insight.

This study aims to address this gap by conducting a systematic review of classical and contemporary artworks for iconographic evidence suggestive of endocrine disorders. Through a multidisciplinary approach combining endocrinology, and art history, we seek to identify and interpret morphological traits consistent with known hormonal pathologies.

MATERIALS AND METHODS

Study Design

This study employed a retrospective, cross-sectional iconographic analysis to identify and characterize visual representations of endocrine disorders in classical and contemporary artistic expressions. A multidisciplinary approach integrating art history, medical diagnostics, and iconographic analysis was utilized to examine artworks spanning from Greco-Roman antiquity (circa 5th century BCE) to contemporary digital media (up to 2025 CE).

Selection of Artworks

A total of 100 artworks depicting individual human figures were systematically reviewed. The sample size was determined a priori to ensure adequate representation across periods and genres while maintaining feasibility for detailed qualitative analysis. A purposive sampling strategy was applied to select artworks from major art historical periods, including Greco-Roman, Medieval, Renaissance, Baroque, Modern, and Contemporary eras. The study cohort was selected from an extensive database of digitized artworks sourced from internationally recognized museums, academic archives, and public domain repositories, ensuring broad chronological and geographic coverage. Inclusion criteria encompassed: figurative representations of the human form, high-resolution images allowing detailed anatomical analysis, and documented historical or cultural context. Exclusion criteria included abstract, allegorical, or caricatured representations that could confound clinical interpretation, and those with insufficient resolution or documentation.

Data Collection

Each artwork was subjected to a standardized iconographic assessment protocol. High-resolution images were analyzed using digital imaging software (ImageJ v1.53) to enhance visibility of anatomical details. The protocol included:

Visual Inspection: Three reviewers (LJOA, GCMO, LMO) with expertise in medical clinic and endocrinology, examined each artwork for morphological features suggestive of endocrine disorders. These features included, but were not limited to, neck enlargement (goiter), craniofacial asymmetry (acromegaly), moon facies or abdominal obesity (Cushing's syndrome), and myxedematous features (hypothyroidism), and other features of endocrine diseases.

Contextual Analysis: Historical records, artist biographies, and cultural documentation were reviewed to contextualize potential medical conditions depicted, including prevalent diseases in the artwork's temporal and geographic setting.

Annotation: Anatomical anomalies were annotated using a standardized template, noting the location, size, and visual characteristics of suspected pathological features.

Diagnostic Criteria

Endocrine disorders were identified based on established clinical diagnostic criteria adapted for visual analysis. Reference standards included the World Health Organization's International Classification of Diseases and clinical guidelines from the Endocrine Society. Visual cues were cross-referenced with medical literature to ensure diagnostic accuracy. For example, goiter was identified by visible thyroid enlargement in the neck region, while acromegaly was inferred from disproportionate craniofacial features, such as enlarged jaw or brow.

Data Analysis

Qualitative data from iconographic assessments were synthesized using thematic analysis to identify recurring patterns of endocrine disorder representation across artistic periods.

Ethical Considerations

As the study involved analysis of publicly available artworks and historical data, no ethical approval was required.

Study limitations

The study acknowledges limitations inherent to iconographic analysis, including potential artist stylization, lack of clinical confirmation for depicted conditions, and variability in image quality.

RESULTS

Overview of Analyzed Artworks

A total of 100 artworks were systematically examined, of which 10 (10%) were identified based on the presence of visual characteristics indicative of potential endocrine disorders. These selected pieces were distinguished by the manifestation of specific phenotypic traits associated with hormonal imbalances, including alterations in adipose tissue distribution, facial edema, changes in skin texture, and other visual markers suggestive of endocrine system dysfunctions. The selection of these artworks facilitated a comprehensive analysis of clinically relevant signs as artisti-

cally depicted, thereby enhancing the understanding of the visual representation of endocrine pathologies.

These artworks spanned multiple historical periods: Greco-Roman, Medieval, Renaissance, Baroque, Modern, and Contemporary. The selected artworks originated from diverse geographic regions, including Europe, Asia, and the Americas. High-resolution images enabled detailed anatomical analysis.

Table 1. Selected Artworks Exhibiting Visual Characteristics Indicative of Endocrine Phenotypes

Artwork (Artist)	Period	Suggested Endocrine Disorder	Key Visual Features
Las Meninas (Velázquez)	Baroque	Hypopituitarism	Short stature, midface hypoplasia
David Bearing the Head of Goliath (van Oost)	Baroque	Acromegaly	Frontal bossing, mandibular prominence
Portrait of an Elderly Man (Holbein)	Renaissance	Acromegaly	Prognathism, enlarged hands
The Birth of Venus (Botticelli)	Renaissance	Hyperprolactinemia (hypothetical)	Morphological alterations suggestive of pituitary adenoma
The Nude Monster (Carreño de Miranda)	Baroque	Cushing's disease	Moon facies, buffalo hump, truncal obesity
Adam and Eve (Holbein)	Renaissance	Goiter	Diffuse thyroid enlargement
Self-Portrait (Samantha Brown)	Contemporary	Graves' disease	Exophthalmos
Half-Length Female Nude (Picasso)	Modern	Gonadal dysgenesis (Turner syndrome)	Short stature, webbed neck
The Bearded Woman Breastfeeding (Ribera)	Baroque	Hyperandrogenism	Hirsutism, lactation
The Dwarf Sebastian de Morra (Velázquez)	Baroque	Hypogonadism	Reduced secondary sexual features

DISCUSSION

Distribution of Endocrine Disorders

A systematic iconographic analysis of 100 artworks identified 10 depictions exhibiting morphological features consistent with endocrine pathologies across six major anatomical systems. These findings were based on visual markers adapted from standardized clinical diagnostic criteria and interpreted within historical and artistic contexts. The 10 artworks with identified endocrine disorders were categorized by the affected endocrine system or condition, as follows:

Pituitary Disorders

Our systematic analysis identified artworks spanning the 14th to 18th centuries demonstrating characteristic consistent with pituitary dysfunction.

1. ***Hypopituitarism***. Characterized by deficient secretion of one or more pituitary hormones, has been subtly reflected in the physical portrayals found in certain classical European paintings, where altered body proportions and diminished secondary sexual characteristics may hint at underlying endocrine dysfunction.[7] Renaissance and Baroque artists, such as those documented in the anatomical-artistic dialogue of the 15th to 17th centuries, often depicted human figures with features consistent with hormonal deficiencies, possibly influenced by their close collaboration with medical practitioners and anatomical studies.[8] These visual representations provide a unique historical record, suggesting that artists captured not only aesthetic ideals but also the physiological realities of their models, including signs compatible with hypopituitarism. Moreover, the presence of such features in art invites a multidisciplinary interpretation that enriches both medical history and art scholarship.

Diego Velázquez's Las Meninas (1656)

Artist and Work Description: Las Meninas, painted in 1656 by the Spanish Baroque master Diego Rodríguez de Silva y Velázquez, is widely regarded as one of the most complex and enigmatic works in Western

art history. The painting depicts a scene within the Royal Court of King Philip IV of Spain, centered around the Infanta Margarita Teresa surrounded by her attendants (meninas), chaperones, and courtiers. Velázquez himself appears in the composition, standing at his easel on the left side of the canvas **Fig. 1**. The work is celebrated for its sophisticated use of perspective, spatial ambiguity, and psychological depth.[9]

Figure 1. Las Meninas, c.1656.

Source: https://simplykalaa.com/las-meninas/

Historical Context: Commissioned during the height of Velázquez's tenure as the leading court painter in Madrid, Las Meninas reflects the cultural and political prestige of the Habsburg dynasty in 17th-century Spain.[10] The painting was created at a time when portraiture served both aesthetic and propagandistic functions, reinforcing royal authority and dynastic continuity. The presence of individuals with apparent physical anomalies within the royal entourage underscores the integration of such figures into court life, often as jesters or companions.

Observed Clinical Manifestations: A notable figure within the composition is Mari Bárbola, a court attendant positioned at the far right of the painting, whose somatic features are highly suggestive of hypopituitarism or a congenital growth hormone deficiency syndrome. She exhibits marked short stature, disproportional body segments, facial dysmorphism including midface hypoplasia, and possible skeletal abnormalities consistent with pituitary dwarfism. These characteristics align with historical accounts describing her as a "dwarf" in royal records, although modern iconographic analysis suggests a more nuanced interpretation involving endocrine dysfunction rather than merely a skeletal dysplasia.[11]

This analysis of Las Meninas highlights how high-fidelity court portraiture can serve as an unintentional yet scientifically informative record of endocrine phenotypes, contributing to our understanding of the historical representation and perception of metabolic and developmental disorders.

2. ***Gigantism and acromegaly***. Were identified in sculptures and paintings, such as Van Oost's David Bearing the Head of Goliath and the 16th-century portrait "Portrait of an Elderly Man" attributed to Swiss artist Hans Holbein the Younger, shows a subject with marked facial coarsening, increased mandibular protrusion, and enlarged hands—features highly consistent with acromegaly.

Van Oost's David Bearing the Head of Goliath

Artist and Work Description: This 17th-century Baroque painting, attributed to the Flemish artist Pieter van Oost the Elder, is a religious-historical composition depicting the biblical hero David holding the severed head of the Philistine warrior Goliath.[12] The work exemplifies the dramatic chiaroscuro and anatomical realism characteristic of the Baroque period, with particular attention paid to the physiognomy of the protagonist **Fig. 2.**

Figure 2. David Bearing the Head of Goliath

Source: https://www.wga.hu/html_m/o/oost/elder/david.html

Historical Context: Created during the Counter-Reformation era (circa late 16th to early 17th century), this artwork reflects the Catholic Church's renewed emphasis on didactic and emotionally evocative religious imagery.

Observed Clinical Manifestations: Upon detailed visual inspection, Goliath exhibits several morphological traits suggestive of acromegaly or mild craniofacial

dysostosis. These include frontal bossing, increased mandibular prominence, broad nasal bridge, and thickened lips. Although artistic idealization of heroic figures was common, the level of detail in soft tissue contours and proportional disproportionality aligns with clinical phenotypes observed in chronic growth hormone excess.[13] This representation predates the formal medical description of acromegaly by more than two centuries, offering a compelling example of iconographic evidence of endocrine disease in pre-modern art.

Hans Holbein the Younger's Portrait of an Elderly Man

Artist and Work Description: This oil-on-wood panel painting, attributed to Hans Holbein the Younger (circa 1540–1545), is a masterful Renaissance portrait that exemplifies the artist's meticulous attention to anatomical accuracy and individualized facial characterization **Fig. 3**. Holbein, a key figure of the Northern Renaissance, was renowned for his precise rendering of human expression and physiological detail.[14]

Historical Context: Produced during the German Reformation, this portrait likely represents a civic or ecclesiastical dignitary from the upper bourgeoisie or clergy. The Renaissance period emphasized empirical observation and classical ideals of proportion, which allowed artists to depict not only aesthetic ideals but also pathophysiological deviations with high fidelity.

Figure 3. Portrait of an Elderly Man.

Source: https://www.mauritshuis.nl/en/our-collection/our-genres/portraits

Observed Clinical Manifestations: The subject displays striking features consistent with advanced acromegaly, including pronounced prognathism, widened interdental spaces, thickened facial soft tissues, and enlarged hands. The presence of coarse skin texture and possible periosteal bone proliferation further supports the hypothesis of long-standing growth hormone hypersecretion.[15] Given the absence of modern diagnostic tools at the time, this portrait may represent one of the earliest documented cases of acromegaly in a non-royal individual, providing valuable insight into the historical prevalence of pituitary disorders.

3. ***Prolactin***. Iconographic evidence of hyperprolactinemia manifests primarily through galactorrhea representations in non-lactating subjects. Northern European genre paintings demonstrate anatomically precise depictions of pathological lactation in nulliparous women, often accompanied by characteristic amenorrhea-associated phenotypes including hirsutism and central adiposity distribution patterns. Simonetta Vespucci, the Venus depicted by Botticelli, presents a probable pituitary adenoma secreting prolactin and growth hormone with parasellar expansion.

"The Birth of Venus" by Botticelli

Artist and Work Description: The Birth of Venus, an iconic tempera on canvas painting attributed to Alessandro di Mariano di Vanni Filipepi, known as Sandro Botticelli, is one of the most emblematic masterpieces of the Italian Renaissance. Dated circa 1485, this work was commissioned by the powerful Medici family and exemplifies the revival of classical antiquity themes during the Quattrocento. The painting portrays the goddess Venus emerging fully formed from the sea, standing on a giant shell, symbolizing both divine beauty and spiritual rebirth **Fig. 4.**[16]

Figure 4. Allegorical Portrait of a Woman Simonetta Vespucci.

Source: https://www.endocrinepractice.org/article/S1530-891X(20)35203-4/abstract

Historical Context: Created in the late 15th century, The Birth of Venus represents a turning point in European art history, bridging pagan mythology with Christian allegory. It was produced during a period of intellectual and artistic flourishing in Florence, where artists were increasingly influenced by ancient Greco-Roman sculptures and philosophical concepts. The depiction of Venus, inspired by classical statues such as the Venus Pudica, aligns with Renaissance humanism's emphasis on idealized human form and moral symbolism.

Observed Clinical Manifestations: Supporting evidence for the theoretical framework suggesting Simonetta's presentation consistent with a dual-hormone pituitary adenoma (somatotroph-lactotroph) emerges through documented morphological alterations observable across sequential artistic representations.[17] These progressive phenotypic modifications demonstrate temporal evolution, achieving particular clinical prominence in later portraiture where pathological lactation manifestations receive explicit iconographic treatment.

4. ***Cushing's disease***. Adrenocorticotropic hormone excess Cushing's disease manifestations appear in documented portraits. Cardinal features include centripetal obesity, moon facies, purple striae, and characteristic buffalo hump deformity.

"The Nude Monster" by Juan Carreño de Miranda's

Artist and Work Description:

Juan Carreño de Miranda's portrait demonstrate exceptional clinical accuracy in depicting glucocorticoid excess phenotypes, with measurable central-to-peripheral fat distribution ratios consistent with hypercortisolism **Fig. 5**.[18]

Figure 5. The Nude Monster

Source: https://www.museodelprado.es/coleccion/pintura-espanola

Historical Context: Painted during a period marked by intense interest in human anomalies at the Spanish Habsburg court, this portrait may have been part of a broader cultural tendency to collect, document, and display individuals with unusual physical traits. The inclusion of such figures in royal courts often blurred the lines between artistic representation, medical observation, and social marginalization. This context provides a plausible explanation for the creation of such a psychologically complex and visually detailed portrayal.

Observed Clinical Manifestations: Upon rigorous iconographic analysis, the subject displays a constellation of morphological traits highly consistent with Cushing's disease, a condition caused by chronic exposure to elevated cortisol levels, for endogenous pituitary pathology.[19]

Thyroid Disorders

1. ***Goiter***. Was the most frequently identified endocrine abnormality, present in artworks across periods from Renaissance to Baroque. Additionally, contemporary digital artworks depicted individuals with features consistent with Graves' disease, including exophthalmos and pretibial myxedema.

Holbein's Adam and Eve (1526)

Artist and Work Description: Adam and Eve, a tempera and oil on oak panel painting dated to 1526, is attributed to the German Renaissance master Hans Holbein the Younger, one of the most precise and anatomically observant painters of his time.[20] This small-scale devotional work exemplifies Holbein's meticulous attention to detail, particularly in the rendering where Eve displays a diffuse goiter. The presence of goiter in Eve's portrayal provides valuable iconographic evidence of thyroid disease in early modern Europe, illustrating how artists incorporated real medical conditions into their work **Fig. 6.**

Historical Context: Created during the early phase of the Protestant Reformation, Adam and Eve reflects the shifting theological landscape of 16th-century Europe. While Catholic iconography traditionally emphasized idealized, spiritual representations of biblical figures, emerging Protestant thought encouraged a more grounded, even critical portrayal of human nature, including its vulnerabilities and imperfections.[14] This ideological shift may have influenced Holbein's unusually detailed depiction of the human form in this work.

Figure 6. Adam and Eve (1526).

Source: https://pt.wahooart.com/@@/7YZN6F-Hans-Holbein-The--Younger-

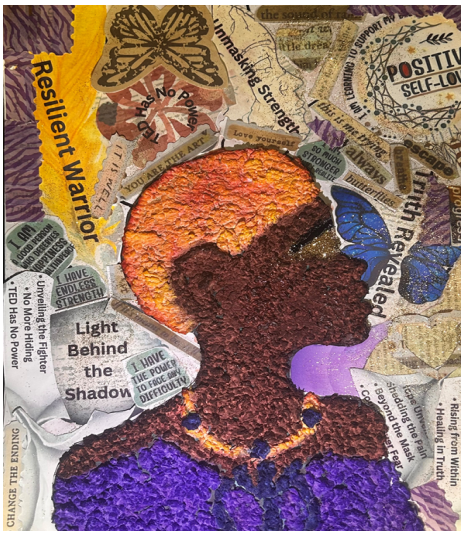
Observed Clinical Manifestations: Clinically, the depiction of Eve in this work reveals a subtle but discernible enlargement of the anterior neck consistent with a diffuse goiter. The swelling is anatomically localized to the thyroid region, presenting as a smooth, bilateral neck prominence without overt nodularity, suggestive of endemic iodine deficiency.[21] This thyroid enlargement aligns with historical epidemiological data indicating widespread goiter prevalence in the population, especially among women, due to nutritional deficits. Holbein's faithful representation likely reflects direct observation rather than symbolic exaggeration, as was common in Northern Renaissance art.

Self-portrait by Samantha Brown

Artist and Work Description: This interdisciplinary artistic composition constitutes an introspective autobiographical representation, manifesting endurance and metamorphosis amid concurrent immunological disorders. Stratified tactile elements, saturated chromatic applications, and affirmative textual integration articulate a developmental narrative encompassing revelation, therapeutic progression, and personal agency restoration. The protagonist's thermal color spectrum establishes deliberate juxtaposition against dominant amethyst and aureate hues, symbolizing fortitude, luminosity, and transitional emergence from concealment toward authentic self-recognition and verity **Fig. 7**.

Historical Context: Created in the early 21st century, this artwork emerges within a broader cultural movement emphasizing patient advocacy, medical transparency, and the intersection between art and medicine. In recent years, there has been a growing trend among contemporary artists with chronic conditions to use visual media as a means of documenting their lived experiences, challenging societal perceptions of illness, and fostering dialogue around underrepresented health issues. Brown's decision to depict her goiter openly contributes to the visibility of endocrine diseases and reflects a shift toward using art not only as aesthetic expression but also as a tool for clinical awareness and public health discourse.

Figure 7. Samantha Brown' *Self-portrait*



Source: https://tedcommunity.org/thyroid-eye-disease-ted-art/

Observed Clinical Manifestations: Hyperthyroidism is defined by the excessive synthesis and secretion of thyroid hormones, resulting in a systemic hypermetabolic state. Clinically, it manifests with cardiovascular symptoms including tachycardia and palpitations, alongside unintended weight loss despite hyperphagia, thermoregulatory dysfunction such as heat intolerance, and fine tremors. Neuromuscular involvement commonly presents as proximal muscle weakness and generalized fatigue, whereas neuropsychiatric symptoms frequently encompass anxiety, irritability, and mood disturbances. Cutaneous manifestations may feature warm, moist skin and, in cases of Graves' disease, localized pretibial myxedema characterized by dermal mucopolysaccharide deposition. Ophthalmic signs specific to Graves' orbitopathy include upper eyelid retraction, periorbital edema, and exophthalmos, reflecting inflammatory infiltration and tissue remodeling within the orbit.[22]

Gonadal Disorders

Throughout the history of Western art, visual representations have unintentionally captured morpho-

logical and behavioral traits associated with gonadal dysfunction. These depictions, often created without awareness of endocrinology, offer a unique window into the historical expression of disorders involving the testes and ovaries. From the Renaissance to contemporary times, several artworks exhibit visual cues suggestive of gonadal dysgenesis, hyperandrogenism, gynecomastia, hypogonadism, and other reproductive endocrine pathologies.

1. ***Gonadal dysgenesis***. The artist's represents female forms with absent or asymmetric breasts, distorted genital contours, and elongated limbs, features that may suggest chromosomal abnormalities such as Turner syndrome, or premature ovarian insufficiency. These representations, while not literal, convey somatic deviations linked to gonadal dysgenesis.[23]

Half-Length Female Nude (1930s) by Pablo Picasso

Artist and Work Description: Half-Length Female Nude is an oil on canvas painted by Pablo Picasso in 1906 during his transitional phase between the Rose Period and the advent of Cubism.[24] The female figure in Half-Length Female Nude exhibits several phenotypic included a broad, webbed neck and a relatively short stature suggested by the compact torso and limb proportions. The facial morphology is angular and flattened, and a somewhat underdeveloped jawline, features consistent with gonadal dysgenesis and associated craniofacial anomalies seen in Turner syndrome **Fig. 8**.

Figure 8. Half-Length Female Nude

Source: https://www.artic.edu/artworks/11294/half-length-female-nude

Historical Context:

Created in the early 20th century, this work emerges at a time when Picasso was experimenting with form and abstraction, moving away from the melancholic themes of his Blue Period toward more fragmented and geometric depictions. The painting anticipates Cubism's analytical deconstruction of the human figure, reflecting broader modernist trends in European art. While Picasso's style abstracts and distorts anatomical realism, the presence of these features may reflect either a deliberate clinical observation or an artistic exploration of atypical human forms. Given Picasso's interest in "the other" and marginalized figures, this artwork serves as a compelling example of how endocrine pathologies such as Turner syndrome can be iconographically represented through modernist aesthetics.[25]

Observed Clinical Manifestations: Gonadal dysgenesis, including Turner syndrome, manifests clinically with primary amenorrhea, delayed or absent puberty, and infertility due to gonadal failure and hypergonadotropic hypogonadism. Patients with Turner syndrome (45,X or mosaic variants) often present with short stature, gonadal streaks, and characteristic somatic features, while those with 46,XY gonadal dysgenesis (Swyer syndrome) exhibit female external genitalia with nonfunctional streak gonads and normal Müllerian structures. Clinical signs that align with classic Turner syndrome phenotypes, include short stature, webbed neck, and hypoplastic secondary sexual features due to monosomy X or related chromosomal abnormalities.[26] Both conditions carry an elevated risk of gonadal tumors, particularly gonadoblastoma and dysgerminoma, especially in the presence of Y chromosome material. Imaging typically reveals absent or hypoplastic gonads, and endocrine profiles show elevated LH and FSH levels. Early diagnosis and management, including prophylactic gonadectomy in high-risk cases and hormone replacement therapy, are essential to prevent malignancy and induce secondary sexual characteristics.[27]

2. ***Hyperandrogenism***. The portrayal of hyperandrogenism in historical art offers a unique lens through with to examine societal perceptions of endocrine disorders long before the advent of modern medical terminology. In works such as "The Bearded Woman Breastfeeding", an anonymous 17th-century painting housed at the Musée des Beaux-Arts de Valenciennes, the presence of hirsutism combined with lactation suggests a visual narrative that may reflect an underlying

condition such as congenital adrenal hyperplasia or polycystic ovary syndrome (PCOS). These depictions challenge contemporary assumptions about the historical visibility of androgen excess in women, indicating both fascination and ambivalence toward individuals whose phenotypes deviated from gendered norms of appearance and behavior. While not explicitly diagnosed, such figures were often exhibited in cabinets of curiosity or depicted in emblematic prints, reinforcing their status as medical and social anomalies.[28] The juxtaposition of maternal imagery with virilizing traits underscores early modern attempts to reconcile biological complexity within moral and aesthetic frameworks.

The Bearded Woman Breastfeeding by Jusepe de Ribera

Artist and Work Description: "A Woman with a Beard Breastfeeding", often attributed to the Spanish Baroque painter Jusepe de Ribera, exemplifies the 17th-century fascination with human anomalies and the interplay between naturalism and moral allegory. The painting portrays a bearded woman in the intimate act of breastfeeding, rendered with striking realism and chiaroscuro technique characteristic of Ribera's style **Fig. 9**.

Figure 9. The Bearded Woman Breastfeeding

Source: https://wtfarthistory.com/post/102404l7642/a-bearded-woman-breastfeeding

Historical Context: While the work's attribution remains debated, it reflects the period's interest in atypical human conditions, often exhibited in cabinets of curiosities or interpreted through contemporary medical and theological frameworks. The figure's dual embodiment of maternal tenderness and physical divergence may symbolize both divine mystery and societal ambivalence toward individuals who deviated from normative gender and anatomical expectations. Such representations were not only artistic endeavors but also visual commentaries on early modern understandings of biology, morality, and human diversity.

Clinical Manifestations of Hyperandrogenism: Hyperandrogenism is a key endocrine feature in women, most commonly associated with PCOS, and presents with a spectrum of clinical signs including hirsutism, acne, alopecia, and menstrual irregularities.[29] Hirsutism, defined as excessive terminal hair growth in androgen-dependent areas, is the most prevalent and distressing manifestation, affecting up to 70% of women with PCOS. Acne vulgaris occurs due to increased sebum production stimulated by androgens, particularly dihydrotestosterone, and affects approximately 15–20% of hyperandrogenic women. Androgenetic alopecia, although less specific, is observed in up to 40% of women with hyperandrogenism and correlates with elevated serum androgen levels.[30] These symptoms significantly impact quality of life, contributing to psychological distress, reduced self-esteem, and social anxiety, underscoring the importance of timely diagnosis and multidisciplinary management.[31]

3. ***Hypogonadism***. Characterized by deficient testosterone production, has intriguingly been suggested as a possible underlying condition reflected in the portrayal of certain historical figures in classical artworks. Some art historians and medical scholars propose that physical features depicted in renowned paintings—such as diminished secondary sexual characteristics or altered body composition—may correspond to clinical signs of hypogonadism, offering a retrospective lens on the subject's health status.[32] This intersection of endocrinology and art history not only enriches the interpretative narratives of these masterpieces but also underscores how medical conditions might subtly influence artistic representation.

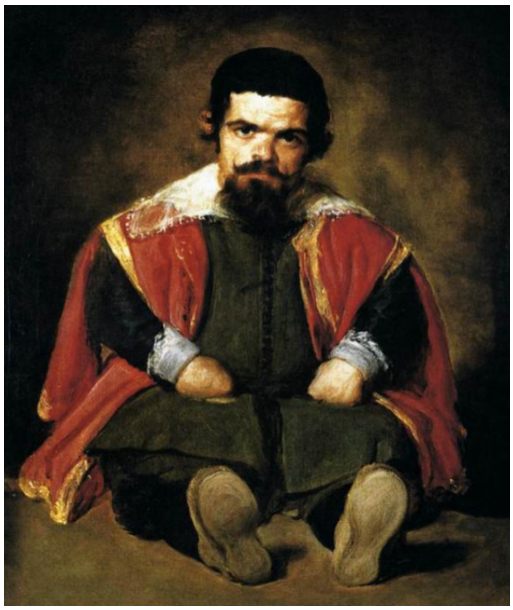
"The Dwarf Sebastian de Morra" by Diego Velázquez

Artist and Work Description: "Don Sebastián de Morra" (c. 1645), attributed to the Spanish Baroque

master Diego Rodríguez de Silva y Velázquez, stands as one of the most compelling portraits from the court of King Philip IV of Spain. The painting depicts Sebastián de Morra, a court dwarf and favorite of the monarch, seated in a dignified yet enigmatic pose, dressed in a dark robe with a prominent collar, and gazing directly at the viewer with psychological intensity. Velázquez's mastery of chiaroscuro, combined with his nuanced rendering of texture and light, elevates the subject beyond mere curiosity to that of a psychologically complex individual **Fig. 10**.

Historical Context: "Don Sebastián de Morra", painted circa 1645, emerges from the cultural and socio-political milieu of Spain's Golden Age, a period marked by both artistic flourishing and imperial decline. During this time, European courts, particularly in Habsburg Spain, maintained individuals with distinctive physical traits—often referred to as "dwarfs" or "court dwarves"—as symbols of curiosity, entertainment, and even political symbolism. Sebastián de Morra, the subject of Velázquez's portrait, was a court attendant in the entourage of King Philip IV, whose patronage of the arts fostered an environment where realism and human complexity were increasingly explored in visual representation. Unlike earlier depictions that emphasized caricature or exoticism, Velázquez's portrayal reflects a nuanced engagement with individuality, dignity, and psychological depth, aligning with broader Renaissance and Baroque shifts toward humanistic representation.

Figure 10. "The Dwarf Sebastian de Morra"



Source: https://www.wikiart.org/en/diego-velazquez/don-sebastian-de-morra

Clinical Manifestations of Hypogonadism: Hypogonadism in adult men encompasses a spectrum of clinical manifestations arising from deficient testosterone production or impaired gonadotropin signaling, often resulting in both sexual and systemic physiological disturbances. Common symptoms include reduced libido, erectile dysfunction, decreased muscle mass, increased visceral adiposity, and diminished energy levels, reflecting the hormone's broad metabolic and androgenic roles. Mood alterations such as depression, irritability, and cognitive decline are also frequently reported, underscoring the influence of testosterone on central nervous system function.[33] Physical signs may include loss of body hair, gynecomastia, and osteopenia or osteoporosis due to the essential role of androgens in bone remodeling.[34] These manifestations vary in severity depending on the age of onset and etiology-whether congenital, as in Klinefelter syndrome, or acquired through disease, medication, or aging—and require a comprehensive diagnostic approach integrating clinical evaluation and biochemical confirmation.[35]

FINAL CONSIDERATIONS

Art has long transcended its aesthetic function, serving as an unintentional yet valuable chronicle of human health and disease. This study highlights how visual representations in classical and contemporary art can offer insightful glimpses into the historical presence of endocrine disorders, often predating clinical descriptions by centuries. By identifying morphological features consistent with known hormonal pathologies, this analysis bridges art history and medical science, reinforcing the value of interdisciplinary approaches in understanding disease evolution. These findings affirm that artistic depictions are not only cultural artifacts but also potential repositories of early clinical observation, enriching our comprehension of endocrinology's historical landscape.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

EVALUATION OF FRUCTOSAMINE OVER 3 MONTHS AND HBA1C AT ENDPOINT: A PROSPECTIVE STUDY OF ESTIMATED MEAN GLUCOSE CONCORDANCE

AVALIAÇÃO DA FRUTOSAMINA AO LONGO DE 3 MESES E DA HBA1C NO ENDPOINT: ESTUDO PROSPECTIVO DA CONCORDÂNCIA DAS GLICEMIAS MÉDIAS ESTIMADAS

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ABSTRACT

Introduction: Glycemic monitoring relies predominantly on hemoglobin A1c (HbA1c), although its accuracy is compromised in patients with hemoglobinopathies or altered erythrocyte turnover. Fructosamine, reflecting 2-3-week glycemic exposure through glycated serum proteins, provides complementary monitoring capabilities. Converting both biomarkers to estimated average glucose (eAG) enhances clinical interpretability, yet limited prospective evidence exists regarding concordance between serial fructosamine-derived eAG and HbA1c-derived eAG measurements. **Objective:** To evaluate concordance between estimated mean glucose values derived from serial fructosamine measurements over three months and those calculated from endpoint HbA1c assessments in diabetic patients. **Methods:** This prospective observational cohort study enrolled 36 adults with diabetes mellitus at the HIPERDIA center in Itabuna, Brazil (July 2024-July 2025). Monthly fructosamine measurements were obtained alongside baseline and endpoint HbA1c determinations. Fructosamine-derived eAG utilized Andrade's equation: eAG(mg/dL) = (0.5157×fructosamine)-20. HbA1c-derived eAG employed Nathan's equation: eAG(mg/dL) = (28.7×HbA1c%)-46.7. Statistical analysis included Pearson correlation, Lin's concordance correlation coefficient, and Bland-Altman analysis. **Results:** Mean participant age was 58.4±12.7 years, with 80.6% having type 2 diabetes. Fructosamine declined from 312.4±45.8 to 305.2±44.6 µmol/L over three months. Strong correlations were observed between fructosamine-derived and HbA1c-derived eAG values (r=0.782-0.805, p<0.001), with substantial concordance (CCC=0.745-0.776). Systematic bias revealed consistent fructosamine underestimation (-34.1 to -35.9 mg/dL), with acceptable Bland-Altman limits of agreement (-78.4 to 6.6 mg/dL). **Conclusion:** Serial fructosamine measurements demonstrate substantial concordance with HbA1c-derived eAG, supporting its utility as complementary glycemic monitoring, particularly when HbA1c reliability is compromised.

Keywords: Fructosamine, HbA1c, Glycemic monitoring, Estimated average glucose, Diabetes mellitus.

RESUMO

Introdução: O monitoramento glicêmico baseia-se predominantemente na hemoglobina A1c (HbA1c), embora sua acurácia seja comprometida em pacientes com hemoglobinopatias ou alterações no turnover eritrocitário. A frutosamina, refletindo a exposição glicêmica de 2-3 semanas através de proteínas séricas glicosiladas, oferece capacidades de monitoramento complementares. A conversão de ambos os biomarcadores em glicose média estimada (GME) aprimora a interpretabilidade clínica, todavia, evidências prospectivas limitadas existem quanto à concordância entre medições seriadas de GME derivada de frutosamina e GME derivada de HbA1c. **Objetivo**: Avaliar a concordância entre valores de glicose média estimada derivados de medições seriadas de frutosamina ao longo de três meses e aqueles calculados a partir de avaliações de HbA1c no endpoint em pacientes diabéticos. **Métodos**: Este estudo prospectivo observacional de coorte incluiu 36 adultos com diabetes mellitus no centro HIPERDIA em Itabuna, Brasil (julho de 2024-julho de 2025). Medições mensais de frutosamina foram obtidas juntamente com determinações de HbA1c basal e no endpoint. A GME derivada de frutosamina utilizou a equação de Andrade: GME(mg/dL) = (0,5157×frutosamina)-20. A GME derivada de HbA1c empregou a equação de Nathan: GME(mg/dL) = (28,7×HbA1c%)-46,7. A análise estatística incluiu correlação de Pearson, coeficiente de correlação de concordância de Lin e análise de Bland-Altman. **Resultados:** A idade média dos participantes foi de 58,4±12,7 anos, com 80,6% apresentando diabetes tipo 2. A frutosamina declinou de 312,4±45,8 para 305,2±44,6 µmol/L ao longo de três meses. Correlações fortes foram observadas entre os valores de GME derivados de frutosamina e de HbA1c (r=0,782-0,805, p<0,001), com concordância substancial (CCC= 0,745-0,776). O viés sistemático revelou subestimação consistente da frutosamina (-34,1 a -35,9 mg/dL), com limites de concordância de Bland-Altman aceitáveis (-78,4 a 6,6 mg/dL). **Conclusão**: Medições seriadas de frutosamina demonstram concordância substancial com GME derivada de HbA1c, sustentando sua utilidade como monitoramento glicêmico complementar, particularmente quando a confiabilidade da HbA1c está comprometida.

Descritores: Frutosamina, HbA1c, Monitoramento glicêmico, Glicose média estimada, Diabetes mellitus.

INTRODUCTION

Glycemic monitoring is essential for effective diabetes management, guiding both therapeutic decisions and long-term outcomes. Hemoglobin A1c (HbA1c) remains the gold standard, reflecting average glycemia over the preceding 2–3 months. However, its accuracy can be compromised in patients with anemia, hemoglobinopathies, or conditions affecting red blood cell turnover, highlighting the need for reliable alternative markers.[1,2]

Fructosamine, which measures glycated serum proteins (primarily albumin), reflects glycemic control over the preceding 2–3 weeks and provides a valuable complement to HbA1c in these clinical scenarios. It is particularly useful for monitoring short-term changes in glucose levels, such as during therapy adjustments or in pregnancy.[3] Studies show moderate to strong correlations between fructosamine and HbA1c (r = 0.75–0.91), supporting its role as a surrogate marker of glycemic exposure.[4]

Converting both biomarkers into estimated average glucose (eAG) enhances clinical interpretability. While HbA1c-derived eAG uses established equations (eAG (mg/dL) = [(28.7 × HbA1c%) – 46.7]),[5] recent work has validated a fructosamine-based formula: eAG (mg/dL) = (0.5157 × fructosamine) − 20, showing strong linearity in diverse populations.[6] This equation, developed and validated in Brazilian cohorts, improves the translation of fructosamine into clinically actionable data.

Despite these advances, limited prospective evidence exists regarding the concordance between eAG derived from serial fructosamine measurements and HbA1c-derived eAG. Most studies rely on single fructosamine assessments, potentially underestimating its value. The integration of multiple fructosamine values—such as monthly measurements over 3 months—

may offer a more stable and accurate eAG estimate, better aligned with the integrated nature of HbA1c.

The objective of this prospective study was to evaluate the concordance between estimated mean glucose values derived from serial fructosamine measurements obtained over a 3-month period and those calculated from endpoint HbA1c assessments in a diverse cohort of patients with diabetes mellitus. Secondary aims included characterizing the temporal patterns of fructosamine-derived eAG estimations, determining optimal mathematical approaches for integrating multiple fructosamine measurements, and assessing the clinical utility of fructosamine series in enhancing glycemic monitoring precision compared to traditional single-point HbA1c-derived eAG calculations.

METHODS

Study Design

This prospective observational cohort study was conducted at the HIPERDIA center in Itabuna, Bahia, Brazil, from July 2024 and July 2025. The study aimed to evaluate the concordance between eAG derived from serial fructosamine measurements over three consecutive months and eAG calculated from HbA1c measured at month 3.

The study was conducted in strict accordance with the principles of the Declaration of Helsinki for ethical research involving human subjects. Comprehensive confidentiality protocols and data protection measures were implemented throughout the study to ensure patient privacy and protect sensitive clinical information. The Ethics Committee of the Reference Center for Diabetes and Hypertension has reviewed the research project, and has determined that it complies with the stipulations of CNS Resolution No. 196/96.

Participants

Participants were adults (≥18 years) with a clinical diagnosis of type 1 or type 2 diabetes mellitus, under stable glycemic treatment for at least 3 months prior to enrollment. Exclusion criteria included: pregnancy or lactation; significant hepatic dysfunction (alanine aminotransferase >3× upper limit of normal); advanced chronic kidney disease (estimated glomerular filtration rate <30 mL/min/1.73 m²); known hemoglobinopathies or conditions affecting hemoglobin structure; active malignancy; recent blood transfusion within 3 months; severe anemia (hemoglobin <8.0 g/dL); and acute illness or hospitalization within 4 weeks of enrollment.

Sample size calculation was performed using G*Power 3.1.9.7 software, assuming a correlation coefficient of 0.80 between fructosamine-derived and HbA1c-derived eAG values, with $\alpha = 0.05$ and power = 90%. The minimum required sample size was determined to be 19 participants. To account for potential dropouts and enhance statistical power, we aimed to recruit 36 participants.

Data Collection Protocol

Participants were followed longitudinally with monthly assessments (Month 1, Month 2, Month 3). Serum fructosamine and HbA1c levels were measured at each medical appointment. Clinical data—including demographics, medical history, current medications, anthropometric measurements, and vital signs—were systematically recorded using standardized forms.

Laboratory Analyses

Fructosamine Measurements

Serum fructosamine concentrations were determined using the nitro blue tetrazolium (NBT) colorimetric reduction method on a Cobas Integra® system (Roche). The assay principle involves the reduction of NBT by fructosamine under alkaline conditions, producing a colored formazan compound measured spectrophotometrically at 530 nm. Quality control was maintained through daily calibration using certified reference materials and participation in external quality assurance programs. The analytical measurement range was 125-750 µmol/L, with intra-assay and inter-assay coefficients of variation of <3% and <5%, respectively.

HbA1c Analysis

HbA1c levels were measured at baseline and endpoint (month 3) using high-performance liquid chromatography (Variant™ II, Bio-Rad) methodology certified by the National Glycohemoglobin Standardization Program (NGSP) and traceable to the Diabetes Control and Complications Trial reference method. The analytical measurement range was 4.0-15.0%, with precision specifications of <2% coefficient of variation.

Additional Laboratory Parameters

Complete blood count, comprehensive metabolic panel, liver function tests, and lipid profile were obtained at baseline and endpoint to characterize the study population and identify potential confounding factors

Estimated Average Glucose Calculations

HbA1c-Derived eAG

Estimated average glucose from HbA1c was calculated using the validated equation established by Nathan et al.: eAG (mg/dL) = (28.7 × HbA1c%) - 46.7.[5]

Fructosamine-Derived eAG

Individual monthly fructosamine-derived eAG values were calculated using the recently validated Brazilian formula: eAG (mg/dL) = (0.5157 × fructosamine µmol/L) - 20.[6]

Multiple integration approaches were employed to derive composite eAG estimates from the three monthly fructosamine measurements: Simple arithmetic mean: $(eAG_1 + eAG_2 + eAG_3) / 3$.

Statistical Analysis

Statistical analyses were performed using R version 4.5.1, and PSPP (public domain software). Normality of continuous variables was assessed using the Shapiro-Wilk test and visual inspection of Q-Q plots. Descriptive statistics were reported as mean ± standard deviation for normally distributed variables, median (interquartile range) for non-parametric data, and frequencies (percentages) for categorical variables.

Primary analysis examined the concordance between fructosamine series-derived eAG and endpoint HbA1c-derived eAG using Pearson correlation coefficients, Lin's concordance correlation coefficient (CCC), and Bland-Altman analysis. The concordance correlation coefficient was interpreted as: <0.90 poor, 0.90-0.95 moderate, 0.95-0.99 substantial, and >0.99 almost perfect agreement.

Secondary analyses included: (1) comparison of different mathematical integration methods using root mean square error and mean absolute error; (2) subgroup analyses stratified by diabetes type, glycemic control status (HbA1c <7% vs ≥7%), and demographic characteristics; (3) temporal trend analysis using repeated measures ANOVA; and (4) multivariable linear regression to identify factors associated with eAG concordance.

Bland-Altman plots were constructed to assess systematic bias and limits of agreement (mean difference ± 1.96 × standard deviation). Clinical significance was defined as mean difference <10 mg/dL and 95% limits of agreement within ±30 mg/dL, based on established glucose monitoring accuracy criteria.

Missing data were handled using multiple imputation techniques when appropriate, with sensitivity analyses performed to assess the impact of missing observations. Statistical significance was set at $p < 0.05$, with Bonferroni correction applied for multiple comparisons when appropriate

RESULTS

Demographics and Clinical Characteristics

Mean age was 58.4 ± 12.7 years, indicating middle-aged participants with moderate variability. Sex distribution was balanced, with males and females each comprising 50.0% of the sample. The diabetes type is predominantly Type 2, accounting for 80.6%, while Type 1 comprises 19.4%. Baseline medications reveal the majority use metformin (72.2%), followed by insulin (38.9%) and sulfonylureas (33.3%), reflecting the therapeutic landscape within this population. The **Fig. 1** presents a horizontal bar chart illustrating key clinical and demographic parameters of the study cohort.

Fructosamine Measurements and Derived eAG Values

Serial fructosamine measurements demonstrated a progressive decline over the 3-month observation period, with mean values decreasing from 312.4 ± 45.8 µmol/L at baseline to 305.2 ± 44.6 µmol/L at month 3, representing a 2.3% reduction in glycated protein concentrations. The corresponding fructosamine-derived estimated average glucose values exhibited parallel decremental trends, declining from 141.0 ± 23.6 mg/dL to 137.4 ± 23.0 mg/dL, indicating improved short-term glycemic control throughout the study period. Median fructosamine concentrations consistently remained below arithmetic means across all time points, suggesting a right-skewed distribution with several patients exhibiting markedly elevated glycated protein levels. The interquartile ranges demonstrated stable variability patterns (approximately 67-70 µmol/L) across monthly assessments, indicating consistent population heterogeneity in glycemic control status. Overall mean fructosamine-derived eAG of 139.2 ± 22.4 mg/dL corresponded to intermediate glycemic control, with the observed temporal reduction suggesting potential therapeutic optimization or improved diabetes self-management during the monitoring period **Table 1.**

HbA1c Measurements and Derived eAG Values

HbA1c levels exhibited a mean of 7.8 ± 1.2 at baseline, decreasing slightly to 7.6 ± 1.1 by the endpoint, with an overall mean of 7.7 ± 1.1; median values and interquartile ranges (IQR) also showed a modest reduc-

Figure 1. Baseline Demographics and Clinical Characteristics.

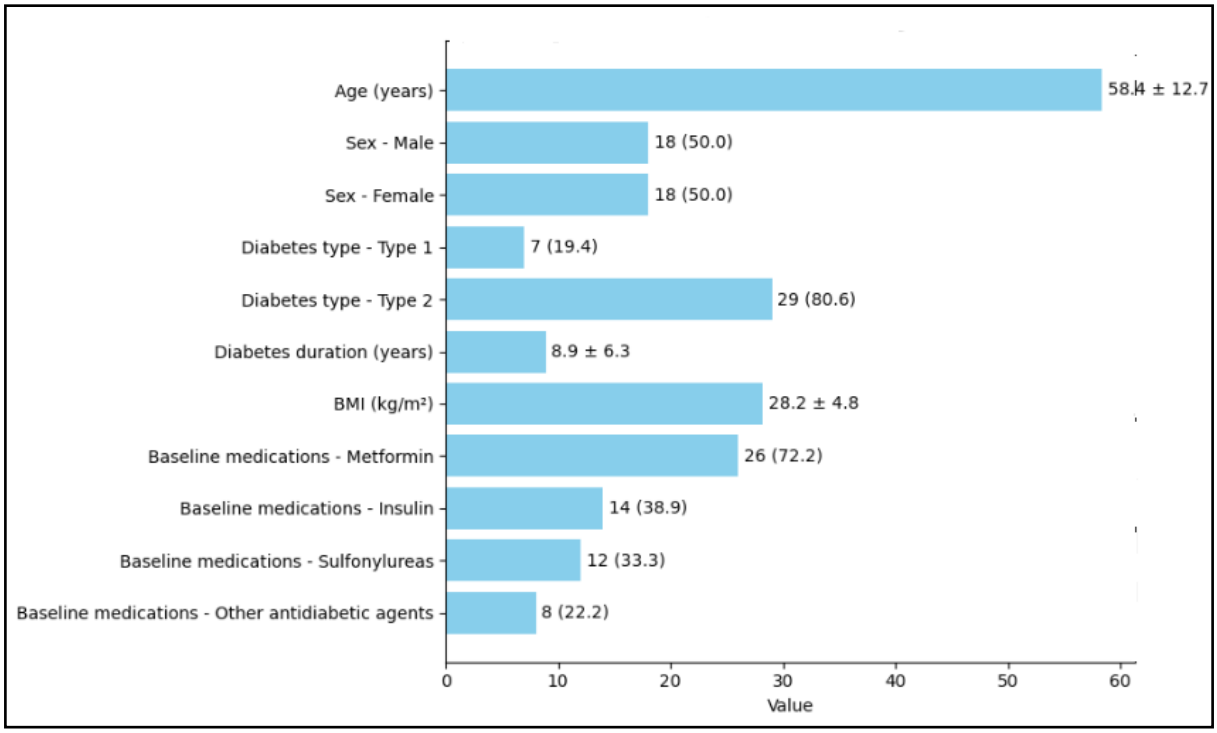

Table 1. Fructosamine Measurements and Derived eAG Values Over Time.

Parameter	Month 1	Month 2	Month 3	Overall Mean
Fructosamine (µmol/L)				
- *Mean ± SD*	312.4 ± 45.8	308.7 ± 42.1	305.2 ± 44.6	308.8 ± 43.5
- *Median (IQR)*	308.0 (278.5–345.0)	305.0 (275.0–340.0)	302.0 (272.0–337.0)	305.3 (275.2–340.7)
- *Range*	235–425	238–418	240–415	237.7–419.3
Fructosamine-derived eAG (mg/dL)[a]				
- *Mean ± SD*	141.0 ± 23.6	139.1 ± 21.7	137.4 ± 23.0	139.2 ± 22.4
- *Median (IQR)*	138.8 (123.6–157.9)	137.3 (121.8–155.3)	135.7 (120.2–153.7)	137.3 (121.9–155.6)
- *Range*	101.1–199.0	102.6–195.4	103.7–193.9	102.4–196.1

tion from 7.6 (6.9–8.5) to 7.4 (6.8–8.2), with a mean IQR of 7.5 (6.9–8.4). The range of HbA1c values narrowed from 5.8–10.4 to 5.9–10.1, averaging 5.9–10.3. Similarly, HbA1c-derived eAG (mg/dL) demonstrated a mean of 177.0 ± 34.4 at baseline, slightly declining to 171.5 ± 31.6 at the endpoint, with an overall mean of 174.3 ± 32.8; median values with IQR decreased from 171.4 (151.3–197.0) to 165.8 (148.6–188.8), averaging 168.6 (151.0–194.2). The eAG range also contracted from 119.8–252.1 to 122.6–243.6, with an overall range of 122.4–248.9, indicating a trend toward improved glycemic control over the study period **Table 2**.

Concordance Analysis Between Fructosamine and HbA1c-Derived eAG

The concordance analysis between fructosamine-derived and HbA1c-derived estimated average glucose values demonstrated strong positive correlations across all temporal comparisons, with Pearson correlation coefficients ranging from 0.782 to 0.805 ($p<0.001$), indicating robust linear relationships between the two glycemic biomarkers. Lin's CCC ranged from 0.745 to 0.776, suggesting substantial agreement between measurement methods, with the highest concordance observed for the mean values comparison (CCC=0.776, 95% CI: 0.651-0.861). Systematic bias analysis revealed consistent underestimation of eAG by fructosamine-derived calculations, with mean differences ranging from -34.1 to -35.9 mg/dL, indicating that fructosamine systematically yielded lower eAG estimates compared to HbA1c-derived values. Bland-Altman analysis demonstrated acceptable limits of agreement within clinically relevant ranges (-78.4 to 6.6 mg/dL), with narrow confidence intervals suggesting reliable measurement precision across the observed glycemic spectrum. The temporal stability of correlations (r=0.782 at month 1 vs r=0.798 at month 3) and the enhanced concordance observed with mean values (r=0.805) support the utility of serial fructosamine measurements for improved eAG estimation accuracy **Table 3.**

Table 2. HbA1c Measurements and Derived eAG Values.

Parameter	Month 1 (Baseline)	Month 3 (Endpoint)	Mean
HbA1c (%)			
- *Mean ± SD*	7.8 ± 1.2	7.6 ± 1.1	7.7 ± 1.1
- *Median (IQR)*	7.6 (6.9–8.5)	7.4 (6.8–8.2)	7.5 (6.9–8.4)
- *Range*	5.8–10.4	5.9–10.1	5.9–10.3
HbA1c-derived eAG (mg/dL)[b]			
- *Mean ± SD*	177.0 ± 34.4	171.5 ± 31.6	174.3 ± 32.8
- *Median (IQR)*	171.4 (151.3–197.0)	165.8 (148.6–188.8)	168.6 (151.0–194.2)
- *Range*	119.8–252.1	122.6–243.6	122.4–248.9

Table 3. Concordance Analysis Between Fructosamine and HbA1c-Derived eAG.

Comparison	Correlation Coefficient (r)	p-value	Lin's CCC	95% CI	Bias (mg/dL)	Limits of Agreement (mg/dL)
Fructosamine eAG (Month 1) vs HbA1c eAG (Month 1)	0.782	<0.001	0.745	0.612–0.836	-35.9	-78.4 to 6.6
Fructosamine eAG (Month 3) vs HbA1c eAG (Month 3)	0.798	<0.001	0.763	0.635–0.851	-34.1	-74.8 to 6.6
Mean Fructosamine eAG vs Mean HbA1c eAG	0.805	<0.001	0.776	0.651–0.861	-35.0	-76.6 to 6.6

Subgroup Analysis by Glycemic Control Status

A subgroup analysis stratified by glycemic control status demonstrated markedly different glycemic profiles between cohorts. The well-controlled group (HbA1c <7%) exhibited significantly lower mean estimated average glucose (eAG) values for both fructosamine and HbA1c ($p<0.001$ for both) compared to the poorly-controlled group (HbA1c ≥7%). Although the correlation (r) between the two eAG measures was strong in both subgroups, the difference in correlation coefficients was not statistically significant ($p=0.285$). Similarly, while a greater mean bias was observed in the poorly-controlled cohort, this difference did not reach statistical significance ($p=0.142$) **Table 4**.

Temporal Trends Analysis (Repeated Measures ANOVA)

Temporal trends analysis using repeated measures ANOVA revealed modest but measurable changes in glycemic parameters over the 3-month observation period, with varying degrees of statistical significance and clinical impact. Fructosamine levels demonstrated a borderline significant temporal trend ($F=2.84$, $p=0.063$, $\eta^2=0.075$), suggesting a small effect size for the observed decline in glycated protein concentrations across monthly assessments. Similarly, fructosamine-derived estimated average glucose values showed comparable temporal dynamics ($F=2.91$, $p=0.059$, $\eta^2=0.077$), approaching statistical significance with a small-to-moderate effect size indicating clinically relevant improvements in short-term glycemic control. In contrast, HbA1c demonstrated a statistically significant reduction from baseline to endpoint ($F=4.12$, $p=0.042$, $\eta^2=0.105$), with a moderate effect size suggesting meaningful improvement in long-term glycemic status over the study duration. The progressively increasing effect sizes from fructosamine parameters ($\eta^2\approx0.075$-0.077) to HbA1c change ($\eta^2=0.105$) reflect the differential sensitivity of these biomarkers to temporal glycemic variations, with HbA1c showing greater responsiveness to sustained metabolic improvements compared to the more dynamic fructosamine measurements **Table 5.**

DISCUSSION

This study provides robust prospective evidence supporting the clinical utility of serial fructosamine measurements as a complementary tool for glycemic monitoring in diabetic patients. Our results demonstrate a substantial level of concordance

Table 4. Subgroup analysis stratified by glycemic control status

Parameter	Well-controlled (HbA1c <7%, n=12)	Poorly-controlled (HbA1c ≥7%, n=24)	p-value
Mean Fructosamine eAG (mg/dL)	122.4 ± 15.8	147.4 ± 20.7	<0.001
Mean HbA1c eAG (mg/dL)	151.2 ± 18.2	186.0 ± 28.4	<0.001
Correlation (r)	0.721	0.798	0.285
Mean bias (mg/dL)	-28.8	-38.6	0.142

Table 5. Temporal Trends Analysis - ANOVA

Parameter	F-statistic	p-value	Effect Size (η^2)
Fructosamine levels over time	2.84	0.063	0.075
Fructosamine-derived eAG over time	2.91	0.059	0.077
HbA1c change (Month 1 to 3)	4.12	0.042	0.105

between estimated average glucose derived from repeated fructosamine assessments and that obtained from hemoglobin A1c, reinforcing the potential of fructosamine to reliably reflect medium-term glycemic control. This is particularly relevant in settings where HbA1c interpretation may be limited, thereby enhancing the precision and individualization of diabetes management.

Fructosamine has emerged as a valuable tool for assessing short-term glycemic control, particularly in clinical settings where HbA1c may be unreliable. The recent validation of the eAG equation using fructosamine has enhanced its clinical interpretability and comparability with HbA1c-derived eAG, demonstrating strong linearity and reliability across diverse diabetic populations.[6] This model aligns with prior evidence supporting moderate to strong correlations between fructosamine and glycemic exposure, reinforcing its utility in monitoring dynamic glucose fluctuations over a 2- to 3-week period.[7] Furthermore, ADA guidelines and international consensus recognize fructosamine as a suitable alternative biomarker when HbA1c interpretation is confounded, emphasizing the importance of standardized eAG conversion for clinical decision-making.[8] Compared with previous studies, our study corroborates the temporal reliability and clinical responsiveness of serial fructosamine assessments, demonstrating consistent directional trends in eAG that reflect mean glycemic levels and supporting the integration of repeated fructosamine measurements into routine monitoring protocols for enhanced glycemic management.

Recent diabetes management guidelines emphasize HbA1c as the primary biomarker for long-term glycemic assessment, with recent systematic reviews demonstrating that modest HbA1c reductions through structured interventions correlate with meaningful improvements in clinical outcomes, while acknowledging inherent limitations in populations with altered hemoglobin kinetics.[9,10] The established linear relationship between HbA1c and eAG, defined by the ADAG study equation, provides the foundation for translating percentage-based measurements into clinically interpretable glucose concentrations that facilitate patient education and therapeutic decision-making.[5] Our results align with established clinical patterns, where the observed baseline HbA1c levels reflect typical presentations in real-world diabetes populations, and the modest temporal improvements parallel effect sizes reported in recent systematic reviews, with corresponding eAG values demonstrating appropriate concordance with the validated equations and supporting the clinical applicability of comparative glycemic biomarker analyses.

Published data demonstrate moderate to strong correlations between fructosamine and HbA1c across diverse populations, with studies reporting correlation coefficients that approach established thresholds for clinical utility. However, the accuracy of these assessments is influenced by albumin concentrations and protein metabolism.[11,12] Systematic patterns of bias between these biomarkers are well-documented, with fructosamine consistently yielding lower glycemic estimates than HbA1c. This discrepancy is attributed to differential glycation kinetics and the shorter time window of fructosamine assessment compared to the integrated nature of HbA1c measurements.[13,14] The clinical significance of concordance analyses between alternative glycemic biomarkers has gained renewed attention in precision diabetes care, particularly in populations where HbA1c reliability may be compromised. Modern laboratory methodologies have demonstrated improved diagnostic performance through biomarker integration strategies.[15] The findings from the present study are aligned with the current published data, which also reports a significant positive correlation and substantial agreement between the two glycemic estimation methods. Our results corroborate the well-documented systematic bias, confirming that fructosamine-derived eAG consistently underestimates values compared to the HbA1c-based method. The acceptable limits of agreement demonstrated through our Bland-Altman analysis fall within clinically relevant ranges established by recent glycemic monitoring accuracy standards, while the temporal stability of correlations observed across our study period provides compelling evidence for the consistency and reproducibility of serial fructosamine measurements in enhancing glycemic assessment precision.

Longitudinal studies demonstrate differential temporal responsiveness between fructosamine and HbA1c, with fructosamine showing superior correlation with short-term glycemic changes, while HbA1c demonstrates greater responsiveness to sustained metabolic improvements over extended periods.[16,17] Recent investigations emphasize the complementary temporal profiles of these biomarkers in clinical monitoring strategies.[18] Our temporal trend analysis aligns with the literature, which demonstrates distinct kinetic responses of fructosamine and HbA1c to glycemic changes over time. The progressively increasing effect sizes of fructosamine parameters for HbA1c changes observed in our study corroborate the established understanding that HbA1c exhibits superior sensitivity to

sustained metabolic improvements due to its longer temporal integration window.

Thus, our analysis indicates that mean glucose estimated from three serial fructosamine measurements demonstrates strong concordance with HbA1c-derived averages, albeit with minor systematic bias. This concordance highlights fructosamine's reliability when integrated longitudinally, rather than as a single measurement, reinforcing its value as a complementary biomarker in diabetes monitoring. Particularly in contexts where HbA1c-based metrics falter, multiple fructosamine results offer reliable and temporally responsive insights into glycemic control. These findings substantiate its role as a complementary biomarker in integrated diabetes management.[19-21]

CONCLUSION

The present study substantiates the significant concordance between serial fructosamine-derived and HbA1c-derived estimated average glucose, reinforcing fructosamine's viability as a complementary biomarker in glycemic monitoring. This approach enhances precision in diabetes management, particularly in clinical scenarios compromising HbA1c reliability, thereby supporting integrated glycemic assessment strategies for optimized therapeutic decision-making.

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ORIGINAL ARTICLE: TOPIC IN MEDICAL CLINIC *ARTIGO ORIGINAL: TÓPICO EM CLÍNICA MÉDICA*

COMPARISON OF METHOTREXATE GASTROINTESTINAL SIDE EFFECTS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND PSORIATIC ARTHRITIS

COMPARAÇÃO DOS EFEITOS COLATERAIS GASTROINTESTINAIS DO METOTREXATO EM PACIENTES COM ARTRITE PSORIÁSICA E PACIENTES COM ARTRITE REUMATOIDE

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ABSTRACT

Introduction: Psoriatic arthritis (PsA) and rheumatoid arthritis (RA) are chronic, inflammatory, autoimmune diseases. Psoriatic arthritis is a type of spondyloarthritis associated with psoriasis that affects the musculoskeletal system and is negative for rheumatoid factor, whereas rheumatoid arthritis affects the synovial membrane of peripheral joints and is characterized by an additive, symmetrical polyarthritis involving both small and large joints. Methotrexate (MTX) is a safe, effective, and low-cost treatment option for both conditions; however, it can lead to side effects, the most common of which are gastrointestinal disturbances. **Objective:** To compare the gastrointestinal adverse effects of methotrexate in patients with psoriatic arthritis and rheumatoid arthritis. **Methods:** This is a cross-sectional, observational analytical study of patients with psoriatic arthritis and rheumatoid arthritis who were undergoing methotrexate treatment. Data collection was carried out through medical chart reviews and administration of the MISS (Methotrexate Intolerance Severity Score) questionnaire. **Results**: A total of 112 patients were studied, including 67 with RA and 45 with PsA. The PsA group demonstrated significantly greater gastrointestinal intolerance compared to the RA group ($p = 0.007$). Among the patients who did not tolerate MTX (37 patients with MISS ≥ 6 points), 62% had PsA. The results also indicated that individuals with a positive qualitative MISS score were nearly four times more likely to experience MTX intolerance ($p = 0.0009$). The average body mass index (BMI) of the group with gastrointestinal intolerance to MTX was higher than that of the group that tolerated MTX ($p = 0.002$). Variables such as age, sex, smoking status, folic acid use and route of administration, and mean corpuscular volume (MCV) in blood tests were not statistically significant in relation to gastrointestinal tolerability of MTX. **Conclusion:** Patients with PsA showed greater intolerance to MTX than patients with RA, and higher BMI values were found in patients with more gastrointestinal side effects related to the medication. However, no association was found between these manifestations and MCV values, MTX dose or administration route, or folic acid use.

Keywords: Rheumatoid Arthritis, Psoriatic Arthritis, Methotrexate.

RESUMO

Introdução: A artrite psoriásica (APSO) e a artrite reumatoide (AR) são doenças inflamatórias, crônicas e autoimunes. A artrite psoriásica é uma espondiloar-

trite relacionada à psoríase que acomete o sistema musculoesquelético e é negativa para o fator reumatoide, enquanto a artrite reumatoide afeta a membrana sinovial das articulações periféricas e se caracteriza por uma poliartrite, aditiva, simétrica em pequenas e grandes articulações. Ambas possuem como opção terapêutica o uso de metotrexato (MTX), um medicamento seguro, eficaz e de baixo custo, mas que possui alguns efeitos colaterais, sendo o mais frequente relacionado a alterações gastrointestinais. **Objetivo:** Comparar os efeitos adversos gastrointestinais do metotrexato em pacientes com artrite psoriásica e artrite reumatoide. **Métodos:** Este é um estudo observacional analítico transversal de pacientes com artrite psoriásica e artrite reumatoide em uso de metotrexato. A coleta de dados foi feita a partir da análise de prontuários e aplicação do questionário MISS (*Methotrexate Intolerance Severity Score*). **Resultados:** Estudaram 112 pacientes, sendo 67 com AR e 45 com APSO. O grupo de pacientes com APSO demonstrou mais intolerância gastrointestinal quando comparado ao grupo da AR (p=0.007). Dos pacientes que não toleraram o MTX (37 pacientes com MISS≥ 6 pontos) 62% possuíam APSO. Os resultados também apontaram que indivíduos com MISS qualitativo positivo têm quase quatro vezes mais chances de não tolerar o MTX (p=0,0009). O índice de massa corporal (IMC) médio do grupo com intolerância gastrointestinal ao MTX foi maior do que o IMC dos que toleram o MTX (p=0,002). As variáveis que analisaram idade, sexo, fumo, uso de ácido fólico e via de administração de ácido fólico, e valor corpuscular médio (VCM) no hemograma não foram estatisticamente relevantes para a tolerabilidade gastrointestinal ao MTX. **Conclusão:** Pacientes com APSO apresentaram mais intolerância ao MTX que pacientes com AR e valores mais elevados de IMC foram encontrados nos pacientes com mais efeitos gastrointestinais relacionados à medicação. Porém, não houve associação entre essas manifestações e os valores do VCM, a dose e via de administração do MTX e ao uso de ácido fólico.
Descritores: Artrite Reumatoide, Artrite Psoriásica, Metotrexato.

INTRODUCTION

Psoriatic arthritis (PsA) is an inflammatory joint disease associated with skin psoriasis that is negative for rheumatoid factor. [1] It has several classification systems, with the most commonly used currently being the CASPAR criteria. [2] The prevalence of PsA in the general population ranges from 0.1% to 1%, while among those with psoriasis, between 8% to 42%. It occurs in individuals of all ages, with a peak incidence in middle age, around 40–50 years, affecting similarly both sexes.[1-3] Although its etiology is not fully understood, it is known to be a multifactorial disease, in which the presence of genes such as HLA-B27, HLA-B38, HLA-B39, and HLA-B08, along with environmental factors, leads to the release of pro-inflammatory cytokines and, consequently, to the clinical manifestations of the disease.[3]

Rheumatoid arthritis (RA) is a systemic autoimmune disease that presents with symmetrical polyarthritis affecting both small and large joints, which can lead to periarticular damage and systemic inflammation with extra-articular manifestations.[4] Its prevalence varies according to ethnic characteristics; it is estimated to affect approximately 1% of the global population.[5] RA prevalence increases with aging, and this disease has a female-to-male ratio of approximately 3:1. Although its pathogenesis is not completely known, individuals with the "shared epitope," including HLA genotypes such as HLA-DR4 or HLA-DRB1, have a higher risk of having RA and of developing a more severe disease. The disease is also associated with environmental risk factors, including smoking and obesity. [4]

Both diseases have methotrexate (MTX) as the first-line treatment due to its safety, efficacy, and low cost.[6] MTX is an antimetabolite, antiproliferative, and anti-inflammatory agent that acts as an antagonist of dihydrofolate reductase, inhibiting folic acid synthesis.[7] Among its adverse effects, gastrointestinal symptoms are the most frequent, especially nausea, diarrhea, vomiting, and abdominal pain.[8] This intolerance can be assessed using the Methotrexate Intolerance Severity Score (MISS), in which a score equal to or

greater than 6 is required for the patient to be considered intolerant to MTX.[6] Other factors may be associated with the presence of these adverse effects, such as folic acid supplementation and mean corpuscular volume (MCV).[9] Although gastrointestinal intolerance occurs in both PsA and RA, few studies have compared the prevalence and characteristics of these adverse effects between the diseases.

This study aimed to compare the gastrointestinal adverse effects of methotrexate in patients with psoriatic arthritis and rheumatoid arthritis; to analyze whether the dose and administration route influence the occurrence of gastrointestinal adverse effects; and to investigate whether elevated MCV is associated with MTX-related gastrointestinal side effects.

METHODS

Study Design

This research is a cross-sectional, analytical, observational study.

Ethical Aspects

The study was approved by the Human Research Ethics Committee (CEP) of the Mackenzie Evangelical University Hospital (HUEM) – Mackenzie Evangelical College of Paraná (FEMPAR), under approval number 6.666.667. All participants signed an informed consent form.

Participants

The total sample consisted of 112 patients—45 with PsA and 67 with RA—attending to the Rheumatology outpatient clinic at HUEM. This was a convenience sample that included all patients using MTX for RA or PsA who met the inclusion and exclusion criteria, agreed to participate in the study, and attended the rheumatology clinic from March 2024 to March 2025.

Inclusion Criteria

Patients of both sexes, aged 18 years or older, diagnosed with psoriatic arthritis (based on CASPAR criteria) or rheumatoid arthritis (according to the 2010 classification criteria of the European League Against Rheumatism and the American College of Rheumatology) were included, provided they complied, signing the informed consent form.

Exclusion Criteria

Patients using non-steroidal anti-inflammatory drugs (NSAIDs) or other medications that could cause gastrointestinal adverse effects, as well as those diagnosed with inflammatory bowel disease, were excluded.

Data collection

The following data were collected from medical records or through patient interviews:

- **a.** Demographic and anthropometric data: age, age at disease onset, sex, race, smoking status, weight and height for body mass index (BMI) calculation.
- **b.** Laboratory data: white blood cell count, mean corpuscular volume (MCV), hematocrit, and platelet count.
- **c.** Clinical data: gastrointestinal manifestations and concurrent medication use, including folic acid.
- **d.** Application of the MISS questionnaire to assess intolerance and gastrointestinal adverse effects related to methotrexate in PsA and RA patients[6]. This questionnaire has 12 questions. In the absence of symptoms, a score of 0 is assigned, and in the presence of symptoms, scores range from 1 (mild) to 3 (severe). The maximum score is 36 (indicating severe gastrointestinal intolerance to MTX), and the minimum is 0 (no GI adverse effects). A score ≥6 indicates MTX intolerance.

Statistical Analysis

Data were collected and stored in a Microsoft Excel spreadsheet. Statistical analyses were performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA). Results were expressed as means, medians, minimum and maximum values, and standard deviations (quantitative variables), or as frequencies and percentages (qualitative variables). Inferential analysis used Chi-square and Fisher's exact tests for categorical data, and Student's t-test or Mann–Whitney test for numerical data. A p-value <0.05 was considered statistically significant.

RESULTS

Sample Analysis

The total sample consisted of 112 patients: 45 with psoriatic arthritis and 67 with rheumatoid arthritis. In both disease groups; the majority were female and of Caucasian ethnicity. Regarding BMI, both groups had a mean value above the ideal weight range: RA patients were classified as overweight, while PsA patients had class I obesity.

Table 1. Description of the Studied Sample.

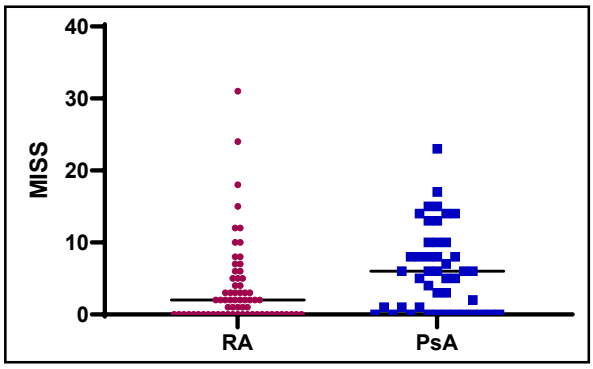
	TOTAL	Rheumatoid arthritis	Psoriatic arthritis
Number	112	67	45
Female gender	82/112	57/67	20/45
Ethnic background			
Caucasians	99/112	59/67	40/45
Afrodescendent	12/112	7/67	5/45
Asian	1/112	1/67	0
Age- years - mean (SD)	57.6(11.5)	58.9 (12.3)	55.5(10.2)
Age at diagnosis –years -mean (SD)	46.8 (11.7)	46.5 (12.2)	47.4 (11.2)
Tobacco exposure – n	7/112	7/67	0
BMI- mean (SD) – Kg/m^2	29.2(5.7)	27.9 (5.3)	31.0 (5.8)
Leucocytes– n/mm3 – median (IQR)	6.960 (5575-8515)	6240 (5350-8280)	7250 (5985-9070)
MCV – fl - median - (IQR)	89.4 (86.0-93.0)	90.1 (87.0-93.0)	88.4 (84.2-92.7)
Hematocrite – % - median (IQR)	40.5 (38.1-43.6)	40.0 (38.1-41.6)	42.9 (38.5-45.9)
Platelets - n/mm3 – median (IQR)	231.950 (194.375-279.375)	226.200 (184.000-291.000)	234.000 (197.500-262.900)
Not using folic acid– (n)	9/110	3/65	6/45
MTX dose - mg/week – median (IQR)	20.0 (15.0-25.0)	20.0 (15.0-25.0)	15.0 (15.0-25.0)
Administration route			
Oral	99/112	60/67	39/45
Subcutaneous	13/112	7/67	6/45
Treatment			
Anti TNF	22/112	10/67	12/45
Secuquinumab	16/112	0	16/45
Risanquizumab	2/112	0	2/45
iJak	7/112	7/67	0
Leflunomide	28/112	22/67	6/45
Hidroxicloroquin	5/112	5/67	0
Sulfassalazin	2/112	2/67	0
Tocilizumab	2/112	2/67	0
Rituximab	5/112	5/67	0
Prednisone	22/112	20/67	2/45

N= number; BMI= body mass index, VCM=mean corpuscular volume; TNF= tumoral necrosis factorl; iJAK- JAK inhibitor; SD= standard deviation; IQR= interquartile range.

MISS Score Comparison

Comparison of MISS scores between RA and PsA patients revealed a statistically significant difference between the two groups. See **Fig. 1.**

Figure 1. Comparison of MISS values between rheumatoid arthritis (RA) and psoriatic arthritis (PsA) patients.



RA: median MISS = 2.0 (0.0–5.0); PsA: median MISS = 6.0 (0.0–23.0); p = 0.007.

In the RA group, 14/67 (20.8%) patients were intolerant to MTX, while in the PsA group, 23/45 (51.1%) were intolerant (p = 0.0009).

Comparison of MTX tolerant and intolerant patients with RA and PsA

The comparison between the two groups is shown in **Table 2**. The only observed significant difference was in BM; higher BMI was associated with greater intolerance.

DISCUSSION

This study demonstrated that patients with PsA, female sex, and higher BMI were more likely to be intolerant to MTX. Almalag et al., in a study with 117 RA patients, showed that among the 55 classified as intolerant to MTX, 94.5% were women.[10] Additionally, Bulatović Calasan et al., in a study of 291 patients (249 with RA and 42 with PsA) also found that female patients had lower MTX tolerability (75%) compared to male patients (25%).[11] Almalag et al. suggested that this could be due to MTX being primarily excreted by the kidneys, and since glomerular filtration rate is lower in women, serum levels may be higher.[10]

Regarding the higher MTX intolerance observed in the current study, the literature remains controversial. In the study by Bulatović Calasan et al., a similar prevalence of gastrointestinal intolerance was observed between groups, although slightly higher in PsA patients (14.3%) compared to RA patients (10.4%).[12] Conversely, Dalkilic et al., in a study of 420 patients (346 with RA and 74 with PsA), concluded that patients in both groups tolerated MTX similarly.[13]

In this sample, PsA patients had a mean BMI of 31 Kg/m^2 (class I obesity), which was higher than that

Table 2. Comparison Between MTX-Tolerant and Intolerant Patients.

	MISS<6	MISS ≥ 6	P
N	75	37	
Age (years)	59.8 (10.9)	55.1 ((12.5)	0.11
Females (n)	27/37	20/75	0.96
Caucasian ethnic background- (n)	3/37	9/74	0.74
Smokers– (n)	1/37	6/75	0.42
BMI– Kg/m2 – mean ±SD	27.7(24.2-30.8)	30.8 (28.3-34.8)	**0.002**
Not using folic acid (n)	4/37	5/74	0.47
Dose -mg/semana	15 (15-21.2)	20 (15-25)	0.32
Subcutaneous administration (n)	5/37	8/75	0.65
Hematocrit (%)	40.6 (39.1-43.1)	40.3 (37.3-43.8)	0.38
Leukocytes – n/mL – median (IQR)	7060 (5540-8836)	6900 (5620-8250)	0.72
Platelets – n/mL-median (IQR)	237.400 (195.700-289.800)	228.900 (193.200-275.000)	0.36
MCV – fl – median (IQR)	88.5 (84.7-92.5)	89.4 (86.8-93.0)	0.26

MCV=median corpuscular volume; MISS= Methotrexate intolerance severity score; n=number; BMI= body mass index; SD= standard deviation; IQR= interquartile range; n= number .

found in RA patients. Supporting this, Wibetoe et al., in a study of 3,517 individuals—1,961 with RA, 835 with spondyloarthritis, and 721 with PsA—found obesity to be more prevalent in PsA than in other forms of arthritis.[14] Similarly, Zohar et al. reported a higher obesity prevalence among PsA patients (34.5%) compared to the general population (23.6%).[15] One hypothesis for this finding is the increased number of adipokines in PsA patients. The literature also suggests that obesity affects both disease activity and therapeutic decisions, as it contributes to a chronic low-grade inflammatory state. [16,17]

With respect to the influence of obesity on MTX treatment, in this study, patients with MISS ≥ 6 had a significantly higher average BMI (30.8 kg/m²) compared to MTX-tolerant patients (27.7 kg/m²; p = 0.0021). Thus, the majority of MTX-intolerant patients had PsA and higher BMI. One possible hypothesis is that since obesity is associated with hepatic steatosis—and the liver is the main site of MTX metabolism—this hepatic alteration may reduce MTX degradation and elimination. Consequently, MTX may remain biologically active for a longer period, increasing the risk of adverse effects, including gastrointestinal symptoms. Furthermore, PsA itself has been linked to an increased risk of liver diseases such as cirrhosis and non-alcoholic fatty liver disease, conditions that may be exacerbated by obesity.[16,17] Hoekstra et al. found that higher BMI was associated with increased hepatotoxicity and MTX discontinuation, although they did not establish a relationship between BMI and gastrointestinal adverse effects.[12]

As in the studies by Bulatović Calasan et al. and Almalag et al., smoking status was not associated with gastrointestinal intolerance to MTX in the present study. However, a limitation here is that only current smoking was considered; past exposure was not evaluated. [10,11]

Regarding the route of administration, 86.48% of patients in this study used oral MTX. Islam et al., in a study of 92 RA patients (half receiving oral and half subcutaneous MTX), found fewer adverse effects in the subcutaneous group. Notably, nausea (63% vs. 37%), vomiting (30% vs. 11%), and dyspepsia (48% vs. 29%) were reduced.[18] Similarly, Tanaka et al., in a study of 102 RA patients (52 subcutaneous, 50 oral), found fewer adverse effects, particularly gastrointestinal, in those using the subcutaneous route.[19] However, Bulatović Calasan et al., when comparing MISS scores between parenteral and oral MTX users, found higher gastrointestinal intolerance in the oral group, which was attributed to a greater prevalence of behavioral symptoms.[11] In the present study, no significant difference in MTX tolerability was observed according to the route of administration.

The median weekly MTX dose in the MISS ≥ 6 group was 20 mg, compared to 15 mg in the tolerant group; however, this difference was not statistically significant. Although MTX dose is generally considered a risk factor for intolerance, both Bulatović Calasan et al. and Almalag et al. found no dose differences between tolerant and intolerant patients, supporting the present findings.[10,11] Conversely, Fatimah et al., in a study of 150 RA patients, found that gastrointestinal intolerance was more common in those taking 20 mg MTX (46.2%) versus 7.5 mg (20%).[20]

Regarding folic acid supplementation, most patients were using it concurrently with MTX, as it is known to reduce MTX side effects such as nausea, indigestion, and diarrhea.[21] Among the 37 patients with gastrointestinal intolerance in this study, only 4 were not taking folic acid. Likewise, Hoekstra et al., in a study of 411 RA patients randomized to receive either folic acid or placebo, found no association between folic acid use and MTX-related GI side effects.[12] In the present study, the number of patients not using folic acid was too small to draw any conclusions.

The mean MCV in patients with MISS ≥ 6 was 88.5 fl, which is within the reference range. The literature suggests that elevated MCV may be associated with increased MTX toxicity.[22] However, this association was not observed in the present study.

CONCLUSIONS

This study showed that patients with PsA experienced more gastrointestinal side effects related to MTX compared to those with RA.

Additionally, patients with MTX intolerance in both groups had higher BMI values, especially in the PsA group, which had a mean BMI consistent with class I obesity.

No differences in MTX tolerability were observed between oral and subcutaneous administration routes. Regarding MTX dose, no statistically significant relationship was found between weekly dose and gastrointestinal side effects.

Although the literature suggests that elevated MCV may indicate increased MTX toxicity, this association was not observed in the present study, and the average MCV values were within normal limits. Likewise, the influence of folic acid on gastrointestinal intolerance could not be evaluated due to the low number of non-users in the sample.

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ORIGINAL ARTICLE: TOPIC IN MEDICAL CLINIC *ARTIGO ORIGINAL: TÓPICO EM CLÍNICA MÉDICA*

MONOFILAMENT TEST OR IPSWICH TEST: COMPARATIVE STUDY IN DIABETIC NEUROPATHY AT THE BEDSIDE

TESTE DO MONOFILAMENTO OU IPSWICH TEST: ESTUDO COMPARATIVO EM NEUROPATIA DIABÉTICA À BEIRA DO LEITO

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ABSTRACT

Introduction: Diabetic neuropathy is a common and underdiagnosed complication of diabetes, associated with high risk of ulcers and amputations. The 10g monofilament is an effective, but less accessible method for screening these complications. The Ipswich Test emerges as a practical and cost-free alternative to detect sensory loss in diabetic feet. **Objectives**: Compare sensitivity, specificity, and effectiveness of the 10g Monofilament Test and the Ipswich Test in screening for peripheral neuropathy in diabetic patients. **Methodology**: A descriptive and interventional study with a qualitative-quantitative approach was conducted between October 2024 and February 2025 at the Hospital Evangélico Mackenzie do Paraná. A total of 92 hospitalized patients diagnosed with diabetes participated. They were assessed using a questionnaire, symptom scoring, and physical examination. The 10g monofilament, tuning fork, wooden stick, and Ipswich Test were applied to standardized points on the feet. **Results**: The average age was 65 years, with a predominance of type 2 diabetes. Only 30.4% had previously had their feet examined, and 47.8% reported neuropathic symptoms. The monofilament showed a sensitivity of 61.36% but lower specificity. The Ipswich Test demonstrated higher specificity (79.15%) and a sensitivity of 43.18%, with a significant association with neuropathic symptoms (p=0.02). **Conclusion:** The low rate of previous foot examinations (30.4%) highlights shortcomings in diabetic neuropathy screening. Although the Ipswich Test has lower sensitivity, its higher specificity and ease of application, requiring minimal training, make it a viable complementary tool—especially in resource-limited settings—to identify advanced casei.

Keywords: Diabetes Mellitus. Diabetic Neuropathy. Diabetic Foot.

RESUMO

Introdução: A neuropatia é uma complicação comum e subdiagnosticada do diabetes, associada à um risco elevado de úlceras e amputações. O monofilamento de 10g é um método eficaz, mas pouco acessível de rastreamento dessas complicações. O Ipswich Test surge como alternativa prática e sem custo para rastrear perda de sensibilidade nos pés diabéticos. **Objetivos:** Comparar a sensibilidade, especificidade e eficácia do Teste do Monofilamento de 10g e do Ipswich Test no rastreio da neuropatia periférica em pacientes com diabetes. **Metodologia:** Estudo descritivo com abordagem quali-quantitativa, realizado entre outubro de 2024 e fevereiro de 2025 no Hospital Evangélico Mackenzie

do Paraná. Participaram 92 pacientes internados com diagnóstico de diabetes, avaliados por escore de sintomas neuropático e exame físico. Foram utilizados monofilamento de 10g, diapasão, palito e Ipswich Test em pontos padronizados dos pés. **Resultados:** A média de idade foi 65 anos, com predominância de diabetes tipo 2. Apenas 30,4% haviam tido seus pés examinados previamente, e 47,8% relataram sintomas de neuropatia. O monofilamento apresentou sensibilidade de 61,36% e menor especificidade. O Ipswich Test teve maior especificidade (79,15%) e sensibilidade de 43,18%, com associação significativa a sintomas neuropáticos (p=0,02). **Conclusão:** A baixa taxa de exames prévios dos pés (30,4%) evidencia falhas no rastreio da neuropatia diabética. Embora o Ipswich Test tenha menor sensibilidade, sua maior especificidade e fácil aplicação, sem exigir amplo treinamento, o tornam uma ferramenta complementar viável, especialmente em locais com recursos limitados para rastrear casos avançados.

Descritores: Diabetes Mellitus. Neuropatia Diabética. Pé Diabético.

INTRODUCTION

Diabetic neuropathy (DNP) is the most prevalent chronic complication of Diabetes Mellitus (DM), affecting 30% of patients with diabetes and more than 50% of individuals over the age of 50 affected by the disease[1]. The disease occurs with dysfunction of the nerves of the peripheral and/or autonomic nervous system, and can affect all types of fibers and have varied clinical presentations[2]. First, sensory and autonomic symptoms appear due to the involvement of fine fibers, progressing to broad sensory fibers and, finally, motor fibers distally in the lower limbs with progression to the upper limbs[2]. The most common symptoms are burning pain, tingling in the limbs, fatigue and cramps[3]. Despite the intense pain and reduced quality of life associated with diabetic neuropathy, it is the most underdiagnosed complication and is difficult to measure directly, requiring a physical examination of the foot for diagnosis.

The Brazilian Diabetes Society states that every patient with diabetes should be screened annually for diabetic neuropathy from the moment of diagnosis in type 2 DM, and after 5 years in type 1 DM[4,5]. Individuals with diabetes have an imminent 15%-25% risk of foot ulcers and a 15 times higher risk of lower limb amputation when compared to individuals without diabetes[1].

The 10g Semmes-Weinstein monofilament test evaluates the coarse fibers and identifies advanced cases of neuropathy, which are already at risk of developing ulcers and all the resulting complications, such as foot amputation[6]. Abnormal results with this method indicate a 7.7-fold increase in the risk of ulceration[7]. However, it is equipment that is not provided by the Unified Health System and is not universally available to examine all patients with diabetes following the recommendations of the guidelines[4]. The Ipswich Test (IPTT) was developed as an alternative to detect loss of plantar sensitivity without any specific equipment or cost[8]. The method consists of lightly touching 6 or 8 places on the patient's foot with the tip of the examiner's index finger. Usually the tips of the first, third and fifth toes of both feet are touched, and additionally the dorsum of both hallux can be touched[8]. Patients are instructed to close their eyes and respond verbally when they feel the examiner's touch. If the patient fails to detect pressure on two or more toes, it means that their foot is at risk of an ulcer[7,9].

This study aimed to comparatively analyze the Monofilament Test and the Ipswich Test, evaluating aspects of the sensitivity, specificity and effectiveness of both tests in screening for peripheral neuropathy.

MATERIAL AND METHODS

This is a descriptive study with a qualitative and quantitative approach approved by the local Ethics Committee (6.817.931) with informed consent, carried out at the Mackenzie Evangelical University Hospital in Paraná from October 2024 to February 2025. The sample consisted of inpatients with a previous diagnosis of diabetes, aged over 18. Patients with decreased lower limb sensitivity due to another previously established condition were excluded.

Data collection included:

A. Epidemiological data: gender, age, education, health insurance, type of diabetes, time since diag-

nosis, medication used to control the disease, history of compensated DM, whether they usually carry out self- inspection of the foot, whether any professional had carried out the examination on them previously, whether they had ever had a lower limb ulcer and whether they had had an amputation in the past.

B. Neuropathic Symptom Score (NSS)[10] to assess typical symptoms, location, intensity, period of greatest pain, whether the patient has ever woken up because of these symptoms and whether they have any pain relief positions.

C. Neurological examination to assess loss of protective sensitivity, evaluating vibration sensitivity with a 128Hz tuning fork, pain sensitivity with a Chinese toothpick, cold sensitivity with an icy tuning fork handle, the 10g Semmes-Weinstein monofilament test and the Ipswich Test.

The monofilament and pain sensitivity tests were applied bilaterally to the head and metatarsals of the 1st, 3rd and 5th toes. Vibration sensitivity was assessed on the medial malleolus and thermal sensitivity was tested on the dorsum of the foot. The Ipswich Test was applied at the same points as the Monofilament Test. Loss of protective sensitivity was considered to be the presence of an altered monofilament test associated with an alteration in at least one other test, including decreased or absent vibration sensitivity, decreased or absent pain sensitivity and decreased or absent cold sensitivity.

Statistical analysis:

The data was summarized by calculating descriptive measures for the quantitative variables and constructing frequency (univariate) and contingency tables for the bivariate analysis of categorical variables. The Chi-square test was used to verify the relationship between categorical variables. The Mann-Whitney test was used to check for differences between groups with regard to quantitative variables. The odds-ratio was calculated for some relationships. A logistic regression model was fitted to predict the presence of symptoms among patients.

RESULTS

Description of the sample

The study sample consisted of 92 hospitalized patients with diabetes mellitus, 96.7% (n=89) without supplementary health insurance. The average age of the group was 65.09± 12.43 years. **Table 1** describes the characteristics of the study population.

Table 1. Epidemiological data of the sample studied.

Variable	f(n=92)	%
Gender (male)	57	61,95
Education level		
Elementary school	47	51,08
High school	29	31,52
Higher education	11	11,95
Health insurance	3	3,26
Type 2 diabetes	88	95,65
Time since diagnosis		
0-9 years	41	44,56
10-19 years	25	27,17
20-29 years	13	14,13
30-39 years	7	7,60
40-49 years	2	2,17
50-59 years	3	3,26
Did not know	1	1,08
Medications used		
Oral medication	41	44,56
Insulin	20	21,73
Combined therapy	31	33,69
Self-reported compensated diabetes	37	40,21
Foot self-examination	44	47,82
Previous foot exam	28	30,43
Active ulcer	13	14,13
Previous ulcer	18	19,56
Previous amputation	4	4,34

Neuropathic Symptoms Score

Symptoms in the lower limbs were present in almost 47.82% (n=44) of the patients, the most frequent being fatigue, cramps and itching, as shown in **Table 2**.

Table 2. Most frequent symptoms.

VARIABLE	f(n=44)	%
Symptom		
Fatigue	28	63,64
Cramps	28	63,64
Itching	28	63,64
Burning sensation	16	36,36
Numbness or tingling	16	36,36

Neuropathy was classified using the neuropathic symptom score as Mild (3-4 points),Moderate (5-6 points) and Severe (7-9 points), as shown in **Table 3**.

Table 3. Neuropathy classification by neuropathic symptom score.

Neuropathic Symptom Score	f(n=44)	%
Mild	19	43,18
Moderate	16	36,36
Severe	9	20,45

Assessment of loss of protective sensitivity (PSP) using monofilament and the IPSWICH test

The statistical analysis comparing the group of symptomatic and asymptomatic patients showed no significant difference in the results of the PSP test (p=1, Odds-ratio=1). However, the test proved to have a sensitivity of 50% and specificity of 50%. The monofilament sensitivity test showed the highest sensitivity, but the lowest specificity. The Ipswich test showed greater specificity and higher predictive value. There was significant agreement (<0.0001) between the tests, according to the contingency coefficient C(C=0.444), **Table 4**.

Table 5 shows the logistic regression model assessing the factors associated with the presence of symptoms. The coefficients (estimates), standard error, Wald statistics and p-values are presented for each independent variable in the model. In this analysis, Gender and the Ipswitch Test were found to be statistically significant predictors of symptoms. Women with diabetes were 2.69 times more likely to have symptoms than men with diabetes. A Yes result on the Ipswitch test indicates 2.89 times more chance of having symptoms than a No result.

Table 5. Logistic regression model adjustment for presence of symptoms.

	Estimate	Standard Error	Wald	p-value
Intercept	0,207	0,271	0,586	0,586
Gender	0,593	0,251	5,564	0,018
Education level	0,318	0,244	1,708	0,191
IPSWICH test	-0,730	0,260	7,875	0,005
Scale	1000	0,000		

DISCUSSION

The survey revealed an important deficiency in the prevention, screening, diagnosis and management of diabetic neuropathy in the population treated at the hospital. Only 28 patients (30.4% of the sample) reported having had their diabetic foot examined by a health professional. Previous studies have reinforced the difficulty of routinely implementing this practice in primary care, indicating that up to 83.63% of patients with DM have never undergone a clinical examination of their feet and more than half have never undergone any type of monitoring or visual inspection of their feet during clinical assessment[11].

The main factors associated with inadequate diabetic foot care include low schooling, unsatisfactory glycemic control and the type of drug treatment. Low schooling, observed in 52.16% of the sample, makes it difficult to access and understand self-care guidelines, increasing the risk of complications.

Although lower than rates reported in other regions of Brazil, where this percentage can exceed 84.5%[12], this socio-cultural condition still represents a significant obstacle to adherence to continuous care. The association between social vulnerability, lack of health education programs and inadequate glycemic control contributes to the increased incidence of ulcerations and amputations in DM patients[13].

We found that only 40.22% of the individuals reported maintaining adequate glycemic control in the period prior to hospitalization, which is compatible with other studies in which 79% of patients do not

Table 4. Comparison between 10g monofilament and Ipswich test.

Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
PSP	50%	50%	47,83%	52,17%
Ipswich	43,18%	79,16%	65,51%	60,32%
10g Monofilament	61,36%	39,58%	61,36%	39,58%

reach the recommended glycemic levels, with discontinuation of medication observed in 25% of them over two years[14]. Although most of the participants were using oral hypoglycemic agents (46.6%), only 31.8% were using combined therapy, which may have contributed to inadequate glycemic control. Previous studies have shown that the isolated use of medication alone accounts for up to 86% of cases[15], despite the pathophysiological complexity of DM, which often requires multimodal treatment[16].

Another relevant aspect observed was the low familiarity of professionals with standardized tests for screening NPD, often compromised by logistical and human resource limitations[9]. Tools such as the Ipswich Touch Test (IPTT) and the Neuropathic Symptom Score can complement the traditional physical examination, increasing diagnostic capacity. In the sample studied, 47.82% of patients had neuropathic symptoms. Those with alterations in at least one point on the IPTT were 2.89 times more likely to report symptoms, which reinforces the usefulness of the test as a clinical screening tool.

When comparing the two main methods for assessing protective sensitivity - the 10 g monofilament and the IPTT - different performances were observed. The monofilament showed higher sensitivity (61.36%) but lower specificity (39.58%), while the IPTT showed higher specificity (79%) and lower sensitivity (43.18%). These results are compatible with findings in the literature, which indicate sensitivity and specificity values of up to 81% and 91% for the monofilament, and 77% and 90% for the IPTT, respectively[2]. The agreement between the tests in this sample was high (k=0.88; p<0.0001), which corroborates their complementary use in NPD screening.

Although the sensitivity and specificity values observed were lower than those described in other studies, this divergence can be partially explained by the nature of the sample - made up of hospitalized patients, often in an unstable clinical state, with a higher risk of functional and cognitive impairment, factors which can affect the performance of diagnostic tests. Even so, the simplicity, low cost and applicability of the IPTT reinforce its viability, especially in primary care settings with limited resources.

These findings highlight the urgent need for integrated health education strategies, professional training and increased access to basic tests for the early detection of NPD. Such measures are fundamental to reducing the risk of serious complications and optimizing the clinical management of DM patients, especially in vulnerable populations.

CONCLUSION

The deficiency of diabetic neuropathy screening was evidenced by the low proportion of patients who had previously undergone foot examination, representing only 30.4% of the sample analyzed. As far as diagnostic tests are concerned, the Ipswich Test showed lower sensitivity and higher specificity, but it is a method that is easy to apply and does not require extensive staff training, which makes it a viable complementary tool in places with limited resources for screening advanced cases of diabetic neuropathy.

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ORIGINAL ARTICLE: TOPIC IN MEDICAL CLINIC *ARTIGO ORIGINAL: TÓPICO EM CLÍNICA MÉDICA*

RHEUMATOID ARTHRITIS PATIENTS HAVE EARLY CHRONOTYPE THAT DOES NOT ASSOCIATE WITH DISEASE ACTIVITY

PACIENTES COM ARTRITE REUMATOIDE TÊM CRONOTIPO PRECOCE QUE NÃO SE ASSOCIA À ATIVIDADE DA DOENÇA

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ABSTRACT

Background: Chronotypes refers to one's preferred schedule for sleep and wakefulness. It is closely linked to circadian rhythm that regulates hormone and cytokines release. The circadian rhythm is altered in several immune mediated diseases. **Objective:** To study chronotypes in rheumatoid arthritis (RA) patients and their possible relationship with disease activity. **Methods:** Cross-sectional study with 77 RA patients and 79 controls. Patients and controls answered the Morning-Evening Questionnaire Self-Assessment Version (MEQ-SA) questionnaire that classifies chronotypes in morning types, evening types and intermediated. Data on disease activity: DAS28 (Disease Activity Index using 28 joints)-ESR (erythrocyte sedimentation rate), DAS28-CRP (C reactive protein), SDAI (Simplified Disease Activity Index), and CDAI (Clinical Disease Activity Index) were collected in RA patients. **Results:** In the RA sample, 55 (71.4%) were classified as morning types; 19 (24.6%) as intermediate and 3 (3.8%) as evening types. In the control group 33 (41.7%) were classified as morning types; 40 (50.6%) in intermediated and 6 (7.5%) in the evening type; p=0.0009. No correlation of values of MEQ-SA with disease activity indexes were found (all with p>0.05). **Conclusion**: RA patients had earlier chronotype than controls but disease activity did not have influence on this preference.

Key words: Rheumatoid arthritis. Chronotype. Disease activity. Inflammation.

RESUMO

Introdução: Cronotipos referem-se ao horário preferido de uma pessoa para dormir e acordar. Está intimamente ligado ao ritmo circadiano que regula a liberação de hormônios e citocinas. O ritmo circadiano está alterado em várias doenças imunomediadas. **Objetivo:** Estudar cronotipos em pacientes com artrite reumatoide (AR) e sua possível relação com a atividade da doença. **Métodos:** Estudo transversal com 77 pacientes com AR e 79 controles. Pacientes e controles responderam ao questionário *Morning-Evening Questionnaire Self-Assessment Version* (MEQ-SA) que classifica os cronotipos em tipos matutino, noturno e intermediário. Dados sobre a atividade da doença: DAS28 (Índice de Atividade da Doença usando 28 articulações) -ESR (velocidade de hemossedimentação), DAS28-CRP (proteína C reativa), SDAI (Índice de Atividade da Doença Simplificado) e CDAI (Índice de Atividade da Doença Clínica) foram coletados em pacientes com AR. **Resultados:** Na amostra de AR, 55 (71,4%) foram classificados como matutinos; 19 (24,6%) como intermediários e 3 (3,8%) como tipos noturnos. No grupo controle, 33 (41,7%) foram classifi-

cados como matutinos; 40 (50,6%) no tipo intermediário e 6 (7,5%) no noturno; p=0,0009. Não foi encontrada correlação dos valores do MEQ-SA com os índices de atividade da doença (todos com p>0,05). **Conclusão:** Os pacientes com AR apresentaram cronotipo mais precoce do que os controles, mas a atividade da doença não influenciou essa preferência.

Descritores: Artrite reumatoide. Cronotipo. Atividade da doença. Inflamação.

INTRODUCTION

The immune system functioning is subject to individuals' circadian rhythm[1]. Circadian rhythm refers to a 24-hours cycles that allow the physiological processes to be optimized by regulating the sleep-wake cycles, hormone release, eating habits, and other functions[2]. It is directed by the suprachiasmatic nucleus in the hypothalamus, that communicates with peripheral cells through hormonal and neuronal connections[2,3]. The observable behaviors influenced by the intrinsic circadian rhythm, which dictate one's preferred schedule for sleep and wakefulness, are referred to as chronotype [4].

The autoimmune diseases may be affected by the body clock in at least two ways: 1 - through its influence in their pathophysiologic process; 2 - in the expression of their symptoms[5].

Pro-inflammatory cytokines are linked to the circadian rhythm. Increased sleepiness during infections episodes, that are seen as beneficial, associates with TNF alpha, IL-2 and interferon γ production[6,7]. LPS-dependent secretion of TNF-α is higher at night compared to day and it is further boosted by melatonin[5]. Animal models with autoimmune diseases such as rheumatoid arthritis, RA[1], psoriasis[8]; inflammatory bowel disease[9], etc., have more severe inflammatory phenotypes when the circadian rhythm is disrupted. Concerning disease's clinical expression, the occurrence of morning pain and stiffness in inflammatory arthritis in parallel with pro-inflammatory cytokine and hormone levels fluctuations is well recognized[10].

In RA some interesting observations about influence of circadian rhythm/chronotype have been done. Disturbances in the hypothalamic-pituitary-adrenal axis, reflected in altered circadian secretion of cortisol, melatonin, interleukin (IL) -6, have been documented on this disease[11]. Neeck et al.[12] reported that in patients with RA, the cortisol levels fluctuated during the day based on the disease activity, being reduced in severe case. Sulli et al.[13] found an altered temporal profile of melatonin in RA patients, with a more rapid increase at the start of the night and with an earlier peak than in healthy controls. Moreover, disruption of clock genes function such as BMAL1 (Brain and Muscle ARNT-like 1), CLOCK (Circadian Locomotor Output Cycles Kaput) Period (PER1, PER2, PER3) and Cryptochrome (CRY1, CRY2) that are part of the circadian rhythm regulatory system, have been linked to development and progression of RA[14]. Curiously, Butler et al.[2] have reported that individuals with morning chronotypes working night shifts had higher probabilities of developing RA when compared to day workers.

Taking into account the mutual influence of the inflammatory process and circadian rhythm it is possible to hypothesized that in RA the inflammatory process may be linked to patient's chronotype.

Herein, we studied RA patients chronotypes comparing them with controls and the possible relationship of their chronotypes with disease activity.

METHODS

This is a cross-sectional study approved by the institutional Committee of Ethics in Research (CAAE: 69974123.6.0000.0103) under protocol 6.120.525 with a convenience sample of RA patients from a single tertiary center that cares for patients from the Public Health System. To be included patients should fulfilled at least six points of classification criteria for RA from EULAR/ACR [15] and be older than 18 years of age. Patients using sleep inducing medications, associated fibromyalgia and other inflammatory comorbidities were excluded. Inclusions were done according to consultation order and willingness to participate in the study. Data collection included:

A. Epidemiological and clinical data: sex, age, tobacco and alcohol use, age at diagnosis, presence of rheumatoid factor, DAS28 (Disease activity score using 28 joints) -CRP (C reactive protein), DAS28 -ESR (erythrocyte sedimentation rate), SDAI (Simplified Disease Activity Index); and CDAI (Clinical Disease Activity Index). DAS28-ESR and CRP are measured taking into account the number of tender and swollen joints out of 28, ESR or CRP and patient's general health or global disease activity measured on a visual analogue scale of 100mm.

CDAI was measured through tender and swollen 28-joint count, patient's global disease activity (from 0-10) and evaluator's global disease activity (from 0-10). SDAI was measured by the arithmetic sum of tender and swollen 28-joint count, patient's and evaluator global assessment (both from 0-10) and CRP[16].

B. Morning-Evening Questionnaire Self-Assessment Version (MEQ-SA)- that is questionnaire to determine morningness-eveningness in human circadian rhythms. It has 19 multiple choice items with 4–5-point numerical scale. The sum gives a score ranging from 16 to 86; scores of 41 and below indicate "evening types", scores of 59 and above indicate "morning types", scores between 42-58 indicate "intermediate types" [17]. Data on MEQ-SA was collected simultaneously with data on disease activity.

As controls, patient's companions paired for sex and age were included.

Data was collected in frequency and contingency tables. Comparison of nominal data was done by chi-square and Fisher tests and of numerical data by the Mann Whitney and unpaired t test. Correlations studies of MEQ SA questionnaire and disease activities index were done by Spearman or Pearson test according to data distribution. The adopted significance was of 5%.

RESULTS

The included sample had 156 individuals (77 RA patients and 79 controls). **Table 1** shows patient's demographic and pairing with controls data.

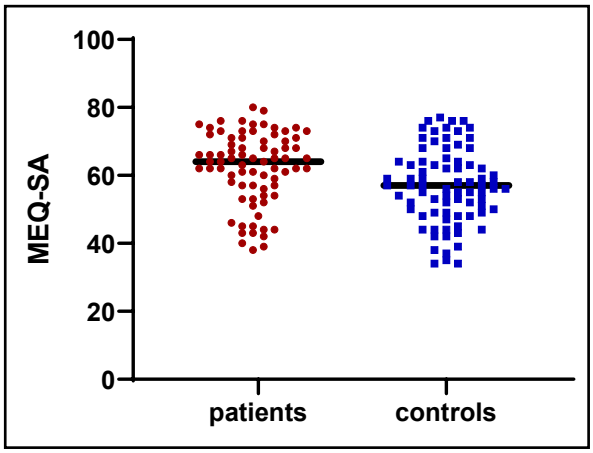
Table 1. Comparison of epidemiological data of rheumatoid arthritis (RA) patients and controls.

	RA Patients n=77	Controls n=79	P
Female sex – n (%)	62 (80.5)	63 (79.7)	0.90
Median age – years – (IQR)	59.0 (52.5-62.0)	54.0 (50.0-63.0)	0.11
Current smokers – n (%)	11 (14.2)	7 (8.8)	0.28

In the RA sample the median disease duration was of 10.0 years (IQR=5.0-14.6) and 58.6% were positive for rheumatoid factor. The median SDAI was 3.19 (0.80-12.80); median CDAI was 4.00 (0-11.90); the median DAS28-ESR was 2.78 (2.15-3.61) and the median DAS28-CRP was 2.18 (1.60-3.33).

The comparative results of MEQ-SA between patients and controls are on **Figure 1**.

Figure 1. Comparison of MEQ-SA results between rheumatoid arthritis (RA) patients and controls**.**



RA= median of 64.0 (56.5-71.0); controls= median of 57.0 (49.0-64.0); p=0.001; MEQ-SA= Morning-Afternoon Questionnaire Self-Assessment Version.

In the RA sample, 55 (71.4%) were classified as morning types;19 (24.6%) as intermediate and 3 (3.8%) as evening types. In the control group 33 (41.7%) were classified as morning types; 40 (50.6%) in intermediated and 6 (7.5%) in the evening type; p=0.0009.

When the results of disease activity indexes were correlated with MEQ-SA the results on **table 2** were found. No correlations were observed.

Table 2. Correlation studies of Morning-Evening Questionnaire Self-Assessment Version with rheumatoid arthritis disease activity indexes.

	r	95% confidence interval	P
SDAI	-0.08	-0.31 to + 0.15	0.49
CDAI	-0.08	-0.31 to + 0.14	0.43
DAS28-ESR	-0.09	-0.32 to + 0.14	0.41
DAS28-CRP	-0.04	-0.27 to + 0.19	0.70

DAS28= Disease activity score using 28 joints; CRP= C reactive protein, ESR= erythrocyte sedimentation rate, SDAI= Simplified Disease Activity Index, CDAI= Clinical Disease Activity Index.

No correlations were found of MEQ with age and disease duration, neither association with sex and presence of FR (all with p>0.05).

DISCUSSION

The results of this study have shown that RA patients have morning chronotype more frequently than controls and that the indexes of MEQ-SA did not correlate with disease activity.

Finding an early chronotype in RA patients is in line with the observation that the circadian rhythm in individuals with this disease has also an early timing. This is considered to be due to altered temporal profile of melatonin with a more rapid increase at the start of the night yielding an earlier peak [13]. Melatonin is a hormone synthesized at the night primarily in the pineal gland and it is considered to have pro-inflammatory properties [7]. Cytokines and cortisol also have circadian fluctuations that are peculiar in RA individuals. Studies with sequential measurements of IL-6 show overnight variations of this cytokine with a peak in the early hours of the morning[18] . In RA the natural morning peak in cortisol level is blunted and the individual becomes unable to mount an appropriately enhanced response to combat inflammation [19]. Such variations could answer by the morning pain and stiffness seen on these patients. However, it was not possible to link the found early chronotype with disease activity indexes. The same was found by Habers et al. [19] that studied 121 RA patients. They also detected a preference for morning chronotypes in RA individuals without associations with inflammatory parameters. Although no explanation for this possible dissociation is found, it is important to note that it is unknown how chronotype and the internal circadian rhythm are related. Factors other than the circadian pacemaker may influence the chronotype such as physical activities, time and amount of exposure to light, social and work activities, genetic factors and even personality traits [20,21].

This study is limited by the small sample and the cross-sectional design. Studies with larger samples and with a prospective design that allow to observe possible changes in chronotype according to changes in disease activity are desired. The study of the rhythmicity of the inflammatory process in RA is important for the personalized chronotherapy by aligning the medication administration to chronotype for better efficacy.

CONCLUSION

RA patients had an earlier chronotype when compared to controls but without association with disease activity.

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SCOPING REVIEW *REVISÃO DE ESCOPO*

AMERICAN THYROID ASSOCIATION (ATA) 2025: KEY UPDATES IN THE TREATMENT AND LONG-TERM MANAGEMENT OF DIFFERENTIATED THYROID CANCER IN ADULTS (DTC)

AMERICAN THYROID ASSOCIATION (ATA) 2025: DESTAQUES DA ATUALIZAÇÃO NO TRATAMENTO E MONITORAMENTO DE LONGO PRAZO DO CÂNCER DIFERENCIADO DE TIREOIDE EM ADULTOS (CDT)

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ABSTRACT

This review summarizes the main highlights of the recent American Thyroid Association (ATA) publication on the management of adult patients with differentiated thyroid cancer (DTC). The previous version, published in 2015, has now been updated in 2025, providing new guidance on treatment, surveillance, and management of recurrence in DTC patients. The present review focuses specifically on treatment and follow-up, with emphasis on active surveillance, radioablation, surgical extent, indications for radioactive iodine therapy, the role of TSH-suppressive therapy, recurrence risk and initial reponse to treatment classifications, and strategies for long-term surveillance. The management of recurrent disease once diagnosed is beyond the scope of this review. The 2025 version introduces advances in therapeutic options for low-risk patients, a new risk classification system incorporating histopathological and molecular features, therapeutic de-escalation strategies for low- and low-intermediate risk patients, subdivision of the intermediate-risk category, the importance of ongoing reassessment of recurrence risk during follow-up, and proposals for surveillance de-escalation in low-risk DTC. This review provides a full translation of the key ATA 2025 recommendations for adult DTC management, including figures extracted from the publication, adapted for clarity and integration into this article.

Keywords: Thyroid cancer, Thyroid carcinoma, Practice guideline, Adults, Scoping review.

RESUMO

Esta revisão reúne os principais destaques da recente publicação da *American Thyroid Association* (ATA), sobre as diretrizes de manejo de pacientes adultos com câncer diferenciado de tireoide (CDT). A última versão publicada em 2015, foi recém atualizada em 2025 e traz as condutas com tratamento, monitoramento e manejo das recidivas de pacientes com CDT. A revisão aqui apresentada, foca no tratamento e monitoramento do CDT, com ênfase na vigilância ativa, radioablação, extensão de cirurgia, indicação de iodoterapia, uso da terapia supressora de TSH, risco de recorrência, classificação de resposta à terapia inicialmente instituída e manejo do monitoramento de longo prazo. Não é escopo desta revisão abordar o tratamento das recidivas uma vez diagnosticadas. A versão de 2025 mostra avanços em opções terapêuticas em pacientes de baixo

risco, a nova classificação de risco baseada em critérios de histopatologia e moleculares, o de-escalonamento terapêutico para pacientes de baixo risco e risco baixo-intermediário, a subdivisão do risco intermediário, a necessidade de constantemente rever a estratificação de risco ao longo do seguimento e propostas para de-escalonamento de seguimento para CDT de baixo risco. O texto desta revisão, traz a tradução na íntegra das principais recomendações do manejo do CDT em adultos da publicação da ATA 2025, incluindo figuras extraídas da publicação, adaptadas em sua estruturação para esta revisão.

Descritores: Câncer de tireoide, Carcinoma diferenciado de tireoide, Diretrizes, Adultos, Revisão de escopo.

INTRODUCTION

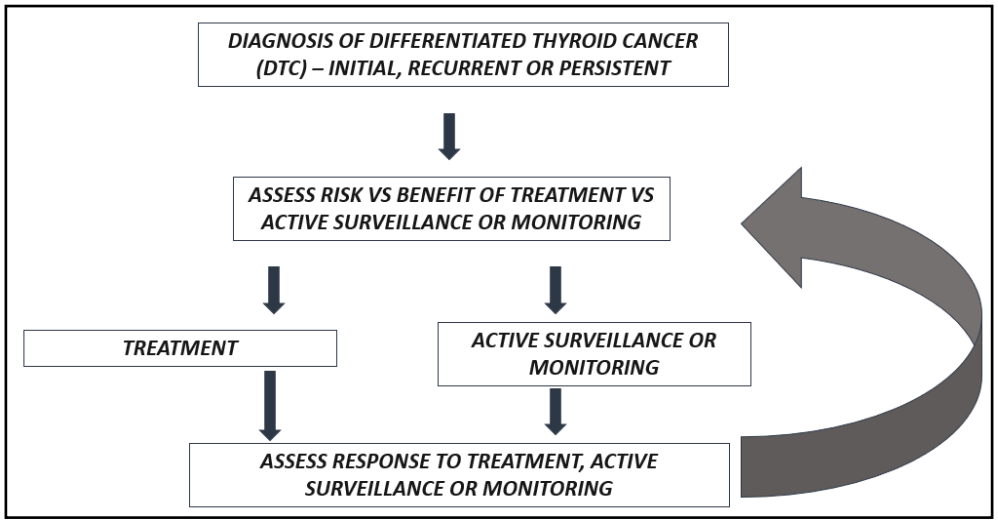
The management of differentiated thyroid cancer (DTC) begins with assessing the risks and benefits of initiating treatment versus opting for active surveillance or monitoring (**Fig. 1**). In the era of active surveillance, the decision to treat must be carefully weighed against the potential risks and benefits of surveillance, as these factors may shift over time, both during surveillance and treatment, in the context of initial diagnosis as well as potential recurrences[1]. It is essential to emphasize that the choice between treatment and surveillance should be based on well-established clinical criteria, always taking patient preferences into account.

In the context of the decision to treat, that is, when surgery is indicated, the primary goal is to resect the tumor and any tissue extending beyond the thyroid gland, as well as lymph nodes identified as involved either preoperatively or intraoperatively. Adequate management with complete resection of all macroscopic tumor tissue is a key factor in disease control. It is important to note that recurrences or persistent disease in the cervical region occur in approximately 74% of cases in lymph nodes, 20% in the thyroid remnant, and 6% in the trachea or other adjacent structures[2].

In addition to surgery, radioiodine therapy (RAI) and TSH-suppressive therapy may also be considered in the management of DTC. However, these modalities must be selected with careful consideration of their risks and benefits, in order to avoid imposing unnecessary therapeutic morbidity in the treatment of an indolent and low-risk tumor[1]. Therefore, it is essential to choose a treatment strategy that provides effective tumor control while minimizing morbidity, within the context of a personalized treatment plan.

Establishing risk stratification helps estimate the likelihood of recurrence in both the short and long term, while accurate staging informs disease-specific mortality estimates[1].

Figure 1. Continuous clinical decision-making flowchart for the clinical management of differentiated thyroid cancer (DTC).



Extracted and adapted from ATA guidelines 2025 publication[1].

Another important consideration is the surgeon's experience with thyroid surgery. A surgeon who performs more than 25–50 thyroidectomies per year is considered experienced in this procedure. In general, surgeries performed by high-volume surgeons are associated with a lower risk of complications and better disease control[3,4].

PREOPERATIVE STAGING WITH LABORATORY AND IMAGING STUDIES

The use of preoperative neck ultrasound (US) to assess the extent of extrathyroidal tumor extension and the presence of cervical lymph node involvement is highly recommended. Fine-needle aspiration (FNA) of suspicious lymph nodes larger than 8–10 mm should be performed when the detection of metastasis would alter the surgical approach[1]. In contrast, thyroglobulin washout from aspirated lymph nodes should be interpreted with caution, as its diagnostic accuracy is reduced in the presence of an intact thyroid gland[1].

Lymph node metastasis is present in up to 50% of cases, even in small, intrathyroidal tumors[5]. Micrometastases (<2 mm) may occur in up to 90% of cases, depending on the diagnostic sensitivity of the method used, but unlike macrometastases, they do not appear to impact survival[6], and when confined to the central compartment, they do not seem to increase the risk of local recurrence[7].

Preoperative ultrasound detects nodal metastases in 20–31% of cases[8], and may alter the surgical strategy in approximately 20% of patients[9], especially when metastases are located in the lateral or posterior cervical compartments (levels II, III, IV, or V). For patients with more extensive extrathyroidal disease, such as suspected tracheal or esophageal invasion, large cervical nodal masses, or possible mediastinal extension, computed tomography (CT) or magnetic resonance imaging (MRI) should be combined with ultrasound to improve staging and assess resectability. Deep cervical nodes, such as retropharyngeal, parapharyngeal, or mediastinal lymph nodes, are best evaluated with CT imaging. Thus, cross-sectional imaging of the neck, chest, and upper abdomen is highly recommended during surgical planning. On the other hand, routine use of 18F-fluorodeoxyglucose PET/CT (^{18}FDG-PET/CT) is not recommended in the preoperative setting[1].

Endoscopic evaluations, such as tracheoscopy and esophagoscopy, may be considered when partial resection of these structures is anticipated based on contrast-enhanced CT or MRI findings. Preoperative laryngoscopy is strongly recommended to document recurrent laryngeal nerve function. Clinical signs such as hoarseness, hemoptysis, dysphagia, or vocal cord paresis/paralysis are highly predictive of cervical organ invasion, including gross neural or endoluminal extrathyroidal extension to the trachea or esophagus[1].

Routine measurement of preoperative thyroglobulin or anti-thyroglobulin antibodies is not recommended. A retrospective study of 422 patients with thyroid cancer found that thyroglobulin levels correlated with gland and tumor size, but not with the presence of metastasis[10]. Similarly, preoperative anti-thyroglobulin antibody levels were not associated with disease stage or overall survival[11].

Regarding preoperative genomic analysis, the 2025 ATA guidelines do not recommend routine testing, but when performed, molecular results should be interpreted alongside with clinical, radiologic, and cytopathologic data to help defining surgical extent. Some studies suggest that combined mutations in TERT and BRAF^{V600E} are associated with poorer prognosis[12,13], although isolated mutations or their presence in small differentiated tumors have not consistently shown aggressive behavior[14]. A higher allelic frequency of BRAF^{V600E} may indicate a more aggressive phenotype[15], especially when coexisting with other mutations[16]. RAS mutations are typically observed in follicular-pattern tumors, and are not specific to malignancy. They may be found in follicular adenomas, noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP), invasive follicular variants of papillary carcinoma, and follicular carcinoma, thus lacking predictive prognostic value when present in isolation[17]. The potential role of molecular testing to determine surgical extent may be relevant in T2N0 tumors, when the decision between lobectomy versus total thyroidectomy remains uncertain. T2 tumors with a high-risk molecular profile may benefit from total thyroidectomy, even though most T2 tumors are generally associated with low-risk molecular profiles[1].

ACTIVE SURVEILLANCE AND THERMAL ABLATION

Although there is limited evidence in the literature regarding local control rates of papillary thyroid

microcarcinomas (PTMCs) treated with thermal ablation, primarily due to the novelty of the technology and the lack of long-term follow-up, it is believed that tumors suitable for ablation may also be managed with active surveillance. The choice between the two depends on clinical information and patient preference. Suitable candidates are patients with intrathyroidal tumors, with a good margin of surrounding normal thyroid tissue, located outside the posterior lobe, without contact with the trachea or the thyroid capsule, and staged as cT1a N0 M0[1].

Studies have shown that active surveillance in this group of tumors results in similar rates of disease-specific survival, distant metastasis, and recurrence compared to patients undergoing surgery[18,19]. For tumors larger than 1 cm, evidence supporting active surveillance is more limited. It is essential that, if active surveillance is chosen, the patient agrees to long-term, indefinite follow-up[1]. Active surveillance is contraindicated in cases of aggressive histology, extrathyroidal extension, lymph node metastasis, or distant metastasis[1].

One of the largest active surveillance cohort comes from Japan, where a study of 1,235 patients suggested that individuals over 60 years old are the best candidates, as their tumors exhibit lower rates of growth ≥3 mm and local or nodal progression compared to younger patients (<40 years old)[19]. A systematic review found that <10% of tumors under surveillance grew >3 mm, about 12% of patients converted to surgery, 0.1% developed distant metastasis, 2.1% had nodal metastases, and no thyroid cancer-related deaths were observed, demonstrating that active surveillance is safe in well-selected DTC patients[20]. Another systematic review found that patients undergoing active surveillance followed by delayed surgery had low disease-specific mortality, low rates of distant metastasis, and low recurrence rates. Tumor growth during surveillance was generally minimal, and conversion to surgery was most often due to patient preference. However, robust conclusions were limited due to studies design heterogeneity and quality[21].

If active surveillance is selected, ultrasound every 6 months for the first 1–2 years is the recommended imaging modality, followed by annual ultrasound thereafter[22]. Routine measurement of thyroglobulin or anti-thyroglobulin antibodies is not recommended. Surgical intervention should be indicated if any of the following occur: tumor growth ≥3 mm, biopsy-proven lymph node metastasis, distant metastasis, extrathyroidal extension, posterior tumor growth, patient anxiety or preference, or inability to maintain appropriate follow-up[1].

Thermal ablation using radiofrequency, microwave or laser, and chemical ablation with ethanol, have been investigated as a treatment option for highly selected patients with low-risk papillary thyroid microcarcinomas. Radiofrequency ablation (RFA) may serve as an alternative for patients who decline surgery or active surveillance. Compared to lobectomy, RFA is associated with a lower incidence of hypothyroidism, but complete tumor eradication cannot be confirmed, and no tissue is available for histopathological analysis[23].

A meta-analysis showed that thermal ablation for cT1a N0 tumors resulted in complete tumor disappearance in ~96% of patients within 1 year, with no detected recurrences or lymph node metastases at 18 months of follow-up[24]. Another study involving 1,613 patients with papillary thyroid carcinoma up to 2 cm treated with RFA and followed for a median of 58.5 months, reported a tumor progression in 4.3%, recurrence in 2.6% and persistente disease in 1.7%. The mean time to tumor progression was 21.5 months. Disease-free survival varied according to tumor size (T1a *vs* T1b), number of tumors (unifocal *vs* multifocal), and the distance from the tumor to the thyroid capsule or trachea (≤2 mm *vs* >2 mm)[25]. The most recent ATA guidelines (2025) recommend caution when considering thermal ablation for T1b tumors or multifocal disease[1].

SURGICAL TREATMENT

Surgical treatment of differentiated thyroid cancer requires careful preoperative planning, particularly regarding vocal function assessment. Subjective evaluation of voice quality by the patient, physician, or family members is insufficient to ensure normal vocal cord function, and does not replace objective laryngoscopic examination. Therefore, laryngoscopy is strongly recommended in patients with pre-existing dysphonia, a history of cervical or thoracic surgery, or known thyroid cancer with gross extrathyroidal extension or bulky nodal metastases in the central or lateral neck compartments[1].

Parathyroid preservation is a critical component of thyroid surgery. Care must be taken to maintain both the glands and their vascular supply to reduce the risk of postoperative hypoparathyroidism. If a parathyroid gland is inadvertently excised or devascularized, it should be confirmed intraoperatively by frozen section and immediately autotransplanted into adjacent skeletal muscle. In cases of total thyroidecto-

my, central neck dissection, or completion thyroidectomy, selective or routine supplementation with calcium and vitamin D based on intraoperative or early postoperative parathyroid hormone (PTH) levels, has been shown to significantly reduce rates of symptomatic hypocalcemia and hospital readmission, compared to serial calcium monitoring alone[1].

For tumors measuring ≤2 cm without gross extrathyroidal extension or metastases (cT1N0M0), lobectomy is considered the treatment of choice, except in cases of bilateral disease or specific indications for contralateral lobe removal. For unilateral tumors between 2 and 4 cm, lobectomy remains an appropriate option, though total thyroidectomy may be considered when postoperative radioiodine therapy is planned, when tumor features require more intensive follow-up, in the presence of suspicious contralateral nodules, or based on patient preference[1]. In patients undergoing lobectomy, it is important to provide preoperative counseling regarding the possibility of intraoperative conversion to total thyroidectomy or the need for completion thyroidectomy based on high-risk intraoperative or postoperative findings.

Total thyroidectomy is indicated in patients with tumors larger than 4 cm (cT3), any tumor with gross extrathyroidal extension (cT3b or cT4), or when lymph node (cN1) or distant metastases (cM1) are present, unless there are contraindications to the procedure. In such cases, resection should include complete removal of the primary tumor and appropriate cervical lymphadenectomy[1].

Regarding lymph node management, the 2025 American Thyroid Association (ATA) guidelines recommend against prophylactic central neck dissection in patients with small (cT1 or cT2) papillary carcinomas without clinical evidence of lymph node involvement (cN0), and for most follicular carcinomas[1]. However, prophylactic central dissection may be considered in patients with more advanced tumors (T3 or T4) or when central compartment pathology is expected to inform further therapeutic decisions. Nevertheless, the risks associated with central dissection, particularly permanent hypoparathyroidism and recurrent laryngeal nerve injury, must be carefully weighed, even in high-volume centers[1].

In patients with confirmed central compartment lymph node metastasis (cN1a), whether identified by preoperative imaging or intraoperative evaluation with or without frozen section, ipsilateral central neck dissection should be performed in combination with total thyroidectomy. For patients with lateral neck metastases (cN1b), confirmed clinically or cytologically, therapeutic dissection of cervical levels IIa, III, IV, and Vb is required[1]. In such cases, ipsilateral central neck dissection is also recommended, even if not clinically involved, as this compartment is typically considered at high risk of occult disease when ipsilateral lateral node metastases are present.

The final surgical pathology report must provide detailed information relevant to staging and prognosis. These include tumor size, margin status, the presence or absence of lymphovascular invasion, number of vessels involved, number of lymph nodes examined and those with metastatic involvement, the size of the largest metastatic lymph node, and the presence or absence of extranodal extension. Histologic subtype should be clearly defined, with particular attention to aggressive variants such as tall cell, columnar cell, *hobnail*, widely invasive follicular or oncocytic carcinomas, poorly differentiated carcinomas, high-grade well-differentiated tumors, or any areas of anaplastic transformation[1].

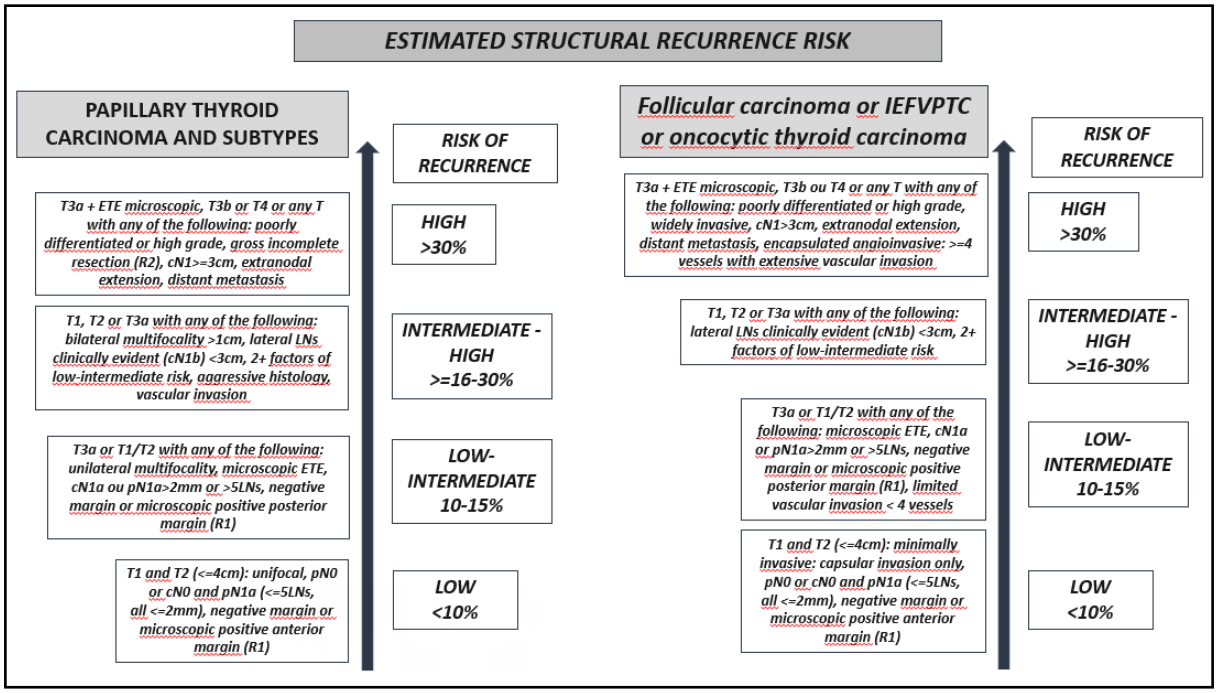
INITIAL RISK STRATIFICATION FOR RECURRENCE

The 2025 American Thyroid Association (ATA) guidelines recommend assessing the risk of persistent or recurrent structural disease, whether locoregional or distant, as well as disease-specific survival in patients with differentiated thyroid carcinoma. This assessment should be based on histopathological features of the tumor, the number of metastatic lymph nodes, pathological staging, postoperative imaging findings, and serum levels of thyroglobulin and anti-thyroglobulin antibodies measured approximately three months after surgery[1].

Routine evaluation of the tumor's molecular profile is not currently recommended for estimating recurrence risk. However, when available, molecular data may be considered as an adjunct to refine risk estimation. **Fig. 2** illustrates the estimated recurrence risk classification for papillary and its variants, follicular, and oncocytic thyroid carcinomas based on histopathological criteria. **Fig. 3** presents recurrence risk stratification according to the molecular risk profile in differentiated thyroid cancer.

The ATA response to therapy system is applied to categorize the outcome of surgical treatment prior to the indication of additional therapies and follow-up, in combination with the ATA estimated recurrence

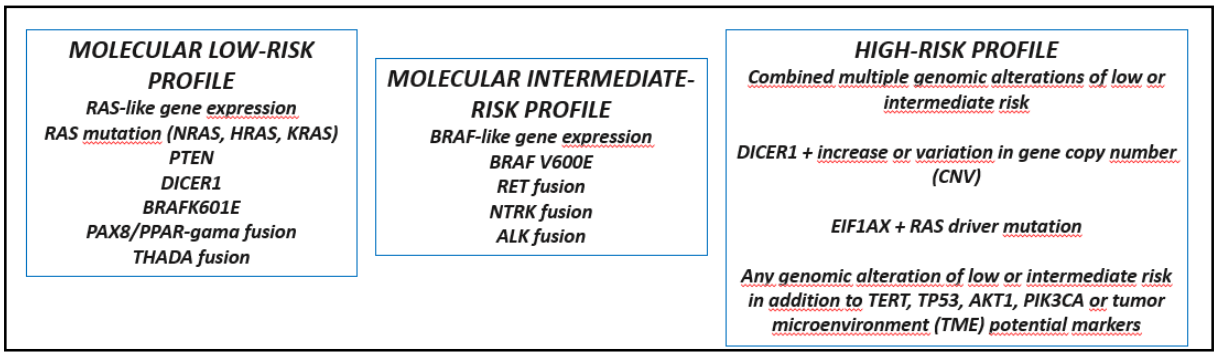
Figure 2. Estimated structural recurrence risk in thyroid cancer based on histopathological criteria.



ETE: extrathyroidal extension; LNs: lymph nodes; IEFVPTC: invasive encapsulated follicular variant of papillary thyroid carcinoma; AJCC/TNM staging system 9th edition[26]: TI – tumor 2 cm or less in greatest dimension, limited to the thyroid; T2 – tumor more than 2 cm but not more than 4 cm in greatest dimension, limited to the thyroid; T3a – tumor more than 4 cm in greatest dimension, limited to the thyroid; T3b – tumor of any size with gross extra-thyroidal extension involving strap muscles or patathyroid gland; T4a – tumor extends beyond the thyroid capsule and invades any of the following: subcutaneous soft tissues, larynx, trachea, oesophagus, recurrent laryngeal nerve or sternocleidomastoid muscle; T4b – tumor invades the prevertebral fascia, mediastinal vessels or encases the carotid artery; N1a – metastasis in level VI (central) or level VII (upper/ superior mediastinum); N1b – metastasis in other unilateral, bilateral or contralateral cervical lymph nodes like level I (submental or submandibular) / level II (upper internal jugular chain) / level III (middle internal jugular chain) / level IV (lower internal jugular chain) / level V (cervical posterior triangle) or at retropharyngeal lymph nodes; c – clinical staging; p – pathological staging.

Extracted and adapted from ATA guidelines 2025 publication[1].

Figure 3. Molecular risk classification of thyroid cancer for estimating structural recurrence risk.



Adapted from ATA guidelines 2025 publication[1].

risk. While recurrence risk stratification and staging provide information on structural recurrence risk and disease-specific mortality, these systems were not designed for therapeutic individualization based on treatment response. Many patients initially classified as intermediate- or high-risk according to baseline recurrence estimates are subsequently reclassified as low-risk following an excellent response to initial therapy[1].

Due to the decreased use of radioactive iodine therapy (RAI) in patients with low recurrence risk and the increased use of lobectomy, the ATA 2025 guide-

lines recommend the first assessment of treatment response at approximately three months after surgery. This initial evaluation provides essential information for decisions regarding the use of RAI and for tailoring its dosage. For example, a patient with multiple lymph node metastases and high recurrence risk may be considered for a lower RAI dose if, at three months after surgery, serum thyroglobulin is undetectable in the absence of anti-thyroglobulin antibodies, while on levothyroxine therapy, and with a neck ultrasound showing no residual or suspicious disease. Conversely, in the same patient, if thyroglobulin levels are detectable, further pulmonary evaluation for metastasis and higher RAI doses may be indicated, thereby altering therapeutic goals[1].

The four initial response to therapy categories were originally proposed by Tuttle et al.[27] to describe the best response to initial therapy during the first two years of follow-up[28]. Currently, however, they are applied at any point during surveillance. The categories include: **excellent response**, defined as no clinical, biochemical, or structural evidence of disease; **indeterminate response**, defined by nonspecific biochemical or structural findings that cannot be classified as benign or malignant, such as stable or declining anti-thyroglobulin antibody levels in the absence of structural disease; **biochemical incomplete response**, defined as abnormal thyroglobulin levels or rising anti-thyroglobulin antibodies without imaging evidence of disease; and **structural incomplete response,** defined as the presence of locoregional or distant disease identified on imaging[1].

The ATA 2025 guidelines recommend serum thyroglobulin measurement 6–12 weeks after total thyroidectomy, either under levothyroxine therapy or following TSH stimulation. This assessment helps guide decisions regarding additional therapy and monitoring[1]. Measurement of thyroglobulin after lobectomy, under normal TSH levels, may be useful to rule out unexpectedly elevated values; however, reference ranges in this clinical setting remain uncertain[1]. Cervical ultrasound to assess the thyroid bed, central, and lateral compartments is strongly recommended by the ATA as part of the evaluation of response to initial therapy. If postoperative thyroglobulin levels are above those expected for an excellent response, or if anti-thyroglobulin antibodies are present, neck ultrasound or cross-sectional imaging with CT or MRI should be performed prior to RAI administration[1].

A repeat cervical ultrasound should be performed 6–12 months after completion of initial therapy, while subsequent imaging studies and timing should depend on the risk of residual or recurrent disease as well as the initial treatment response[1]. Suspicious lymph nodes or lesions <10 mm may be monitored without cytological evaluation, unless they enlarge or threaten vital structures such as vessels, nerves, the trachea, or the esophagus[1]. If cytological confirmation of recurrent or metastatic disease would alter management, lymph nodes or lesions ≥10 mm should be aspirated for cytology and thyroglobulin washout measurement[1].

In cases when thyroglobulin or anti-thyroglobulin antibody levels rise after total thyroidectomy for differentiated thyroid cancer, but cervical ultrasound reveals no structural disease, additional imaging such as CT or MRI should be considered to evaluate common metastatic sites, including the lungs and bones. In this same clinical setting, but for patients with oncocytic or poorly differentiated carcinoma, ^{18}FDG-PET/CT may also be considered[1].

USE OF RADIOIODINE THERAPY (RAI) IN THE MANAGEMENT OF DIFFERENTIATED THYROID CARCINOMA

Routine remnant ablation is not recommended after total thyroidectomy in patients with low-risk differentiated thyroid carcinoma[1]. Adjuvant RAI may be considered after total thyroidectomy in patients classified as low- to high-intermediate risk of recurrence, whereas it is strongly recommended in patients with high risk of recurrence[1]. In patients undergoing total thyroidectomy with known distant metastasis, therapeutic RAI is routinely performed[1]. The use of RAI in oncocytic carcinoma remains controversial[1]. In cases of uncertainty regarding the actual need for adjuvant RAI in this context, a diagnostic whole-body scan may be considered prior to administering an empirical dose.

For patients receiving adjuvant or empirical RAI, preparation with recombinant human TSH is preferable to levothyroxine withdrawal, particularly in those with significant comorbidities that may increase the risks associated with long-term hypothyroidism. If levothyroxine withdrawal is chosen, it should be discontinued for 3 to 4 weeks, with the aim of raising TSH to levels >30 mIU/L prior to RAI administration. In patients with known metastatic disease, preparation for therapeutic RAI may be accomplished either by levothyroxine withdrawal or by recombinant human TSH[1].

A low-iodine diet is recommended for 1 to 2 weeks before RAI ablation, adjuvant therapy, or therapeutic dosing with I-131, in order to reduce exogenous iodine exposure that could interfere with I-131 uptake by metastasis. In most cases, treatment with I-131 is performed without prior diagnostic whole-body scanning using I-123 or low-dose I-131, although such imaging may be considered at the discretion of the physician to guide therapeutic planning[1]. Post-treatment whole-body scanning should be routinely performed 2 to 10 days after ablation, adjuvant, or therapeutic RAI, to identify foci of uptake corresponding to residual disease or metastatic sites. At the time of the post-therapy scan, stimulated measurements of thyroglobulin, anti-thyroglobulin antibodies, and TSH should be obtained, with TSH ideally >30 mIU/L[1].

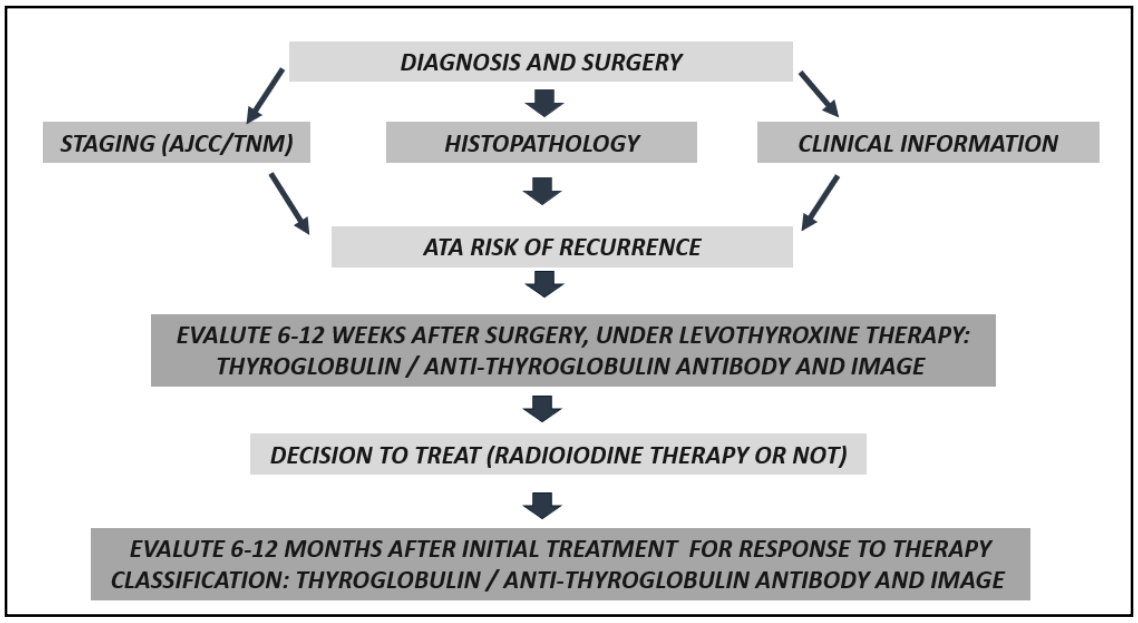
Adjuvant external beam radiotherapy may be considered in selected patients with high-risk disease characterized by gross extrathyroidal extension, visceral invasion, positive margins, or aggressive histological variants, particularly when surgical salvage would not be feasible in the event of recurrence[1]. The potential benefit of improved locoregional recurrence-free survival must be balanced against the absence of evidence for improved overall survival and the significant risk of toxicity. In selected cases of incomplete resection or unresectable disease, concurrent chemoradiation may be considered to enhance locoregional control, though this approach requires careful weighing of the acute and late toxicities associated with combined treatment[1].

LONG-TERM MANAGEMENT OF DIFFERENTIATED THYROID CARCINOMA AND TSH-SUPPRESSIVE THERAPY WITH LEVOTHYROXINE

The long-term management of patients with differentiated thyroid carcinoma (DTC) is guided by two main principles: monitoring for the detection of clinical recurrence and identification of tumor progression in patients with suspected or residual disease. Suppression of thyroid-stimulating hormone (TSH) with levothyroxine remains a cornerstone of therapy in this setting, as it reduces tumor stimulation and the likelihood of recurrence[1]. **Fig. 4** illustrates the framework for long-term management in these patients.

High-specificity diagnostic tools allow the identification of patients with a minimal likelihood of recurrence, enabling the use of less aggressive treatments, thereby reducing toxicity and improving cost-effectiveness[1]. De-escalation of follow-up intensity is possible in low-risk patients several years after initial therapy, provided they maintain a persistent excellent re-

Figure 4. Framework for the management of differentiated thyroid cancer after initial therapy, aimed at guiding treatment decisions and follow-up based on a dynamic risk stratification for recurrence.



AJCC: American Joint Committee on Cancer; TNM: Tumor/ Nodes/ Metastasis[26].
Extracted and adapted from ATA guidelines 2025 publication[1].

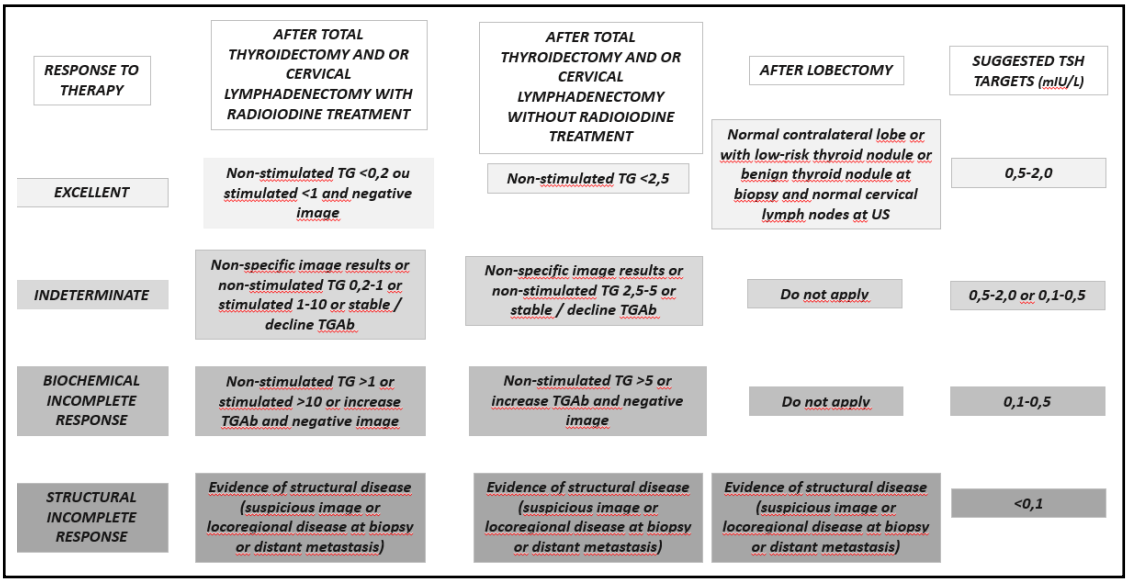
sponse to treatment[1]. Conversely, patients at high risk of recurrence should undergo closer surveillance, as early detection of recurrence provides the best opportunity for an effective therapeutic response[1]. A shared decision-making model should be established with the patient, considering survival outcomes, response to therapy, short- and long-term toxicities, and the financial implications of both treatment and surveillance, as well as their sequelae.

In patients who have undergone total thyroidectomy, the most sensitive biomarker for recurrence detection is serum thyroglobulin (Tg). For this reason, Tg should be measured post-treatment and throughout follow-up in patients with differentiated thyroid carcinoma, in conjunction with anti-thyroglobulin antibody (TgAb) levels[1]. Measurement of Tg while patients are receiving levothyroxine replacement, after total thyroidectomy with or without RAI, is recommended to evaluate initial therapeutic response and to detect recurrence[1]. The first Tg measurement should be obtained approximately 3 months postoperatively, and thereafter every 6–12 months, particularly in intermediate- to high-risk patients[1]. Routine Tg measurement after lobectomy in patients on levothyroxine is generally not recommended[1].

In patients with circulating TgAb, serial trends of antibody levels, measured with the same assay, may be useful for disease monitoring. However, both immunometric and radioimmunometric Tg assays are affected by TgAb interference[1]. Therefore, in this setting, surveillance for residual or recurrent disease should not rely exclusively on biochemical markers, but primarily on imaging studies[1]. **Fig. 5** summarizes the desirable ranges of stimulated and non-stimulated Tg, along with TgAb trends and their integration with imaging findings, according to treatment modality and initial therapeutic response.

The indication, target values, and duration of TSH suppression therapy must balance potential benefits and risks. Patients at high risk of recurrence, particularly those with confirmed metastatic disease, derive the greatest benefit from maintaining subnormal TSH levels[1]. Conversely, TSH suppression is not indicated in low-risk patients or in intermediate-risk patients without biochemical or structural evidence of recurrence[1]. The risks and benefits of suppression, as well as TSH goals, should be continuously reassessed during follow-up, in accordance with dynamic risk stratification[1]. In patients with comorbidities such as osteoporosis or atrial fibrillation, TSH suppression targets should be applied cautiously, even in high-risk settings such as biochemical incomplete response to therapy and even in structural incomplete response to therapy, in order to avoid treatment-related morbidity that may outweigh the risks of tumor progression or recurrence. **Fig. 5** outlines the definitions of treatment response according to the type of therapy performed, including the corresponding TSH suppression targets.

Figure 5. Definitions of therapeutic response according to the type of treatment administered and recommended TSH levels for each response to therapy category.



TG: thyroglobulin; TGAb: anti-thyroglobulin antibody; US: ultrasound; thyroglobulin values are presented in ng/mL.
Extracted and adapted from ATA guidelines 2025 publication[1].

Patients with low-risk differentiated thyroid cancer (DTC) treated with total thyroidectomy, with or without radioactive iodine therapy (RAI), and who maintain an excellent response for 5 to 8 years following initial therapy, may discontinue routine neck ultrasound[1]. In such cases, follow-up can be conducted using biochemical markers alone, assessed every 1 to 2 years. If the excellent response persists for 10 to 15 years, these patients are considered to be in complete remission and do not require further serum thyroglobulin (Tg) or anti-thyroglobulin antibody (TgAb) measurements[1].

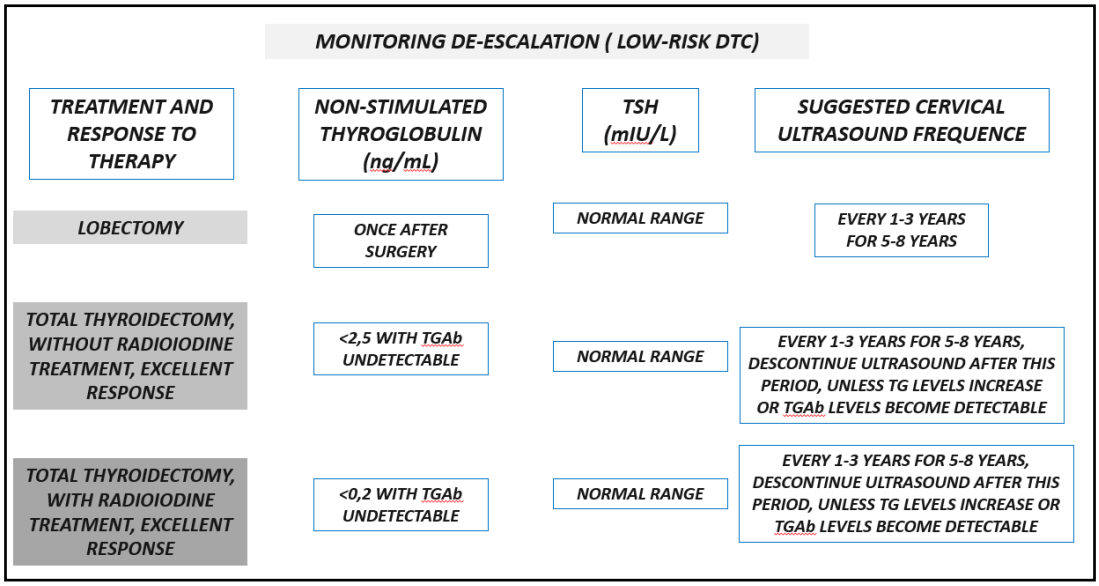
For low-risk patients treated with lobectomy alone, if the baseline ultrasound is negative, subsequent imaging may be performed every 1 to 3 years for a period of 5 to 8 years post-treatment. Any nodules detected in the contralateral lobe should be managed according to the American Thyroid Association (ATA) guidelines for thyroid nodule evaluation. In patients treated with lobectomy whose serum thyroglobulin was measured 3 months postoperatively and was not significantly elevated, further Tg testing during follow-up is not recommended[1]. **Fig. 6** outlines the recommended de-escalation strategies for monitoring patients with low-risk differentiated thyroid carcinoma who demonstrate an excellent response to initial therapy.

Patients treated with lobectomy or total thyroidectomy without radioactive iodine therapy (RAI), as well as those classified as low- or low-intermediate risk of recurrence who demonstrate an excellent response to therapy, do not require diagnostic whole-body scans (DxWBS) with iodine during follow-up[1]. In contrast, patients classified as high-intermediate or high risk of recurrence may undergo DxWBS to assess iodine avidity when there is clinical suspicion of recurrence[1]. When indicated, the diagnostic scan may be performed using either low-dose iodine-131 or iodine-123.

The use of ^{18}FDG PET/CT may be appropriate in high-risk differentiated thyroid cancer patients with elevated serum thyroglobulin levels, particularly in those with oncocytic carcinoma, aggressive histological variants, or in cases with negative imaging following a diagnostic or empiric RAI therapy dose[1]. ^{18}FDG-PET/CT may also serve as a prognostic tool in patients at high risk of disease progression or cancer-related mortality, and can be useful for evaluating the response to local or systemic treatment of invasive disease[1].

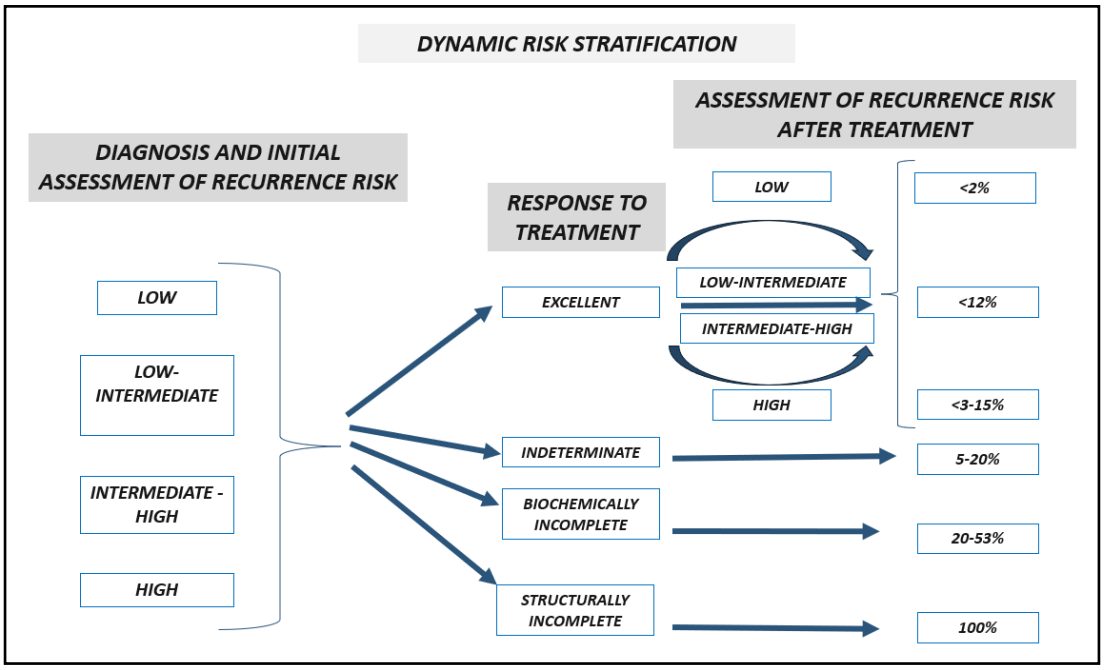
When combined with the initial recurrence risk stratification, dynamic risk assessment allows for individualized management, as the estimated risk evolves over time with continued follow-up. This approach helps guide the intensity, frequency, and type of surveillance testing[1]. **Fig. 7** outlines this dynamic risk classification following initial therapy. Initial pathology, imaging, and clinical evaluation are used to estimate the risk of recurrence and inform therapeutic deci-

Figure 6. Recommended de-escalation strategies for surveillance in patients with low-risk differentiated thyroid carcinoma (DTC) showing an excellent response to initial therapy.



TG: thyroglobulin; TGAb: anti-thyroglobulin antibody.
Extracted and adapted from ATA guidelines 2025 publication[1].

Figure 7. Dynamic risk stratification to guide ongoing long-term surveillance in patients with differentiated thyroid cancer.



Extracted and adapted from ATA guidelines 2025 publication[1].

sions. Subsequent assessment of treatment response leads to a revised recurrence risk estimate, which then informs long-term follow-up strategies.

CONCLUSION

The most recent American Thyroid Association (ATA) publication introduces important updates regarding the need for treatment and surveillance de-escalation in patients with low-risk of recurrence, as well as the potential for active surveillance in selected low-risk tumors. Radioablation has been discussed as an alternative to active surveillance in highly selected cases; however, long-term studies are still lacking to establish its efficacy and safety in the management of papillary thyroid microcarcinoma. Furthermore, the new stratification of intermediate-risk tumors and the emphasis on dynamic risk assessment throughout follow-up, based on the patient's response to therapy, enable tailored de-escalation of monitoring, particularly in low-risk patients. These recommendations are of critical importance, as they aim to reduce treatment-related morbidity in a cancer that is generally indolent, highly curable, and increasingly overdiagnosed, which in turn contributes to overtreatment and unnecessary long-term surveillance testing and costs.

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CASE REPORT *RELATO DE CASO*

POLYMORPHIC ERUPTION OF PREGNANCY IN A FEMALE PATIENT, FIRST-TIME MOTHER, AND WITHOUT POLYMORPHISM IN CUTANEOUS LESIONS

ERUPÇÃO POLIMÓRFICA ESPECÍFICA DA GESTAÇÃO EM PACIENTE COM GRAVIDEZ ÚNICA DE SEXO FEMININO E SEM POLIMORFISMO EM LESÕES CUTÂNEAS

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ABSTRACT

Polymorphic eruption of pregnancy is a specific dermatosis of pregnancy with an unknown, mechanically induced etiology. It is more observed in multiparous women, first-time mother, and during the third trimester of pregnancy. Polymorphic eruption of pregnancy presents a broad range of differential diagnoses among gestational dermatoses, making clinical knowledge essential for proper investigation and timely diagnosis. This study reports a case involving a 34-week pregnant woman, first-time mother, which presents certain deviations from the classic epidemiological profile of the disease.

Keywords: PUPPP, polymorphic eruption of pregnancy, eruption, dermatosis, dermatology, pregnancy.

RESUMO

A erupção polimórfica específica da gravidez é uma dermatose específica da gestação de desconhecida etiologia, mecanicamente induzida, mais comum em multíparas, primigestas e no terceiro trimestre da gestação. Apresenta ampla gama de diagnósticos diferenciais de dermatoses gestacionais, sendo necessário seu conhecimento clínico para correta investigação e diagnóstico oportuno. O presente trabalho é um relato de caso de gestante primigesta, de 34 semanas, com feto feminino, o que apresenta algumas diferenças na clássica epidemiologia da doença.

Descritores: PUPP, erupção polimórfica específica da gestação, dermatose, dermatologia, gestação.

INTRODUCTION

Polymorphic eruption of pregnancy (PUPP) is a specific dermatosis of pregnancy with unknown etiology. It presents with variable clinical manifestations and has a favorable prognosis, typically resolving spontaneously in the postpartum period within an average of[7-10] days, without causing fetal complications[1,2]. It is the most prevalent specific dermatosis of pregnancy, affecting approximately 0.5% of women first-time mother, up to 16% of twin pregnancies, and up to 17% of triplet pregnanciests[1,3] occurrence is more frequent in first-time moth-

er particularly during the third trimester[1,3,4]. Regarding fetal sex, it is twice as common in pregnancies with male fetuses compared to female fetuses. There is no association between the condition and a personal or family history of PUPPP or autoimmune diseases.

CASE DESCRIPTION

We report the case of a 31-year-old female patient, first-time mother, at 34 weeks of gestation (female fetus), referred to secondary outpatient dermatologic care due to cutaneous complaints identified during a routine prenatal obstetric consultation in January 2024. The patient reported the onset of intense pruritus, most pronounced in the area of abdominal striae gravidarum, with progressive involvement of the lower (LL) and upper limbs (UL), persisting for 15 days without any relieving or aggravating factors. She reported prior use of loratadine, with no symptomatic improvement, and denied any similar previous episodes. Her past medical history was notable for gestational diabetes mellitus, for which she was continuously using folic acid and ferrous sulfate. She denied allergies, as well as any personal or family history of dermatoses, atopy, or skin cancer. The patient was a former smoker.

Physical examination revealed erythematous and widened striae on the abdomen and the proximal third of both thighs, along with multiple erythematous papules distributed across the entirety of the lower limbs and forearms, some of which coalesced into plaques **(Fig. 1).**

Treatment consisted of topical mometasone mixed in equal parts with a moisturizing cream, along with oral loratadine once daily for symptomatic relief. The patient was educated about the association of the dermatosis with pregnancy, its benign nature, favorable prognosis, and typical spontaneous resolution after delivery. Clinical follow-up was advised, with a return visit scheduled in 15 days or earlier if needed.

At follow-up, at 36 weeks of gestation, the patient reported partial improvement, with persistent pruritus on the abdomen and thighs. She denied any changes in the lesion pattern, development of vesiculobullous lesions, or generalized eczema **(Fig. 2)**. Continuation of the topical regimen and oral loratadine was recommended. The patient experienced spontaneous resolution postpartum and was advised to discontinue treatment after symptomatic improvement. She did not return for further dermatological or obstetric follow-up.

Figure 1. A. PUPPP lesions on striae gravidarum in the abdominal region of pregnancy, sparing the periumbilical area at the first consultation (01/23/2024).B. Lesions in the inguinal region and anterior thigh at the first consultation. C and D. Lesions on the extensor surfaces of the left and right arm and forearm, respectively, at the first consultation.

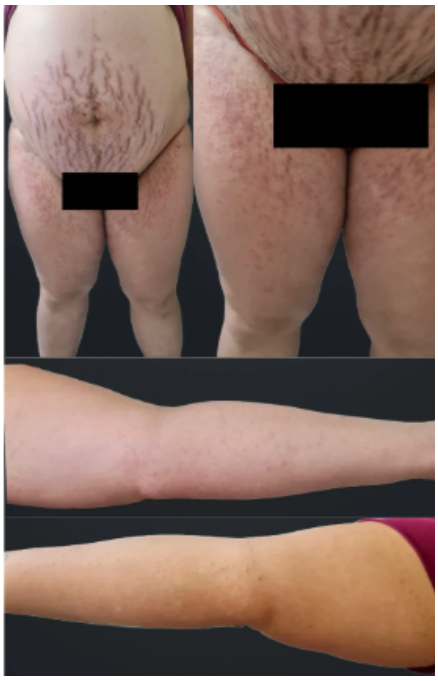
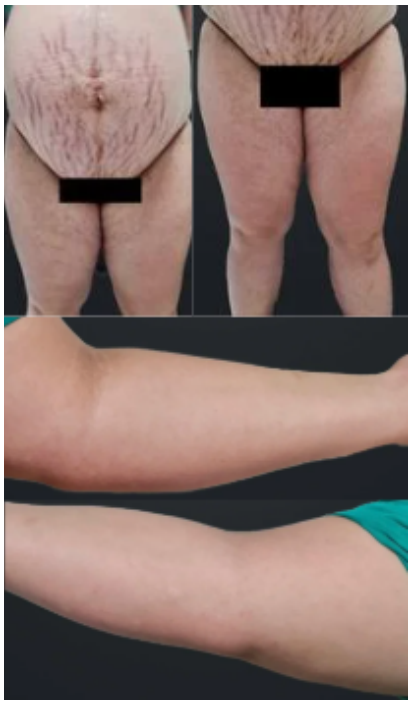


Figure 2. Complete resolution of PUPPP lesions on striae gravidarum, lower and upper limbs, at follow-up consultation (02/07/2024).



DISCUSSION

PUPPP is an inflammatory condition of uncertain etiology. However, its onset has been associated with abdominal distension, hormonal changes—especially elevated progesterone levels—placental factors, and fetal DNA. Studies have shown that 90% of cases result from damage to connective tissue within striae gravidarum, exposing collagen antigens that trigger an allergic response and the development of eruptions over the striae[1,2].

The pathophysiology is particularly associated with abdominal distension, such that women with excessive gestational weight gain have a higher incidence of the disease[1,2,3]. Furthermore, this mechanism does not appear to be associated with any specific HLA subtype.

Another proposed trigger for PUPPP is peripheral chimerism, which results from contact with fetal tissue, particularly in the third trimester. This exposure may lead to collagen damage and vascular alterations, potentially triggering an immune response[4].

Although the pathophysiology of PUPPP remains unclear, studies have shown that it is not an autoimmune disorder and is not associated with a specific human leukocyte antigen (HLA). The mechanism through which this condition becomes systemic is also unknown, but it is hypothesized to involve cross-reactivity between collagen and intact skin, as abdominal distension alone does not fully explain the disease—especially since unaffected, non-distended areas can also be involved[1,4,5].

Lesions typically appear in the third trimester, particularly after the 36th week of gestation and even during the immediate postpartum period[3,4,6,7].

This case involved an earlier presentation than is typical in the literature, occurring at 34 weeks. In terms of fetal sex, the condition is twice as common in pregnancies with male fetuses, which differs from the case presented here.

On physical examination, PUPPP initially presents as small, erythematous, edematous, and pruritic papules, beginning in the striae gravidarum and sparing the periumbilical region, with no mucosal involvement. Lesions may coalesce into urticarial plaques with a pale halo. Over time, the eruption may extend to the trunk and limbs, and rarely to the face and distal extremities[2,3]. In the present case, the patient's lesions and their progression matched the classic clinical presentation. As the disease progresses, most cases develop polymorphis[1,2] including generalized erythema, target lesions, small vesicles (up to 2 mm), and eczematous patches[1,2,8,9].

In this case, however, no lesion polymorphism was observed; the condition remained limited to erythematous, pruritic papules and plaques—unlike the majority of cases in the literature. Only a few cases report large bullous lesions[2,1,11]. No post-inflammatory pigmentation or scarring occurs following spontaneous resolution.

Diagnosis is clinical, as histological and immunofluorescence findings are nonspecific, and laboratory tests remain within normal limits[1,2]. Biopsy may be necessary in selected cases to exclude other gestational dermatoses[9]. In this case, the classic presentation made biopsy unnecessary. Differential diagnoses include atopic eruption of pregnancy, intrahepatic cholestasis of pregnancy, and gestational pemphigoid (also known as *herpes gestationis*).[1,2,9,11]

The most relevant differential diagnosis is gestational pemphigoid (PG), due to its clinical similarity. Both conditions present with erythematous lesions that are nearly indistinguishable, except for PG's typical periumbilical involvement. Despite the clinical resemblance, their progression and outcomes differ. PG usually arises in the second or third trimester and is a true autoimmune disease involving autoantibodies against BP180, a placental antigen. PG is associated with fetal complications such as prematurity and intrauterine growth restriction. Additionally, PG tends to recur, often earlier and more severely in subsequent pregnancies—unlike PUPPP, where recurrence is rare. Definitive differentiation between these conditions is achieved by direct immunofluorescence, which in PG reveals linear C3 and IgG deposits[2,9].

Among other differentials, atopic eruption of pregnancy is the most common dermatosis during pregnancy. It typically begins in the first or second trimester and occurs in 20% of patients with a history of atopic dermatitis—or, more commonly, as a first-time idiopathic presentation[2,9]. Eczema tends to involve a personal or family history and manifests as pruritic erythematous lesions in flexural areas, differing from the case described. Other possibilities include drug eruptions, urticaria, and viral or bacterial infections.

PUPPP is a benign condition with a good prognosis, resolving spontaneously in most cases and rarely recurring in subsequent pregnancies—possibly due to the development of immunological tolerance. When recurrence occurs (in approximately 15% of cases), it tends to be milder and less symptomatic[2,9]. The condition does not cause fetal morbidity, and neonatal skin is typically unaffected. Therefore, PUPPP is not a formal indication for preterm delivery[1,5].

Due to its self-limited nature, treatment is symptomatic. In the present case, the patient had previously used loratadine with no relief. This may be attributed to the lower potency of second-generation antihistamines, despite their better safety profile, and to the higher intensity of pruritus at the time. Studies have shown better efficacy with first-generation antihistamines, though these cross the blood-brain barrier and often cause drowsiness[2,9]. Consequently, topical mometasone was prescribed with a moisturizing cream to enhance the therapeutic response, as corticosteroids are known to improve immune control. Other symptomatic options include topical corticosteroids and emollients. In refractory or more severe cases, systemic prednisone may be used, with gradual tapering following clinical improvement[1,2].

This study aims to inform the medical and academic community about a common complaint that presents a wide range of dermatological differential diagnoses in a pregnant patient, who is often followed by physicians from other specialties not related to dermatology, from prenatal care to delivery. Awareness of the existence of such a condition allows for the establishment of a diagnostic hypothesis and reduces patient suffering. The main limitation of this study is the fact that only one patient was included in the report.

CONCLUSION

This case report presents features that diverge from the most commonly described epidemiological profile of PUPPP, including a female fetus, earlier-than-usual gestational age at onset, first-time mother, and absence of the typical polymorphic nature of the lesions. Nonetheless, the clinical presentation is consistent with the classical description in the literature and reinforces the primarily clinical nature of the diagnosis, as well as the favorable response to symptomatic treatment in most cases.

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CASE REPORT *RELATO DE CASO*

BILATERAL TESTICULAR ADRENAL REST TUMOR IN A YOUNG ADULT WITH POORLY CONTROLLED CONGENITAL ADRENAL HYPERPLASIA

TUMOR BILATERAL DE REMANESCENTE ADRENAL TESTICULAR EM UM ADULTO JOVEM COM HIPERPLASIA ADRENAL CONGÊNITA MAL CONTROLADA

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ABSTRACT

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive genetic disorder that affects adrenal steroidogenesis, compromising the production of hormones such as cortisol and, in some cases, aldosterone. More than 90% of cases are caused by a deficiency of the 21-hydroxylase enzyme, but rarer forms can occur due to the failure of other enzymes, such as 11β-hydroxylase or 3β-hydroxysteroid dehydrogenase. The reduction in cortisol synthesis leads to a chronic increase in adrenocorticotropic hormone (ACTH), due to the loss of negative feedback in the hypothalamic-pituitary-adrenal axis. This stimulates hyperplasia of the adrenal glands and excessive production of androgens. In male patients, a relevant complication is the development of Testicular Adrenal Rest Tumors (TARTs), with an average prevalence of around 40%, which can mechanically obstruct the seminiferous tubules and cause infertility. The treatment of CAH consists of chronic glucocorticoid replacement, with the aim of suppressing ACTH, reducing adrenal hyperplasia and preventing the growth of TARTs.

Keywords: Steroidogenesis dysfunction, male infertility, scrotal mass, adrenal insufficiency, hormonal impairment.

RESUMO

A Hiperplasia Adrenal Congênita (HAC) é um distúrbio genético autossômico recessivo que afeta a esteroidogênese adrenal, comprometendo a produção de hormônios como o cortisol e, em alguns casos, a aldosterona. Mais de 90% dos casos são causados por deficiência da enzima 21-hidroxilase, mas formas mais raras podem ocorrer devido à falha de outras enzimas, como a 11β-hidroxilase ou a 3β-hidroxiesteroide desidrogenase. A redução na síntese de cortisol leva ao aumento crônico do hormônio adrenocorticotrófico (ACTH), pela perda do feedback negativo no eixo hipotálamo-hipófise-adrenal. Isso estimula a hiperplasia das glândulas adrenais e a produção excessiva de andrógenos. Em pacientes do sexo masculino, uma complicação relevante é o desenvolvimento de Tumores de Restos Adrenais Testiculares (TARTs), com prevalência média em torno de 40%, os quais podem obstruir mecanicamente os túbulos seminíferos e causar infertilidade. O tratamento da HAC consiste na reposição crônica de glicocorticoides, com o objetivo de suprimir o ACTH, reduzir a hiperplasia adrenal e prevenir o crescimento dos TARTs.

Descritores: Disfunção da esteroidogênese, infertilidade masculina, massa escrotal, insuficiência adrenal, comprometimento hormonal.

INTRODUCTION

Congenital Adrenal Hyperplasia (CAH) is a group of autosomal recessive genetic conditions that affect steroid synthesis by the adrenal glands. Deficiency of the enzyme 21- hydroxylase is the most common cause, accounting for more than 90% of cases[1,2], but there are less frequent forms caused by defects in other enzymes, such as 11β-hydroxylase, 17α-hydroxylase, 3β-hydroxysteroid dehydrogenase and 17, 20-lyase. This enzyme deficiency compromises the production of cortisol and, in some cases, aldosterone[2,3,4]. As a result, the lack of cortisol activates a negative feedback mechanism, resulting in increased levels of adrenocorticotropic hormone (ACTH) by the pituitary gland[5,4], leading to adrenal hyperplasia and overproduction of androgens[1].

An important complication in male patients, especially in the classic form of CAH, is the development of Testicular Adrenal Rest Tumors (TARTs)[6,7,8]. Although these tumors are mostly benign[1,6], their presence is associated with low fertility in affected individuals[9,5]. TARTs are formed by remnants of adrenocortical tissue that have moved to the testes during embryonic development[8]. Stimulated by high levels of ACTH, these adrenal remnants grow and multiply. In addition, the increase in luteinizing hormone (LH) during puberty is also suggested as a factor contributing to the growth of these lesions[1,8].

TARTs are often bilateral and located within the rete testis[1,7,5]. The main complication associated with these tumors is infertility, which occurs due to mechanical compression on the seminiferous tubules, especially when these masses reach a significant size. This type of damage can result in hypospermatogenesis or obstructive azoospermia, and irreversible damage to the testicles, such as peritubular fibrosis and tubular hyalinization, can persist even after the tumor formation has been removed[1,8,4].

Ultrasound is the most widely used imaging method to detect and monitor TARTs, due to its accessibility and efficiency in identifying lesions that are not yet palpable on physical examination. Typical features include bilaterality, good delimitation, location close to mediastinum testis, with hypoechogenicity and variable vascularization. An important factor in follow-up is the possibility of changes in the size or echogenicity of the lesions after starting treatment with glucocorticoids[1,5,4]. Another challenge is the differentiation between TARTs and Leydig cell tumours (LCTs), since both have similar morphological characteristics. Histology and clinical context, such as a history of CAH and the bilaterality of the lesions, become fundamental for this distinction[1,8,4].

Treatment of TARTs focuses on controlling the underlying CAH and preserving reproductive function[4]. The initial medical approach consists of glucocorticoid therapy in order to suppress ACTH production and promote a reduction in tumor size. However, the response to treatment is variable and does not always result in complete regression of the tumors or restoration of fertility. In selected cases, surgery may be well indicated, especially to relieve symptoms such as severe pain. However, early detection and strict hormonal control from childhood remain the main strategies for preventing permanent damage to the testicles and minimizing the reproductive consequences of this condition[8,3,4].

Considering the occurrence of TARTs in adolescents, their increased prevalence during puberty, the severity associated with inadequate hormonal control and the lasting impacts on gonadal function, it is essential to understand how this condition manifests itself and how it should be managed in this population.

CASE REPORT

Male patient, 24-year-old, Caucasian, currently on leave from his job as a security guard. He was followed up at the Endocrinology and Metabology outpatient clinic at the Federal University of Paraná, in Toledo - PR, due to congenital adrenal hyperplasia (CAH), diagnosed in childhood. Despite having undergone biological newborn screening (heel prick test) at the appropriate time, no alterations were identified. The first clinical signs appeared at 3 months of age, with pubic hair and penile enlargement, when treatment for the condition was also started. There were no reports of perinatal complications associated with the condition. In terms of family history, a sister died at 45 days of age due to the classic form of CAH, with ambiguous genitalia and signs of dehydration.

Since the age of 8, the patient had been severely obese, weighing around 90 kg, and had been under continuous medical supervision. At the age of 14, he stopped treatment and his weight stabilized, with no evidence of clinical complications. At the age of 22, he was treated at another clinic and started taking prednisone 20 mg/day, fludrocortisone acetate 0.1 mg/day, spironolactone 25 mg/day and ketoconazole 200 mg/day. In October 2024, at the age of 23, he was seen at this outpatient clinic with grade 3 obesity, cushing's syndrome, obstructive sleep apnea and hypopnea syndrome (OSAHS), systemic arterial hypertension (SAH) and polyglobulia.

Physical examination revealed cushingoid facies, facial plethora, severe obesity (weight: 183 kg; height: 1.61 m; BMI: 70.60 kg/m²), cyanosis of the extremities, dorsal gibbosity, ochre dermatitis on the lower limbs and paronychia on both hallux. Evaluation of the genitals showed a 9 cm penis, topical testicles, painless on palpation, ultrasound with the presence of nodules suggestive of adrenal rests (hypoechoic, heterogeneous and infiltrative lesion in both testicles, without delimited borders, suggestive of TARTs).

On his return visit in 30 days, with tests results requested at the first appointment, the laboratory results showed hemoglobin (Hb) of 20.85 g/dL (VR: 13,5 - 17,5 g/dL), hematocrit (Ht) of 65.61% (VR: 41% - 50%), sodium of 139 mEq/L (VR: 135 - 145 mEq/L), potassium of 4.87 mEq/L (VR: 3,5 - 5,1 mEq/L), hormonal profile with cortisol of 2.27 µg/dL (VR: 3,7 - 19,4 µg/dL), ACTH of 56 pg/mL (VR: 9 - 52 pg/mL), androstenedione above 10 ng/mL (VR: 0,4 - 3,1 ng/dL), 17α-hydroxyprogesterone (17-OHP) of 10.000 ng/dL (VR: 50 - 210 ng/dL). With these data, the initial diagnosis of classic form CAH was confirmed. The therapeutic plan established included nutritional counseling, suspension of spironolactone, prednisone and fludrocortisone, associated with therapeutic phlebotomy and 11-deoxycorticosterone (DOCA) dosage, in order to allow for a subsequent diagnostic reassessment.

Approximately 60 days later, after therapeutic phlebotomy (2 sessions of 350 mL), a loss of 8 kg and blood pressure control with losartan and hydrochlorothiazide (50mg + 12.5mg), the patient reported clinical improvement, especially related to sleepiness. Baseline tests showed ACTH of 58 pg/mL, 17-OHP of 7,820 ng/dL, total testosterone of 251 ng/dL (VR: 249 - 836 ng/dL) and DOCA of 480 pg/mL (VR: ≤ 16 ng/dL), findings compatible with the classic form of non- salt-losing CAH, with a strong suspicion of 11β-hydroxylase deficiency. Repeated testicular ultrasound showed a bilateral tumor with slight progression. As a result, the prescrition included semaglutide (1.0 mg/week), prednisone was reintroduced at 2.5 mg/day and serial therapeutic phlebotomy was maintained (350 mL per session).

Four months after resuming drug treatment, laboratory tests revealed an Hb of 18.6 g/dL and Ht of 62%, total testosterone of 286 ng/dL and androstenedione above 10 ng/mL. During the same period, there was a weight loss of 13 kg, with a BMI of 65.58 kg/m². A repeat testicular ultrasound kept the findings unchanged. In view of this, the importance of adherence to prednisone therapy and maintenance of bloodletting was reinforced. The dose of semaglutide was increased to 1.2 mg/week in order to optimize metabolic control.

The last clinical assessment, carried out 8 months after the first consultation, showed a weight loss of 44 kg, with a BMI of 53.62 kg/m². Laboratory tests showed an Ht of 61%, maintaining the indication for periodic therapeutic phlebotomy, while the other parameters showed no relevant changes. Clinically, there was a significant improvement in daytime sleepiness, and polysomnography was requested for further assessment, but not yet carried out. The therapeutic regimen was maintained with adjustments, replacing semaglutide with tirzepatide 10 mg/week, prednisone 5 mg/day and 350 mL bloodletting. The major therapeutic challenge in this case is to control the CAH and the progression of TART, in order to prevent it from evolving into Cushing's syndrome, as well as adjusting weight and managing associated comorbidities.

DISCUSSION

Congenital adrenal hyperplasia (CAH) is a heterogeneous group of diseases that compromise the synthesis of adrenocortical steroids. Among its variants, the form resulting from 11β-hydroxylase deficiency is rare, with a prevalence of approximately 1:100,000 live births, predominating in North African and Middle Eastern populations, while 21- hydroxylase deficiency is more frequent and occurs mainly in Eastern Europe and among Jews[10]. 11β-hydroxylase deficiency stands out for its association with hypertension, polyglobulia and the accumulation of hormone precursors such as 11-deoxycorticosterone (DOCA)[10,11].

In the case reported, although the initial diagnosis pointed to the classic form due to 21-hydroxylase deficiency, the presence of chronic hypertension associated with markedly elevated levels of DOCA and the occurrence of refractory polyglobulia strongly suggest the possibility of an 11β-hydroxylase deficit. The family history reinforces this suspicion, as the patient's sister had typical female manifestations, such as ambiguous genitalia and dehydration, highlighting the hereditary nature and severity of the condition.

Since childhood, the patient has shown classic signs of hyperandrogenism, including precocious puberty at eight months, with pubic hair and testicular enlargement, compatible with ACTH hyperstimulation due to cortisol deficiency. The development of severe obesity also fits into the context of hormonal dysfunctions typical of CAH. Although treatment with glucocorticoids is essential, its adverse effects, such as increased BMI and higher blood pressure, are well documented[10]. In the present case, prolonged periods

of interruption in follow-up and iatrogenic use of high doses of glucocorticoids contributed to chronic complications, including excessive weight gain, systemic arterial hypertension and the development of testicular adrenal rest tumors (TARTs).

TARTs are common benign tumors in men with CAH, even under appropriate treatment[3]. They are usually bilateral and close to the testicular mediastinum[6,8], and can cause obstruction of the seminiferous tubules, azoospermia and irreversible local damage[7]. Usually diagnosed after the age of 10, their growth is stimulated by pubertal hormones, with ACTH being the main tumor inducer[8]. Differential diagnosis with Leydig cell tumors (LCTs) is essential, since TARTs are bilateral in around 80% of cases, while Leydig tumors are mostly unilateral (around 97%)[2]. Early treatment can reduce tumor volume, although its effectiveness is not fully established[7], and surgery is usually reserved for cases with significant pain or discomfort[8]. Testicular Doppler ultrasound is the imaging method of choice, identifying hypoechogenic and vascularized masses[1]. Despite their potential impact, TARTs can be asymptomatic[4].

Another relevant point in the case is the presence of severe polyglobulia that is refractory to clinical treatment, with a hematocrit frequently above 60%. This finding is more characteristic of 11β-hydroxylase deficiency than of the classic form due to 21- hydroxylase deficiency, and seems to be related to both the excess of androgens and the action of DOCA on erythropoiesis. The introduction of therapeutic phlebotomy provided partial relief of symptoms, although without normalizing the hematimetric parameters. Severe obesity, with a BMI of over 60 kg/m², was a major aggravating factor. The introduction of treatment resulted in significant weight loss within a few months, promoting significant functional improvement and potentially having a positive impact on obstructive sleep apnea and hypertension control.

Irregular adherence to treatment contributed significantly to the worsening of the clinical condition, highlighting the importance of strict hormonal monitoring, continuous health education and multidisciplinary follow-up. These aspects are fundamental to preventing complications of CAH, reinforcing the importance of early diagnosis and intervention. The case illustrates the complexity of managing classic CAH, especially when associated with less prevalent forms, such as 11β-hydroxylase deficiency. The integration of early diagnosis, appropriate therapy, ongoing screening for complications and patient adherence to treatment is essential to minimize long-term morbidities, including infertility, testicular dysfunction, cardiovascular complications and metabolic disorders.

CONCLUSION

This clinical case highlights the complexity of Congenital Adrenal Hyperplasia (CAH) and its potential long-term systemic complications, particularly the development of residual testicular adrenal tumors (TARTs). The history of chronic hormonal dyscontrol, demonstrated by persistently high levels of 17-hydroxyprogesterone (17-OHP) and ACTH, not only favored the formation and progression of TARTs, but also contributed to an aggravated metabolic condition, marked by severe obesity and systemic hypertension.

The management of these patients requires a multidisciplinary approach, with close monitoring and consistent adherence to glucocorticoid therapy, with the aim of adequately suppressing ACTH and possibly containing tumor growth. Serial testicular ultrasound stands out as an essential tool for early screening, follow-up and differentiation between TARTs and other testicular masses. Male infertility, mainly due to compression of the seminiferous tubules by the tumors, is one of the most serious complications, with a risk of irreversible testicular damage, since even testicular preservation surgery does not guarantee recovery of reproductive function.

In short, this case reinforces the importance of early diagnosis of CAH, adherence to hormone treatment and continuous clinical follow-up to control complications. Patient education and awareness of the long-term risks, especially infertility, are essential to enable shared decisions, timely interventions and, consequently, a better quality of life and prognosis.

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CASE REPORT *RELATO DE CASO*

AN ENDOCRINE PUZZLE: A CASE REPORT OF AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 2

UM QUEBRA-CABEÇA ENDÓCRINO: RELATO DE CASO DE SÍNDROME POLIGLANDULAR AUTOIMUNE TIPO 2

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ABSTRACT

Autoimmune polyglandular syndrome type 2 is an autoimmune endocrine condition characterized by Addison's disease (autoimmune primary adrenal insufficiency) associated with autoimmune thyroid disease and/or type 1 diabetes. Additionally, it may be associated with other autoimmune conditions. It presents a diagnostic challenge, leads to increased morbidity and mortality, and reduces quality of life. We present the clinical case of a 31-year-old man diagnosed with type 1 diabetes and vitiligo since childhood. During outpatient follow-up, around the age of 30, he developed clinical and laboratory criteria for Addison's disease and seronegative hypothyroidism. The case highlights the need for screening for autoantibodies in patients with autoimmune endocrinopathies, due to the increased risk of new autoimmune conditions.

Keywords: Autoimmune polyglandular syndrome type 2; Addison Disease; Diabetes Mellitus, Type 1; Thyroiditis.

RESUMO

A síndrome poliglandular autoimune tipo 2 é uma condição endócrina autoimune caracterizada por Doença de Addison (insuficiência adrenal primária autoimune) associada a doença tireoidiana autoimune e/ou diabetes tipo 1. Além disso, pode estar associada a outras condições autoimunes. Apresenta um desafio diagnóstico, gera aumento da morbimortalidade e redução da qualidade de vida. Apresentamos o caso clínico de um homem de 31 anos com diagnóstico de diabetes tipo 1 e vitiligo desde a infância. Durante o seguimento ambulatorial, por volta dos 30 anos, evoluiu com critérios clínicos e laboratoriais de doença de Addison e hipotireoidismo soronegativo. O caso reforça a necessidade de rastreamento de autoanticorpos em pacientes com endocrinopatias autoimunes, devido ao risco aumentado de novas condições autoimunes.

Descritores: Síndrome Poliglandular Autoimune Tipo II; Doença de Addison; Diabetes Mellitus Tipo I; Tireoidite.

INTRODUCTION

Autoimmune Polyglandular Syndrome Type 2 (APS Type 2) is an autoimmune endocrine disorder characterized by the dysfunction of multiple glands (adrenal, thyroid, pancreas). First described in 1926, its understanding has evolved to recognize it as a complex, polygenic condition resulting from a loss of immunological tolerance.[1–3] Although rare, with an estimated prevalence between 1:10,000 and 1:20,000 in

the general population, its importance lies in the risk of potentially fatal crises, especially those related to adrenal insufficiency.[2,4,5]

APS Type 2 requires the obligatory presence of Addison's disease (autoimmune primary adrenal insufficiency), associated with autoimmune thyroid disease (such as Hashimoto's Thyroiditis or Graves' disease), which is present in 69-82% of patients, and/or type 1 diabetes mellitus (T1DM), present in 30-52% of cases.[1,3,4] The most common combination is Addison's disease with autoimmune thyroid disease, which is known as Schmidt's Syndrome.[1] The clinical presentation is usually variable, with an insidious onset and slow progression. In addition to the main endocrinopathies, the syndrome can be associated with other autoimmune conditions, such as hyperparathyroidism, vitiligo, autoimmune gastritis with vitamin B12 deficiency, chronic autoimmune hepatitis, alopecia, myasthenia gravis, rheumatoid arthritis, Sjögren's syndrome, celiac disease, and hypergonadotropic hypogonadism.[2–4,6]

The pathophysiology of APS Type 2 involves a complex interaction between genetic and environmental factors. There is a strong association with specific Human Leukocyte Antigen (HLA-DR3 and/or HLA-DR4) haplotypes, as well as non-HLA genes like CTLA-4 and PTPN22. The disease is more common in women, with a 3:1 ratio compared to men, and its incidence is highest between the second and fifth decades of life.[1,7]

CASE PRESENTATION

We present the case of a 31-year-old male patient, E.P., with type 1 diabetes mellitus since the age of 10, admitted to Hospital Universitário Evangélico Mackenzie with abdominal pain, weight loss, nausea, vomiting, hyperglycemia, and positive ketonemia (>3 mmol/L). With no fever or signs of infection, he had irregular insulin use, constituting diabetic ketoacidosis secondary to poor adherence to the therapeutic treatment. He was already being followed by primary care and an endocrinologist. There were a history of vitiligo and a family history of diabetes mellitus, vitiligo, and pheochromocytoma. During hospitalization, he achieved glycemic stabilization with intensive insulin therapy. Infectious screening exams revealed no changes, allowing for hospital discharge with referral for outpatient follow-up.

In outpatient follow-up, chronic hyperglycemia persisted, and he already presented complications such as paresthesia in hands and feet, reduced visual acuity, and impaired renal function. Extreme glycemic variability, low adherence to carbohydrate counting, and irregular use of insulin in fixed doses were observed, with repeated elevations of glycated hemoglobin.

Tests for thyroid dysfunction were requested: anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies, both negative, but with elevated TSH and reduced free T4, establishing the diagnosis of seronegative hypothyroidism.

After a few months, he was readmitted to the hospital with fatigue, postural hypotension, tachycardia, and cutaneous hyperpigmentation in areas of vitiligo. Serum cortisol at 8 a.m. was 3.6 ng/dL (reference range: 5–25 ng/dL), confirming primary adrenal insufficiency.

Given the association of type 1 diabetes mellitus, hypothyroidism, vitiligo, and primary adrenal insufficiency, the diagnosis of autoimmune polyglandular syndrome type 2 was confirmed. After in-hospital treatment with hydrocortisone 50 mg every 6 hours, fluid and electrolyte replacement for the adrenal crisis, he continued outpatient treatment with levothyroxine and hydrocortisone at a dose of 7.5 mg, orally, distributed in a dose of 5 mg in the morning and 2.5 mg in the afternoon, with adequate clinical response and metabolic stabilization.

Table 1. Patient's laboratory chemistry analysis.

Blood Tests	Result	Unit	Normal Values
TSH	8.7	µUI/mL	0,38-5,33
Cortisol 8 a.m.	3.8	µg/dL	6,70- 22,60
Free T4	0.48	ng/dL	0,54- 1,24
Vitamin B12	918	pg/mL	200-900
Sodium	132	mEq/L	135-145
Vitamin D	48	ng/mL	30-100
Anti-TPO antibody	2,4	U/mL	<9
Testosterona	100	ng/dL	300-1000
BUN	66	mg/dL	10-50
Creatine	3.73	mg/dL	0.7-1.3
HbA1c	8.1	%	<7
PTH	437	pg/mL	12-88
C peptide	0.02	ng/ml	0.5-2
DHEA-S	139	ug/dL	18-391

Despite this, poor adherence to T1DM treatment led to irregular glycemic control and progression of

diabetic complications: retinopathy progressing to bilateral amaurosis, primary hypogonadism, diabetic gastroparesis, diabetic foot, and chronic renal failure on peritoneal dialysis. He had multiple hospitalizations for adrenal crisis, aggravated by poor absorption of glucocorticoids due to gastroparesis and infections related to the dialysis catheter, and episodes of diabetic ketoacidosis. In the last hospitalization, he presented with acute obstructive abdomen, evolving to death.

DISCUSSION

APS type 2 is rare and potentially fatal if not diagnosed and treated early.[4] There are currently four main categories of autoimmune polyglandular syndromes (types 1-4), based on clinical features and inheritance patterns. APS type 2 is more common than APS type 1 but remains a rare condition.[7]

In the presented case, the patient started with type 1 diabetes and vitiligo in childhood and, about 20 years later, developed adrenal insufficiency and clinical hypothyroidism. While some endocrinopathies can emerge in close sequence, the interval between them is often years.[4,8] The clinical presentation of this case is compatible with the literature, although the diagnosis is frequently challenging.[3] The diagnosis of APS involves specific serological autoantibody tests and subsequent functional tests.[1,4] The case reinforces the need to screen for autoantibodies in patients with autoimmune endocrinopathies due to the increased risk of new associations.[1,4,8]

Primary adrenal insufficiency, or Addison's disease, results from the autoimmune destruction of the adrenal cortex. It is characterized by a deficiency of glucocorticoids and often mineralocorticoids. Symptoms include fatigue, weakness, fever, anorexia, nausea, vomiting, hyponatremia, and hyperkalemia. An adrenal crisis can lead to potentially fatal shock. Management should be immediate, without waiting for lab results, with fluid replacement and intravenous glucocorticoids.[2,5] In the described patient, complications from T1DM, such as gastroparesis and kidney failure, compromised the absorption and effectiveness of glucocorticoids, favoring the recurrence of adrenal crises.

Another relevant finding was seronegative hypothyroidism, a less common condition. This occurs in cases where antibody production takes place predominantly in the thyroid tissue itself rather than in the serum. Rare situations have been described where the thyroid tissue produces antibodies locally, identified in histological tissue after a thyroidectomy, where lymphoid cells removed directly from the gland produced anti-Tg and anti-microsomal antibodies *in vitro*.[9]

The treatment for APS type 2 is based on hormone replacement and managing complications.[3] The simultaneous management of adrenal insufficiency and T1DM is challenging because glucocorticoids raise blood glucose levels.[1,2] These patients are at a higher risk for hospitalizations, hypoglycemia, and ketoacidosis.[1] The case illustrates the complexity of managing multiple associated autoimmune endocrinopathies, especially in patients with poor therapeutic adherence and severe diabetes complications that directly impact the effectiveness of hormone replacement.

Early recognition of APS type 2 in patients with T1DM is essential to prevent adrenal crises and diabetic ketoacidosis. A timely diagnosis, combined with rigorous control of associated autoimmune diseases, can reduce complications and improve prognosis.[1,4]

CONCLUSION

Autoimmune polyglandular syndrome type 2 is a rare and multifactorial condition characterized by the progression of autoimmune endocrinopathies. The described case reinforces the importance of systematic diagnosis and endocrinological follow-up to prevent serious complications. Given the predisposition to multiple autoimmune diseases, physicians should maintain a high index of diagnostic suspicion in patients with an isolated autoimmune disease and atypical symptoms. Screening for organ-specific autoantibodies can assist in early detection. Proper management involves hormone replacement, insulin therapy, and corticotherapy, with continuous and multidisciplinary follow-up being essential. This report contributes to the medical literature by documenting a rare combination of autoimmune diseases that started in childhood and manifested more broadly in adulthood, highlighting the need for constant clinical vigilance.

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