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Treatment of children and adolescents with type 1 diabetes involves a combination of education, support, empowerment, and open communication to promote emotional well - being and enhance quality of life.

EDITORIAL

TREATMENT AND CONTINUING EDUCATION IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES: A UTOPIA?

Treating and educating children and adolescents with type 1 diabetes is certainly challenging, but I wouldn't call it a utopia. It's an ongoing process that requires a multidisciplinary approach involving healthcare providers, educators, caregivers, and the patients themselves.

Effective treatment involves not just managing blood sugar levels but also addressing the emotional and psychological aspects of living with a chronic condition. Continuous education is vital for both the patients and their families to understand the disease, its management, and how to navigate daily challenges.

Some key components of successful treatment and education in type 1 diabetes for children and adolescents include:

1. **Comprehensive Diabetes Care Team:** This includes endocrinologists, diabetes educators, dietitians, mental health professionals, and other specialists as needed. A team approach ensures that all aspects of the disease are addressed.
2. **Individualized Treatment Plans:** Every child is different, and their treatment plans should be tailored to their specific needs, lifestyle, and preferences. This includes insulin therapy, diet plans, physical activity recommendations, and monitoring schedules.
3. **Patient and Family Education:** Teaching patients and their families about diabetes management is crucial. This includes understanding insulin administration, monitoring blood glucose levels, recognizing and managing hypoglycemia and hyperglycemia, and making healthy lifestyle choices.
4. **Psychosocial Support:** Living with a chronic condition can be emotionally challenging, especially for children and adolescents. Providing access to mental health professionals, support groups, and resources for coping with stress and anxiety is essential.
5. **Technology Integration:** Advancements in technology, such as continuous glucose monitors (CGMs) and insulin pumps, can significantly improve diabetes management. Educating patients and caregivers on how to use these technologies effectively is important.
6. **Transition to Adulthood:** As children with type 1 diabetes transition into adulthood, they need support in navigating healthcare transitions, managing their diabetes independently, and understanding the long-term implications of the disease.

While achieving ideal outcomes for every child with type 1 diabetes may not be attainable in every case, striving for optimal care through ongoing education, support, and collaboration among healthcare providers, families, and patients can significantly improve outcomes and quality of life.

AND THE FEAR OF DISEASE?

The fear of disease, especially chronic conditions like type 1 diabetes, can be a profound and complex experience for both patients and their loved ones. It often stems from uncertainty about the future, concerns about managing symptoms and complications, and the emotional toll of living with a condition that requires constant attention and care.

For children and adolescents with type 1 diabetes, fear can manifest in various ways:

1. **Fear of Hypoglycemia:** The risk of low blood sugar episodes (hypoglycemia) can be frightening for both children and parents. The fear of losing consciousness or experiencing severe symptoms can lead to anxiety and reluctance to engage in activities that might lower blood sugar levels.
2. **Fear of Hyperglycemia and Complications:** On the other hand, consistently high blood sugar levels (hyperglycemia) can lead to long-term complications such as nerve damage, kidney disease, and vision problems. The fear of these complications can motivate adherence to treatment but can also create anxiety about the future.

3. **Fear of Social Stigma:** Children and adolescents may also experience fear related to social acceptance and stigma. Managing diabetes in social settings, dealing with questions or misunderstandings from peers, and feeling different can contribute to anxiety and self-consciousness.
4. **Fear of Losing Control:** Diabetes requires constant monitoring and decision-making regarding food, insulin doses, physical activity, and other factors. The fear of making a mistake or losing control over blood sugar levels can be overwhelming at times.
5. **Fear of the Unknown:** Living with a chronic condition like type 1 diabetes means facing uncertainties about future health outcomes, advancements in treatment, and the impact of the disease on various aspects of life. This fear of the unknown can be challenging to cope with.

Addressing the fear of disease in children and adolescents with type 1 diabetes requires a holistic approach:

- **Education:** Providing comprehensive education about the disease, its management, and potential outcomes can empower patients and their families to better understand and cope with their fears.
- **Psychological Support:** Access to mental health professionals, support groups, and counseling services can help individuals navigate their emotions, anxiety, and fears related to diabetes.
- **Empowerment:** Encouraging patients to take an active role in their diabetes management, make informed decisions, and develop coping strategies can reduce feelings of helplessness and increase confidence.
- **Open Communication:** Creating a supportive environment where patients feel comfortable expressing their fears, asking questions, and seeking help is essential for building trust and resilience.
- Overall, addressing the fear of disease in children and adolescents with type 1 diabetes involves a combination of education, support, empowerment, and open communication to promote emotional well-being and enhance quality of life.

Overall, addressing the fear of disease in children and adolescents with type 1 diabetes involves a combination of education, support, empowerment, and open communication to promote emotional well-being and enhance quality of life.

DIABETES CARE TEAM OF STUDY PROJETO DOCE (**D**iabetes **O**bjetivando **C**ontrole e **E**ducação)

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

IDENTIFICATION OF METABOLIC PATHWAYS ALTERED IN THYROID CANCER PROGRESSION AND METASTASIS

IDENTIFICAÇÃO DAS VIAS METABÓLICAS ALTERADAS NA PROGRESSÃO E METÁSTASE DO CÂNCER DE TIREOIDE

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Introduction: Thyroid cancer is a common endocrine malignancy with a rising incidence. However, to improve patient outcomes, it is essential to understand the molecular mechanisms driving its progression and metastasis, and the metabolomics can unveil alterations in metabolic pathways that contribute to thyroid cancer. **Objective**: To identify the metabolic pathways altered in thyroid cancer progression and metastasis. **Methods**: Multiple bioinformatics tools were employed in the research. Gene expression data was obtained from the *Gene Expression Omnibus and The Cancer Genome Atlas*. Functional assessment of the expressed genes in thyroid cancer was performed using gene set enrichment analysis. The *Kyoto Encyclopedia of Genes* and Genomes database was utilized to identify the metabolic pathway involved in thyroid cancer progression and metastasis. A computational algorithm was developed to estimate the activity levels of the identified metabolic pathways and construct a signaling pathway. **Results**: The altered metabolic pathways in thyroid cancer progression and metastasis were identified based on the following algorithm: activation of growth factor signaling, activation of multiple signaling pathways, regulation by transcription factors, dysregulation of *downstream* signaling cascades, changes in cellular metabolism, tumor progression, invasion and metastasis, and feedback regulation. **Conclusion**: By applying an algorithm, was possible to evaluate key molecular events driving the aggressive behavior of thyroid cancer. These findings provide insights into the underlying mechanisms of thyroid cancer progression and metastasis.
Keywords: Thyroid cancer, metabolic pathways, metastasis.

Introdução: O câncer de tireoide é o mais comum câncer endócrino, com uma incidência crescente. No entanto, para melhorar os resultados dos pacientes, é essencial entender os mecanismos moleculares que impulsionam sua progressão e metástase, e a metabolômica pode revelar alterações nas vias metabólicas que contribuem para o seu desencadeamento. **Objetivo**: Identificar as vias metabólicas alteradas na progressão e metástase do câncer de tireoide. **Métodos**: Foram utilizadas várias ferramentas de bioinformática na pesquisa. Os dados de expressão gênica foram obtidos do *Gene Expression Omnibus* e do *The Cancer Genome Atlas*. A avaliação funcional dos genes expressos no câncer de tireoide foi realizada por meio da análise de enriquecimento de conjuntos de genes. O banco de dados *Kyoto Encyclopedia of Genes and Genomes* foi utilizado para identificar a via metabólica envolvida na progressão e metástase do câncer de tireoide. Um algoritmo computacional foi desenvolvido para estimar os níveis de atividade das vias metabólicas identificadas e construir uma via de sinalização.

Resultados: As vias metabólicas alteradas na progressão e metástase do câncer de tireoide foram identificadas com base no seguinte algoritmo: ativação do sinal de fator de crescimento, ativação de várias vias de sinalização, regulação por fatores de transcrição, desregulação de cascatas de sinalização *downstream*, alterações no metabolismo celular, progressão tumoral, invasão e metástase, e regulação de feedback. **Conclusão:** Ao aplicar um algoritmo, foi possível avaliar eventos moleculares-chave que impulsionam o comportamento agressivo do câncer de tireoide. Esses achados fornecem insights sobre os mecanismos subjacentes da progressão e metástase do câncer de tireoide.

Descritores: Câncer de tireoide; Vias metabólicas; Metástase.

INTRODUCTION

Thyroid cancer is a common endocrine malignancy with a rising incidence[1]. To improve patient outcomes, it is essential to understand the molecular mechanisms driving its progression and metastasis. Metabolomics can unveil alterations in metabolic pathways that contribute to thyroid cancer's aggressive behavior, potentially leading to the discovery of therapeutic targets[2].

Thyroid cancer cells undergo metabolic reprogramming to sustain their energy demands and promote tumor growth[3]. Dysregulated glucose utilization, facilitated by upregulated glucose transporters (GLUT) like GLUT1 and GLUT3, fuels various metabolic pathways, including glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle[4]. These pathways generate ATP and biomass required for cellular proliferation and invasion. Lipid metabolism alterations, characterized by increased lipogenesis and lipid uptake, have also been implicated in thyroid cancer progression. Fatty acid oxidation (FAO), an alternative energy source utilized by cancer cells under stress conditions, plays a role in thyroid cancer cell survival and metastatic potential[5].

There is a close relationship between altered metabolism and signaling pathways involved in thyroid cancer progression. The BRAF V600E mutation is associated with increased glycolysis and altered mitochondrial metabolism[6]. Activation of the PI3K/Akt pathway promotes glycolysis and the pentose phosphate pathway, facilitating tumor growth and metastasis[7]. Dysregulated signaling through the mTOR pathway is also linked to perturbed lipid metabolism in thyroid cancer cells[8].

Metabolites have emerged as potential diagnostic and prognostic markers for thyroid cancer progression and metastasis. Elevated lactate levels, a byproduct of glycolysis, correlate with advanced disease stages and poor prognosis[9]. Certain amino acid changes, like glutamine and serine, are linked to increased aggressiveness and metastatic potential in thyroid cancer[10]. By identifying these metabolic pathways, a better understanding of thyroid cancer's aggressive behavior can guide the development of targeted therapies and personalized treatment approaches.

In this context, the objective of this study is to identify the metabolic pathways altered in thyroid cancer progression and metastasis.

METHOD

Publicly available transcriptomic data sets of thyroid cancer were retrieved from The Cancer Genome Atlas (TCGA) (//cancergenome.nih.gov/), and Gene Expression Omnibus (GEO) (//www.ncbi.nlm.nih.gov/geo/). These data sets consist of RNA sequencing data from tumor samples and matched normal thyroid tissue samples.

The Kyoto Encyclopedia of Genes and Genomes database was utilized to identify the metabolic pathway involved in thyroid cancer progression and metastasis.

Raw RNA sequencing data was preprocessed, which involved quality control, adapter trimming, and removal of low-quality reads. The processed data was then aligned to the reference genome using a well-established alignment algorithm. Subsequently, gene expression levels were quantified by counting the number of reads mapped to each gene. Differential gene expression analysis was performed using bioinformatics tools to identify genes showing significant expression differences between tumor and normal samples.

To identify altered metabolic pathways in thyroid cancer, pathway enrichment analysis was conducted. Gene Set Enrichment Analysis (GSEA) or other similar tools were utilized to assess whether specific metabolic pathways were significantly enriched or depleted

based on the differential expression of genes involved in these pathways.

Metabolic network analysis was performed to understand the interconnectedness and alterations in metabolic pathways in thyroid cancer. Bioinformatics tools, such as Metabolic Network Analysis, were employed to reconstruct metabolic networks using the differentially expressed genes.

Selected metabolic pathways identified through bioinformatics analysis were validated using independent thyroid cancer transcriptomic data sets.

A computational algorithm, based on data from the GEO and TCGA database, was designed for the analysis of signaling pathway activity and for scrutinizing the fluctuations within the gene set. To accomplish this, the following algorithm was developed:

1. Begin the program.
2. Initialize the variables and parameters.
3. Define the main components of the signaling pathway, such as receptors, ligands, and downstream effectors.
4. Set the initial conditions of the pathway, including the activation or inhibition status of the components.
5. Iterate through each step of the pathway:
   - Check for ligand-receptor binding.
   - If binding occurs, activate the downstream effectors.
   - Determine any feedback loops or cross-talk between different signaling pathways.
   - Update the activation status of the components based on the interactions and regulations.
6. Repeat the iterations until the desired end point is reached.
7. Output the final status of the pathway, including the activation levels of key components.
8. End the program.

Statistical analyses were performed using appropriate software R. p-value < 0.05 was considered statistically significant.

The study primarily relied on computational analysis of publicly available genomic data and did not involve any direct interaction with living organisms. Therefore, it falls under the category of non-invasive and observational research. Nonetheless, all data used in the study were obtained from publicly accessible databases that adhere to ethical guidelines and regulations regarding data sharing and privacy. Since no human subjects or animal experiments were involved, ethical approval was waived.

RESULTS

Based on data from TCGA and GEO, a total 100 tumor samples and matched normal thyroid tissue samples, 45 genes displaying significant expression differences ($p < 0.05$) between the two groups was identified.

Through pathway enrichment analysis, it was found that the glycolysis/gluconeogenesis pathway was significantly enriched ($p < 0.001$) in thyroid cancer samples, indicating an increased glucose metabolism in cancer cells.

Additionally, the citric acid cycle (TCA cycle) and oxidative phosphorylation pathways showed a significant depletion ($p < 0.01$), suggesting impaired mitochondrial function in thyroid cancer cells.

Utilizing metabolic network analysis, the metabolic networks specific to thyroid cancer were reconstructed by integrating the differentially expressed genes and their interactions.

The reconstructed network highlighted key dysregulated nodes such as Hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2), indicating their crucial roles in altered glucose metabolism.

The identified altered metabolic pathway was validated using an independent transcriptomic dataset consisting of 100 thyroid cancer samples taken from TCGA and GEO database.

The replication of the results across this dataset further confirmed the significance of the enriched glycolysis/gluconeogenesis pathway and the depleted TCA cycle and oxidative phosphorylation pathways.

Detailed description of the signaling pathway in text format and in graphic format (**Figure 1**) of identification of metabolic pathways altered in thyroid cancer progression and metastasis was developed with the algorithm based on data from the GEO and TCGA database:

Growth Factor Signaling Activation

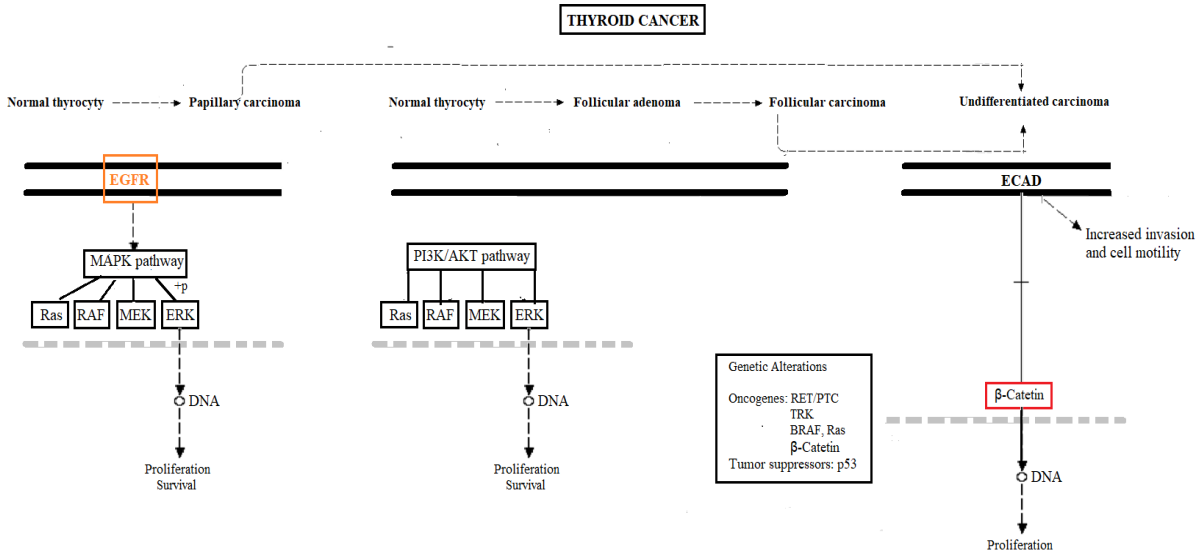
Increased expression or activation of growth factors, such as Epidermal Growth Factor (EGFR), and E-cadherin (ECAD) triggers downstream signaling pathways.

Activation of multiple signaling pathways

MAPK pathway: EGFR activates the MAPK pathway by activating Ras, RAF, MEK, and ERK. This pathway promotes cell proliferation.

PI3K/AKT pathway: EGFR activates the PI3K/AKT pathway, promoting cell survival and inhibiting apoptosis.

Figure 1. Thyroid Cancer Progression and Metastasis Signaling Pathway



Source: Search result

Transcription factors

Transcription of genes related to cell proliferation, such as c-myc and cyclin D1, is activated by transcription factors activated by MAPK and PI3K/AKT.

Dysregulation of Downstream Signaling Cascades

Activation of receptor tyrosine kinases, including the EGFR family, PI3K/AKT/mTOR, and RAS/RAF/MEK/ERK pathways. Mutations in key genes like BRAF, RAS, and PTEN contribute to dysregulated signaling and tumor progression.

Alterations in Cellular Metabolism

Glycolysis Activation: Increased expression of glycolytic enzymes, such as HK2 and PKM2, promotes enhanced glucose uptake and metabolism, providing energy and biosynthetic precursors for tumor growth.

Altered Mitochondrial Respiration: Dysfunctional mitochondria and decreased oxidative phosphorylation activity lead to increased reliance on glycolysis for energy production. Lipid Metabolism Reprogramming: Enhanced fatty acid synthesis and lipid uptake support the rapid proliferation and metastatic potential of thyroid cancer cells.

Increased cell proliferation, survival, and resistance to apoptosis promote tumor growth. Loss of cell adhesion molecules and increased expression of matrix metalloproteinases (MMPs) facilitate invasion and metastasis. Tumor cells secrete angiogenic factors, such as Vascular Endothelial Growth Factor (VEGF). They stimulate the growth of new blood vessels, aiding in tumor nourishment. Activation of pro-angiogenic factors promotes the formation of new blood vessels to supply nutrients and oxygen to growing tumors.

Activation of the Wnt pathway leads to the accumulation of β-catenin in the cell nucleus, resulting in the transcription of genes associated with invasion and metastasis, such as c-Met and MMPs and activation of the TGF-β pathway leads to the phosphorylation of SMADs, which act as transcription factors inducing the transcription of genes related to invasion and metastasis, such as Snail and MMPs.

Negative feedback loops, involving proteins like TSC1/2 and mTOR, may provide some regulatory control over the pathway to prevent excessive proliferation.

DISCUSSION

Understanding the underlying molecular alterations and dysregulated metabolic pathways in thyroid cancer is crucial for developing targeted therapeutic strategies. In this study it was identified the specific metabolic pathways that are dysregulated in thyroid cancer, using a comprehensive analysis of transcriptomic data from TCGA and GEO databases, and by comparing tumor samples with matched normal thyroid tissue samples; several differentially expressed genes were identified and pathway enrichment analysis was

carried out to uncover the altered metabolic pathways in thyroid cancer. When a comprehensive approach to metabolomics data is utilized, the essential metabolic events driving the metastatic behavior of thyroid cancer cells is addressed.

A study utilizing the TCGA and GEO databases for analysis of neoplastic thyroid tissue identified 134 differentially expressed genes (DEGs) with upregulation and 106 DEGs with downregulation. The analysis revealed that these DEGs were enriched in thyroid hormone synthesis and metabolic pathways[11]. The current findings on differentially expressed genes in thyroid cancer are consistent with previous studies. Several genes that were identified have been frequently reported as key drivers of thyroid cancer development and progression. Additionally, the results also highlighted the dysregulation of metabolic pathways, including the PI3K/AKT/mTOR pathway and the MAPK signaling pathway, which have been implicated in the pathogenesis of thyroid cancer.

Studies have shown a positive regulation of glycolysis and a negative regulation of oxidative phosphorylation in thyroid cancer, supporting the notion of altered metabolism in this disease[12]. One study demonstrated that thyroid cancer cells exhibited increased glycolysis and reduced oxidative phosphorylation, and these metabolic changes were associated with altered gene expression patterns, highlighting the role of metabolic remodeling in thyroid cancer progression[13].

Specifically, the pathway enrichment analysis carried out revealed a significant enrichment of the glycolysis/gluconeogenesis pathway, indicating an increased glucose metabolism in thyroid cancer samples. This aligns with previous research demonstrating increased glycolysis in thyroid cancer cells. Furthermore, it was found a significant depletion of the TCA cycle and oxidative phosphorylation pathways, suggesting impaired mitochondrial function in thyroid cancer cells. This finding is consistent with literature reports of reduced oxidative phosphorylation in thyroid cancer cells. Together, these findings emphasize the importance of metabolic remodeling in thyroid cancer progression.

It has been reported that the expression levels of GLUT-1[14,15] and MCT-4[16,17] are correlated with aggressiveness in various types of cancer. A study was conducted to investigate the expression of proteins related to glycolysis in thyroid cancer where it was demonstrated that the expression of GLUT-1 and MCT-4 was significantly higher in cases of anaplastic carcinoma compared to other subtypes of thyroid cancer[18]. Additionally, previous studies have shown that increased expression of GLUT-1[19,20], hexokinase II[21-23], and MCT-4[16] correlate with a poor prognosis in thyroid cancer. Therefore, proteins related to glycolysis appear to play the role of regulators in tumor aggressiveness. The dysregulated nodes in the reconstructed metabolic network, focusing on HK2 and PKM2 was investigated and the results demonstrate the significant roles of these enzymes in altered glucose metabolism, and the evidences regarding the dysregulated glucose metabolism in thyroid cancer.

These results were validated by separate transcriptomic datasets through bioinformatics analysis using independent thyroid cancer transcriptomic data sets, underscoring the significance of the glycolysis/gluconeogenesis pathway, the depleted TCA cycle, and the oxidative phosphorylation pathways.

Bioinformatics algorithms have been used for cancer target gene prediction such as TargetScan[24], miRSystem[25], DIANA[26], miRanda[27], and starBase[28]. To develop the research, a computational algorithm was devised and evaluated, based on data from the GEO and TCGA database, designed for the analysis of signaling pathway activity and for scrutinizing the fluctuations within the gene set.

In light of the growing incidence and prevalence rates associated with thyroid cancer progression and metastasis, there is a pressing need to identify the metabolic alterations underlying the aggressive phenotype. Thus, the investigation of the changes in metabolic pathways that occur during different stages of thyroid cancer, from tumor initiation to metastatic dissemination, is of fundamental importance, and a comprehensive analysis of metabolomics data can identify key metabolic alterations that contribute to the aggressive behavior of thyroid cancer cells.

Furthermore, the discovery of potential molecular targets within these pathways can be exploited for the development of novel therapeutic strategies for the management of advanced thyroid cancer.

CONCLUSION

The metabolic and molecular pathways that are significantly altered in thyroid cancer progression and metastasis have been thoroughly discussed. Through the analysis of multiomics data, the key molecular mechanisms driving tumor aggressiveness was analyzed and the potential of these metabolic alterations as novel prognostic markers or therapeutic targets is promising.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in relation to this article

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

COMPARISON OF HEMOGLOBIN A1C IN NON-DIABETIC PSORIASIS PATIENTS WITH AND WITHOUT ARTHRITIS

COMPARAÇÃO DE NÍVEIS DE HEMOGLOBINA A1C EM PACIENTES PSORIÁSICOS NÃO-DIABÉTICOS COM E SEM ARTRITE

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Introduction: Psoriasis is a common erythematous-scaly dermatosis caused by epidermal keratinocyte hyperplasia. Comorbidities such as Crohn's disease, atherosclerosis, hypertension, dyslipidemia, hyperhomocysteinemia, among others, are common in psoriasis, which increases cardiovascular risks and metabolic disorders such as diabetes mellitus (DM) and metabolic syndrome. Psoriasis patients can develop Psoriatic Arthritis (PA). Similar to purely cutaneous psoriasis, PA is associated with comorbidities like DM2, hypertension, obesity, hyperlipidemia, and metabolic syndrome. However, it is unknown whether the association with diabetes differs in individuals with psoriasis with and without PA. **Objectives:** To comparatively study the prevalence of HbA1c alterations in non-diabetic individuals with psoriasis, with and without psoriatic arthritis. **Methodology**: A cross-sectional observational study involving 49 individuals with psoriasis and/or psoriatic arthritis. Epidemiological, clinical, treatment, and skin inflammatory activity data were collected using the Psoriasis Area Severity Index (PASI) and joint activity using the Disease Activity Index for Psoriatic Arthritis (DAPSA). Subsequently, medical records were reviewed for HbA1c, ESR, and C-reactive protein values. **Results**: The total sample evaluated consisted of 49 patients, with 23 being female and with ages ranging from 19 to 77 years (median of 53). The analysis revealed elevated HbA1c values within the population with associated arthritis (p=0,02) when compared with those without it, and as well as in patients using secukinumab (p=0,05).No associations with inflammatory activity could be proven. **Conclusions:** Non-diabetic patients with psoriatic arthritis have higher HbA1c values than those with psoriasis but without arthritis. Furthermore, an increase in HbA1c levels was observed in patients using secukinumab medication. Additionally, this study did not demonstrate an association between HbA1c values and skin activity scores (PASI) or joint activity scores (DAPSA).
Keywords: Psoriasis. Psoriatic arthritis. Diabetes mellitus. Blood glucose.

Introdução: A psoríase é uma dermatose comum, eritemato-escamosa causada pela hiperplasia de queratinócitos da epiderme. Na psoríase são comuns comorbidades como doença de Crohn, aterosclerose, hipertensão, dislipidemia, hiper-homocisteinemia, entre outras, que potencializam os riscos cardiovasculares e desordens metabólicas como a diabetes mellitus (DM) e síndrome metabólica. Pacientes com psoríase podem desenvolver Artrite Psoriásica (AP). Semelhante à psoríase puramente cutânea, a AP apresenta relação a comorbidades como a DM2, hipertensão, obesidade, hiperlipidemia e síndrome metabólica. Todavia não se sabe se a associação com diabetes difere nos indivíduos com psoríase com e sem AP. **Objetivos:** Estudar comparativamente a prevalência de alterações de HbA1c em indivíduos não diabéticos com psoríase, com e sem artrite psoriásica. **Metodologia:** Estudo observacional transversal

envolvendo 49 indivíduos com psoríase e/ou artrite psoriásica. Foram coletados dados epidemiológicos, clínicos, de tratamento e de atividade inflamatória da pele pelo PASI (*Psoriasis Area Severity Index*) e articular pelo DAPSA (*Disease Activity index for Psoriatic Arthritis*). A seguir os prontuários foram revisados para valores de HbA1c, VHS e Proteína C Reativa. **Resultados:** A amostra total avaliada foi de 49 paciente, sendo 23 do sexo feminino, com idades variando de 19 a 77 anos (mediana de 53). Na análise foi possível encontrar valores elevados de HbA1c dentro da população que apresentava quadro de artrite associada (p=0,02) quando comaprada com os sem artrite, assim como, ela também foi encontrada elevada em pacientes que faziam uso do medicamento secuquinumabe (p=0,05). Nenhuma associação com atividade inflamatória pode ser comprovada. **Conclusões:** Pacientes não diabéticos com artrite psoriásica apresentaram valores de HbA1c mais elevados dos que os com psoríase mas sem artrite. Além disso, foi observado um aumento nos níveis de HbaA1c nos pacientes que faziam uso do medicamento secuquinumabe. Neste estudo não foi possível demonstrar associação entre valores de HbA1c com índices de atividade de pele (PASI), nem com índices de atividade articular (DAPSA).
Palavras-chave: Psoríase. Artrite psoriásica. Diabetes mellitus. Glicemia.

INTRODUCTION

Psoriasis (Pso) is an erythematous-squamous dermatosis that associated with keratinocyte hyperplasia of the epidermis, affecting the population worldwide with approximately more than 100 million of individuals affected[1,2]. Pso etiology has not yet been clarified; however, it is known that this disease has a genetic base and that some environmental factors are related to its onset[1].

The clinical presentation is variable; not only the skin manifestations are diverse and may present as plaque psoriasis, psoriasis guttata, palmo-plantar psoriasis, erythrodermic form, nail involvement, etc. but also, in 30 % of the cases, psoriatic arthritis (PsoA) may appear[3]. The evolution of psoriasis to PsA may occur in stages, although the mechanisms are unclear. In many patients, there may be little or no association between severity of musculoskeletal involvement and severity of skin or nail psoriasis[3].

Several comorbidities have been found in psoriasis patients; among than atherosclerosis, arterial hypertension, obesity, dyslipidemia and diabetes that contribute to morbimortality on these patients. Such comorbidities have been linked to the ongoing chronic inflammatory process and they appear in both: Pso and PsoA[1,2,4]. Nevertheless, it is unknown if they affect patients with and without arthritis in the same proportion. It is reasonable to hypothesize that patients with PSoA have an extra inflammatory burden that could increase the prevalence of such comorbidities.

Herein an analysis of hemoglobin (hb) A1c in a sample of Brazilian patients with Pso without known history of diabetes mellitus was done, aiming to compare the values of those with and without arthritis.

MATERIAL AND METHODS

This is a cross-sectional study approved by the local committee of ethics in research (CAAE = 65159 522.8.0000.0103) under protocol=5.773.665. All participants signed consent.

To be included patients should be older than 18 years of age, of either sex and have psoriasis diagnosed by biopsy or by a certified dermatologist. Patients unable to understand the consent form, with associated inflammatory diseases and with diabetes mellitus were excluded.

Data collection included:

a. Epidemiological and anthropometrical data (age, use of tobacco, sex, age at diagnosis, auto-declared ethnic background, weight and heigh and waist measurement).

b. Clinical and laboratory data: data on psoriasis (form of skin disease, nail involvement, presence of psoriatic arthritis, psoriasis skin severity measured by Psoriasis Area Severity Index or PASI[5,6], of articular inflammation by DAPSA or Disease activity index for psoriatic arthritis)[7], presence of comorbidities, used treatment and results of hemoglobin A1c, fasting blood glucose, ESR (erythrocyte sedimentation rate) and CRP (or C reactive protein).

Statistical evaluation:

Data was grouped in frequency and contingency tables. Distribution of numerical data was evaluated by Shapiro-Wilks test and expressed in mean and standard deviation if normal or median and interquartile range if non parametric.

Comparison of HbA1c according to studied variables was done by unpaired t test or Mann Whitney test according to normal or non-normal distribution. Correlation studies of HbA1c with ESR and C reactive protein, age and BMI were done by Spearman or by Pearson tests. The adopted significance was 5%. The tests were calculated with help of the software Graph Pad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

RESULTS

a. Description of studied sample:

The sample had 49 patients. Most of them were Caucasians, middle aged and obese or overweight. Details of epidemiological, clinical and treatment data are on **Table 1**.

b. Comparison of HbA1c values according to studied variables.

Table 2 displays the comparison of HbA1c Values according to the studied variables. This table shows that gender, ethnic background and form of psoriasis did not associate with Hb1Ac values. Arterial hypertension and secuquinumab use associated with higher values of hbA1c.

Table 1. Description of studied sample (n=39 psoriasis patients)

Sex – n	female	23/49 (48%)
	male	26/49 (52%)
Ethnic background- n	Caucasians	36/49 (73.4%)
	Afro descendants	12/49 (24.4%)
	Asians	1/49 (2.0%)
Mean age (IQR)- years		50.7±14.4
Mean age at disease onset (SD)- years		36.9±0.31
Smokers (n)		17/49 (34.6%)
BMI categorical - n	Normal	10/49 (20.4%)
	Overweight	21/49 (42.8%)
	Obesity 1	10/49 (20.4%)
	Obesity 2	6/49 (12.2%)
	Obesity 3	2/49 (4.0%)
Median abdominal circumference - cm		98.0 (88.5-103.5)
	Males	97.5±14.0
	Females	97.4±20.6
Psoriasis form (n)	Plaque	42/49 (85.7%)
	Palmo-plantar	1/49 (2.0%)
	Inverted	3/49 (6.1%)
	Pustulous	1/49 (2.0%)
	Erythrodermic	1/49 (2.0%)
	Guttate	1/49 (2.0%)
Arthritis (n)		22/49 (44.8%)
Median PASI (IQR)		1 (0-3.8)
Mean DAPSA (SD)		9.9 ± 11.2
Median C reactive protein (IQR) mg/dL		2.3 (1.66-6.20)
Median erythrocyte sedimentation rate (IQR) - mm		19.0 (10-33)

Mean fasting glucose (SD) – mg/dL		91.0±11.2
Median hemoglobin A1c (IQR)- %		5.4 (5.1-5.5)
Co morbidities -n	Arterial hypertension	7/49 (14.2%)
	Dyslipidemia	5/49 (10.2%)
	Hypothyreoidism	5/49 (10.2%)
	Fibromyalgia	18/49 (36.7%)
Medications - n	Methotrexate	14/49 (28.5%)
	Leflunomide	3/49 (6.1%)
	Adalimumab (anti TNF)	11/49 (22.4%)
	Risanquisumab (anti IL-23)	7/49 (14.2%)
	Secuquinumab (anti IL-17A)	8/49 (16.3%)
	Ustequinumab (anti IL12/23)	5/49 (10.2%)
	Only topical treatment	8/49 (16.3%)

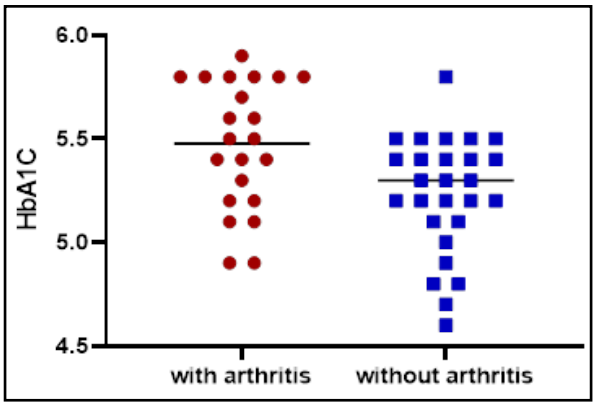
N= number; IQR= interquartile range; SD= standard deviation; BMI= body mass index; DAPSA= Disease activity index for psoriatic arthritis; PASI= Psoriasis Area Severity Index.

Table 2. Hemoglobin (Hb) A1c values according to the studied variables

	HbA1c values (%) with the variable	HbA1c values (%) without the variable	P
Female sex	5.3±0.30	5,3±0.30	0,27
Caucasian ethnic background (*)	5.3±0.38	5.4±0.25	0.13
Tabacco use	5.3±0.33	5.3±0.33	0.74
Plaque psoriasis (**)	5.3±0.32	5.4±0.36	0.19
Comorbidities			
Arterial hypertension	5.4±0.28	5.2±0.32	0.05
Dyslipidemia	5.5±0.37	5.3±0.31	0.10
Fibromyalgia	5.4±0.37	5.3±0.31	0.57
Hypothyroidism	5.5±0.36	5.3±0.30	0.19
Treatment			
Methotrexate	5.2±0.25	5.3±0.34	0.43
Adalimumab	5.2±0.39	5.3±0.32	0.35
Risanquizumab	5.4±0.34	5.3±0.32	0.30
Secuquinumab	5.7 (5.2-5.8)	5.3(5.1-5.5)	0.05

(*) refers to Caucasians versus afro descendants; (**)- refers to plaque psoriasis versus- other forms;

Figure 1. Displays the HbA1c values in psoriasis patients with and without arthritis.



Hb A1c values in patients with arthritis =5.5±0.31 %
HbA1c values in patients without arthritis = 5.2±0.38 %; p=0.02.

The results of correlation studies of HbA1c values with numerical variables are on **Table 3** that shows a modest correlation of HbA1c with age and age at psoriasis diagnosis.

Table 3. Correlation studies of hemoblobin A1c with the studied numerical variables

	R	95%CI	p
Age (*)	0.40	0.13 to 0.61	**0.004**
Age at diagnosis (*)	0.28	0.007 to 0.52	**0.04**
Body mass index (**)	0.19	-0.09 to 0.46	0.17
PASI (**)	-0.14	-0.41 to 0.15	0.32
DAPSA (*)	0.29	-0.15 to 0.64	0.19
Erythrocyte sedimentation rate (**)	0.23	-0.05 to 0.49	0.09
C reactive protein (**)	0.28	-0,008 to 0.52	0.05

(*)- Pearson test; (**)-Spearman test.
DAPSA= Disease activity index for psoriatic arthritis;
PASI= Psoriasis Area Severity Index

DISCUSSION

The results of present analysis showed that psoriasis patients without history of diabetes with hypertension, using secuquinumab and with arthritis had higher values of hbA1c than those without these characteristics. It also showed a correlation of HbA1c values with age and age at disease diagnosis.

Ikumi et al.[8] studying glycemic variations in 39 patients with psoriasis observed that the severity of skin involvement but not the presence of arthritis associated with HbA1c values, the opposite of present findings. The small number of studied patients and possible ethnic variations may explain the found differences. There are some possible explanations for the present finding of arthritis association with higher levels of HbA1c: one of them is that patients with arthritis are less active physically due to joint pain and deformities and these may be associated to higher BMI and glucose levels. Unluckily, no data on physical activity was obtained presently, so no further considerations can be done on this issue. Another possible explanation is that arthritis patients receive glucocorticoid more frequently as part of treatment of the musculoskeletal symptoms. In this sample, none of the patients was using glucocorticoid at the time of data collection but there is a possibility that may have used in a near past.

An unexpected finding was that secuquinumab (anti IL-17) use associated with higher levels of HbA1c. The literature results on this aspect are controversial. Experimentally, the administration of anti-IL-17A monoclonal antibody improved hyperglycemia in imiquimod-treated mice with psoriasiform features[9]. A small study found that the psoriasis treatment with anti-IL-17A (secukinumab and ixekizumab) lowered HbA1c after 4 months of treatment in 14/39 (35.8%) patients[8]. However, a study by Schwarz et al.[10] that followed 44 psoriasis patients treated with this drug for 120 days could not detect alterations in HbA1c in secukinumab users. This latter study was corroborated by the results of a post-hoc analysis of 3,010 patients with psoriasis, which investigated the effects of secukinumab, etanercept, and placebo on metabolic parameters, in which no effect of secukinumab on fasting plasma glucose was found[11]. Herein, it was found that secuquinumab users had higher levels of HBA1c. However, it is necessary to observe that the number of patients using this drug presently was quite small (n=8) and this may have caused some bias in the results.

Inflammatory markers (such as ESR, and DAPSA) and index of psoriasis severity (PASI) could not be linked to hbA1c levels presently. CRP values showed a positive correlation that was nullified by the confidence interval. However, the levels of inflammatory markers on the studied sample were low, pointing that the sample had a good control of the disease, and this could have precluded possible associations.so further studies are needed to understand this aspect.

CONCLUSIONS

Non-diabetic patients with psoriatic arthritis had higher levels of hbA1c than psoriasis patients with-

out arthritis. Also, a higher level of hbA1c was found in those using secuquinumab. In this study it was not possible to associate hbA1c values to inflammatory parameters.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

THE PRESENCE OF ANTIBODIES, AND ANTI-IDIOTYPIC ANTIBODIES IN CHRONIC HEPATITIS C TREATMENT-NAÏVE PATIENTS

A PRESENÇA DE ANTICORPOS E ANTICORPOS ANTI-IDIOTÍPICOS EM PACIENTES COM HEPATITE C CRÔNICA SEM TRATAMENTO

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Introduction: In the patients with hepatitis C virus (HCV) various immune-mediated phenomena are described, and antibodies, and anti-idiotypic antibodies (AIAs) in particular are common. **Objective**: The aim of the present study was to investigate the antibodies and AIAs prevalence in chronic hepatitis. **Method:** Sera from 76 consecutive HCV-treated patients at the beginning of the direct-acting antiviral (DAA) era were considered eligible for this study for evaluation of Anti-nuclear (ANA), anti-smooth muscle (SMA), anti-mitochondrial (AMA), anti-neutrophil-cytoplasmatic (ANCA), cytoplasmic ANCA (cANCA), perinuclear ANCA (pANCA), and anti-liver/kidney (LMK) microsomal antibodies. Criteria of eligibility were serum anti-HCV antibody and HCV RNA positivity, chronic inflammation revealed by histological analysis of the liver, genotyping, treatment-naïve patients, and no have the diagnosis of probable or definite autoimmune hepatitis. **Results**: Mean chronological age for the 76 patients (44 females and 32 males) was 51.3 ± 13,9 years (range: 20-67 years). Nineteen patients (25.0%) infected with HCV had detectable levels of antibodies and AIAs at before antiviral treatment. SMA was present in 16 (21.0%) of 76 patients, ANA in 2 patients (2.6%), and pANCA in 1 patients (1.3%). No patient had specimens reactive to AMA, LMK, or cANCA. **Conclusion**: In this study, we show the antibodies and AIAs positivity in chronic hepatitis C patients. We could attribute this to high METAVIR score leading to a release of intracellular antigens at the time of hepatocellular injury and triggering immune responses in the form of autoantibody production.
Keywords: Hepatitis C; Antibodies; Chronic liver disease.

Introdução: Pacientes com o vírus da hepatite C (HCV) frequentemente apresentam fenômenos imunomediados, sendo comum a presença de anticorpos, particularmente os anticorpos anti-idiotípicos (AIAs). **Objetivo**: Investigar a prevalência de anticorpos e AIAs em pacientes portadores de hepatite C crônica. **Metodologia**: Foram avaliados soros de 76 pacientes consecutivos tratados para HCV no início da era dos antivirais de ação direta (DAA). Os anticorpos analisados foram: antinuclear (ANA), anti-músculo liso (SMA), anti-mitocondrial (AMA), anti-citoplasma de neutrófilo (ANCA), ANCA citoplasmático (cANCA), ANCA perinuclear (pANCA) e anti-microssomal fígado/rim (LMK). Os critérios de elegibilidade incluíram: soropositividade para o anticorpo anti-HCV e RNA do HCV, inflamação crônica hepática evidenciada por análise histológica, genotipagem viral, pacientes virgem de tratamento e sem diagnóstico

prévio de hepatite autoimune provável ou definida. **Resultados**: A média de idade dos 76 pacientes (44 mulheres e 32 homens) foi de 51,3 ± 13,9 anos (variação: 20-67 anos). Dezenove pacientes (25,0%) infectados com HCV apresentaram níveis detectáveis de anticorpos e AIAs antes do tratamento antiviral. SMA estava presente em 16 (21,0%) pacientes, ANA em 2 pacientes (2,6%) e pANCA em 1 paciente (1,3%). Nenhum paciente apresentou amostras reativas a AMA, LMK ou cANCA. **Conclusão**: Este estudo demonstra a positividade de anticorpos e AIAs em pacientes com hepatite C crônica antes do tratamento com DAA. A alta pontuação METAVIR, indicativa de inflamação hepática severa, possivelmente leva à liberação de antígenos intracelulares, desencadeando respostas imunológicas na forma de produção de autoanticorpos.
Descritores: Hepatite C; Anticorpos; Doença hepática crônica.

INTRODUCTION

In the patients chronically infected by hepatitis C virus (HCV) various immune-mediated phenomena have been frequently described, antibodies, and anti-idiotypic antibodies (AIAs) in particular are common examples of autoreactivity in a considerable number of individuals with acute and chronic hepatitis C[1-3]. The antibodies and AIAs include anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (SMA), anti-mitochondrial antibodies (AMA), anti-neutophil-cytoplasmatic antibodies (ANCA), and anti-liver/kidney microsomal antibodies (LKM).

The frequency of antibodies and AIAs in HCV-related chronic hepatitis ranges from 10% to 21% for ANA, 10% to 55% for SMA, 0 to 8% for AMA, 0 to 39% for ANCA and 0% to 6% for LKM1[1], and is associated with both cirrhosis and older age[4]. The high prevalence of antibodies and AIAs may reflect a chronic antigenic stimulation or a direct infection of immunocytes by the HCV[5,6].

The aim of the present study was to investigate the antibodies and AIAs in patients chronically infected by HCV before treatment.

METHODS

Study patients

Sera from 76 consecutive HCV-treated patients in the beginning of the direct-acting antiviral (DAA) era were considered eligible for this study for evaluation. Criteria of eligibility were serum anti-HCV antibody and HCV RNA positivity, chronic inflammation revealed by histological analysis of the liver, genotyping, treatment-naïve patients, and no have the diagnosis of probable or definite autoimmune hepatitis.

All the patients gave their informed consent to participate to the study, and the study was approved by the ethical committee of State University of Santa Cruz, Bahia, Brazil in accordance to the Declaration of Helsinki.

Virological assays

The detection of antibodies to HCV (Anti-HCV) was measured by means of an enzyme-linked immunosorbent assay, using structural and non-structural HCV antigens (AXSYM System; Abbott Laboratories, Chicago, IL, USA). HCV-RNA load were available by quantitative polymerase chain reaction (PCR) using primers derived from the highly conserved 5' noncoding region of the viral genome (Amplicor® HCV Detection KIT V2.0; Roche Molecular Systems Inc., Somerville, NJ, USA). HCV-genotypes were performed by PCR amplification of the core region of the HCV genome with specific antisense primers (Applied Biosystems, Foster City, CA). The classification of the genotypes was carried out according to Simmonds et al[7].

Liver Histology

All subjects who were found positive for HCV-RNA underwent percutaneous liver biopsy and histological parameters were classified according to the score system of inflammation activity and fibrosis.

The degree of histological fibrosis was using the METAVIR score. The fibrosis is graded on a 5-point scale from 0 to 4. The activity, which is the amount of inflammation (specifically, the intensity of necro-inflammatory lesions), is graded on a 4-point scale from A0 to A3. Fibrosis score F0= absence of fibrosis; F1= fibrous expansion of portal areas; F2= portal to portal bridging fibrous tracts; F3= portal-central bridging fibrous septa; F4= cirrhosis (bridging fibrous septa with parenchymal nodules). Activity score: 0 = no activity;

A1 = mild activity; A2 = moderate activity; A3 = severe activity (8,9).

Antibodies and AIAs determination

The presence of ANA, SMA, AMA and LKM1 were detected by indirect immunofluorescence (IIF) at serum dilutions with positive title ≥ 1:40. Tests for detection of ANCA were detected by IIF on ethanol-fixed granulocytes with positive title ≥ 1:20. The specificity, reproducibility and optimal conditions of these assays have been determined in preliminary experiments. Autoantibody titers were quantified with commercially available kits for IIF (INOVA, San Diego, CA).

Statistical analysis

The comparison of the association between categorical groups was performed using chi-square or Fischer's exact test. The nonparametric Mann–Whitney test was used for comparison the difference between groups. A p-value < 0.05 was considered significant.

RESULTS

Mean chronological age for the 76 patients (44 females and 32 males) was 51.3 ± 13,9 years (range: 20-67 years).

Liver biopsies were performed in all patient, the results on inflammatory activity and fibrosis (METAVIR score) are shown in **Table 1**.

Table 1. Histopathological features of patients positive for HCV-RNA

Liver biopsy	Patients (*n*)
Inflammatory activity	
0	22
1	20
2	29
3	5
Fibrosis	
F0	2
F1	16
F2	27
F3	24
F4	7

Nineteen patients (25.0%) infected with HCV had detectable levels of antibodies and AIAs at before antiviral treatment, within established laboratory criteria. SMA was present in 16 (21.0%) of 76 patients, ANA in 2 patients (2.6%), and pANCA (perinuclear ANCA) in 1 patients (1.3%), and is reported in **Table 2**. No patient had specimens reactive to AMA, LMK, or cANCA (cytoplasmic ANCA).

Table 2. Prevalence of antibodies and AIAs in patients positive for HCV-RNA

	ANA	SMA	AMA	pANCA	cANCA	LKM
N (%)	2 (2.6)	16 (21.0)	0	1(1.3)	0	0

ANA, anti-nuclear antibodies; **SMA**, anti-smooth muscle anibodies; **AMA**, anti-mitochondrial antibodies, **ANCA**, anti-neutophil-cytoplasmatic antibodies; **LKM**, anti liver/kidney microsomal antibodies.

In the HCV identification, genotype 1 showed a frequency of 69.23%, genotype 3 a frequency of 29.23%, and genotype 2 a frequency of 1.54%.

The relationship between HCV genotypes and presence of antibodies and AIAs demonstrates that HCV-genotype 1 and 1b was significantly more frequent in antibodies and AIAs positive subjects, and is reported in **Table 3**.

Table 3. Antibodies and AIAs - HCV genotype

	ANA	SMA	AMA	pANCA	cANCA	LKM
Genotype 1	-	4	-	1	-	-
Genotype 1a	-	1	-	-	-	-
Genotype 1b	1	5	-	-	-	-
Genotype 2a	-	2	-	-	-	-
Genotype 3	1	2	-	-	-	-
Genotype 3a	-	2	-	-	-	-

ANA, anti-nuclear antibodies; **SMA**, anti-smooth muscle anibodies; **AMA**, anti-mitochondrial antibodies, **ANCA**, anti-neutophil-cytoplasmatic antibodies; **LKM**, anti liver/kidney microsomal antibodies.

The correlation between antibodies and AIAs positivity and degree of inflammatory activity and the degree of fibrosis upon histological examination of the liver biopsies reveal a highly significant statistic (p= 0,002, and p= 0,001), while no significant correlation has been found between antibodies and AIAs positivity and age, sex.

DISCUSSION

Antibodies and AIAs have been frequently detected in sera of HCV-infected patients as reported by several authors[1,10,11]. In this study, we show that the prevalence of antibodies and AIAs in patient with chronic hepatitis C treatment-naïve was of 25.0%. Our study corroborated that antibodies and AIAs is usually found at in HCV subjects. Furthermore, previous study by Chrétien and colleagues[12], have demonstrated that these autoantibodies seem to be part of the natural course of chronic hepatitis C. Nevertheless, previous studies conducted in Europe and the United States of America the frequency of antibodies and AIAs was a little higher than in our study[13,14].

In our study, the subjects had SMA with highest prevalence rates (21.0%), while ANA and pANCA showed low prevalence rates (2.6% and 1.3%, respectively). The prevalence of SMA in our study is comparable to previous studies, which demonstrated these antibodies to be present at similar frequencies[3,15,16]. In contrast with some previous reports LKM were not detected in our report, most likely due to the sample size (Han et al., 2017; Adeyemi et al., 2005)[17,18]. AMA and cANCA, as shown in our study is comparable to previous reports have been rarely found in patients chronically infected by hepatitis C virus[19,20].

The relationship between HCV genotypes and presence of antibodies and AIAs in this study demonstrates that HCV-genotype 1 and 1b was significantly more frequent in antibodies and AIAs positive subjects. Lenzi and colleagues[21], demonstrated a link between the presence of reactivity for SMA and HCV genotype 1b.

The association between patients chronically infected by HCV and the presence of antibodies and AIAs is still controversial. Cassani and colleagues[22], found an association between antibodies and AIAs (mostly ANA and SMA) and necro-inflammatory activity in liver biopsies. These results are strengthened by the observations obtained in this study, that show the correlation between antibodies and AIAs positivity and degree of inflammatory activity and the degree of fibrosis upon histological examination of the liver biopsies showing a highly significant statistic. The study of Cassani and colleagues[22], also found an association between antibodies and AIAs and female gender, in our study no significant correlation has been found between antibodies and AIAs positivity and age, sex.

Therefore, previous studies[15,22] along with our findings, strengthens the argument that continuous liver damage and hepatocyte necrosis in patients with chronic hepatitis C favors autoimmune abnormalities, and antibodies and AIAs also play an relevant role in the progression of stratification of liver damage, but urgent need of sufficient data with several prospective studies in order to definitely clarify to provide an answer.

CONCLUSIONS

In this study, we show the antibodies and AIAs positivity in chronic hepatitis c treatment-naïve patients. We could attribute this to the high METAVIR score that leads to a release of intracellular antigens at the time of hepatocellular injury and triggers immune responses in the form of autoantibody production. However, since our sample size was small, studies with larger numbers of patients are needed to observe the relevance of our results.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

TREATMENT ADHERENCE IN LUPUS NEPHRITIS PATIENTS IS ASSOCIATED TO MOOD DISORDERS

A ADESÃO AO TRATAMENTO EM PACIENTES COM NEFRITE LÚPICA ESTÁ ASSOCIADA AO TRANSTORNO DE HUMOR

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Background: Lupus nephritis (LN) is a serious complication of Systemic Lupus Erythematosus that has a low rate of complete remission. Treatment adherence in this context is essential. **Aim:** To study treatment adherence in LN patients and its possible association with mood disorders. **Methods:** Cross-sectional study of 69 LN females with application of BMQ (brief medication questionnaire), Center for epidemiological studies- depression questionnaire (CES-D) and Beck anxiety inventory (BAI). Epidemiological and clinical data were collected through chart review. **Results:** Low adherence was found in 13/69 (18.8%); probably low adherence in 30/69 (43.3%); probable adhesion in 23/69 (33,3%) and adhesion in 3/69 (4.3%). About 32/69 (46.3%) individuals scored positive in the BMQ "regimen screen"; 26/69 (37.6%) scored positive in the "belief screen" and 64/69 (92.7%) scored positive in the "recall screen". Anxiety ($p < 0.0001$) and depression ($p=0.001$) but not epidemiological variables (all with p=ns) associated with low adherence. **Conclusions:** There is a high degree of treatment non-adherence among LN patients. Mood disorders associated negatively with adherence rate.
Key words: Treatment Adherence. Lupus Nephritis. Depression. Anxiety.

Justificativa: Nefrite lúpica (NL) é uma complicação grave dos lúpus eritematoso sistêmico que atinge uma baixa taxa de remissão completa. Aderência ao tratamento é fundamental neste contexto. **Objetivo**: estudar a aderência ao tratamento em pacientes com NL e sua possível associação com desordens de humor. **Método**: estudo transversal com 69 pacientes com NL com aplicação do questionário BMQ (*Brief Medication Questionnaire*), questionários CES-D (*Center For Epidemiological Studies- Depression*) e BAI (*Beck Anxiety Questionnaire*). Dados epidemiológicos foram obtidos por revisão de prontuários. **Resultados:** Baixa adesão foi encontrada em 13/69 (18,8%); provavelmente baixa adesão em 30/69 (43,3%); provável adesão em 23/69 (33,3%) e adesão em 3/69 (4,3%). Cerca de 32/69 (46,3%) dos indivíduos obtiveram pontuação positiva no domínio " regime" do BMQ; 26/69 (37,6%) obtiveram pontuação positiva no domínio "crenças" e 64/69 (92,7%) obtiveram nota positiva no domínio "lembrança". Ansiedade ($p < 0,0001$) e depressão ($p=0,001$) estiveram associadas a baixas taxas de adesão, mas não com variáveis epidemiológicas (todas com p=ns). **Conclusões:** Há um alto grau de não adesão do tratamento entre os pacientes da NL. Transtornos de humor estão associados negativamente à taxa de adesão.
Descritores: Aderência a tratamento; Nefrite lúpica, Depressão, Ansiedade.

INTRODUCTION

Lupus nephritis (LN) is one most common manifestations of systemic lupus erythematosus (SLE) occurring in 40 to 50% of patients and causing considerable morbidity and even mortality.[1] Higher risk for kidney involvement is seen among young individuals, male patients and in those with anti-ds DNA.[2,3]

LN is an important predictor of poor outcome and its treatment is often challenging.[1,4] Prompt treatment decreases the risk of end-stage renal disease[5] but, in general, the obtained response is far from satisfactory; the rate of complete remission is estimated in 20% to 30% after receiving six months of standard therapy.[6] Cyclophosphamide, mycophenolate mofetil, azathioprine and calcineurin inhibitors are the main used drugs in the current armamentarium, and recently newer drugs such as voclosporin and belimumab have been added.[2,4,5,7]

"Drugs don't work in patients who don't take them."[4] So, treatment adherence acquires an important role in the prognosis of lupus nephritis as it is fundamental to achieve the best possible results from available resources. Several studies have been done about this problem and shows rates that range from 3% to 76% depending on the assessment toll and population background.[8,9] Most of them evaluate antimalarials, as this drug is very important to avoid the SLE flare.[8,9]

Treatment non-adherence can lead the attending doctor to believe the patient is refractory to treatment and to progress to unnecessary drug scalation, exposing the patient to more potent medications and its side effects. Patient's education, social and economic background, psychological conditions, and doctor-patients relationship may exert influence on this context.[8,9]

Herein we studied treatment adherence in patients with LN from a rheumatology center that cares for SLE patients from the Brazilian Public Health System in order to know the magnitude of the problem and its possible association with depression and anxiety in a local sample.

METHODS

This is a cross sectional study with a convenience sample from a single rheumatology center that cares for lupus patients from the Public Brazilian Health System and that came for regular appointments during the period of march, 2021 to march, 2022 and agreed to participate in the study signing the consent. The Public Brazilian Health System provides, for free, all medications needed in this context: antimalarials, glucocorticoids, immunosuppressors (azathioprine, mycophenolate mofetil, cyclophosphamide, tacrolimus, and cyclosporine), anti-hypertensive and lipid-lowering drugs.

To be included the patients should fulfil the 2019 ACR/EULAR classification criteria for SLE[10] and have nephritis. This study was approved by the local Committee of Ethics in Research and all participants signed consent. Patients unable to understand the consent form were excluded.

- **Data collection:** It was obtained through chart review or upon direct questioning and included: epidemiological data (sex, age, auto declared ethnic background, years of formal study, marital status), clinical and treatment data (disease duration, results from kidney biopsy, number of nephritis flares and used medication).

Patients were invited to answer the following questionnaires:

A. Brief Medication questionnaire (BMQ). This tool includes 3 domains: 1- the "regimen screen" (with 5 questions) that enquires patients how they took each medicine in the past week and measures the adherence; 2- the "belief screen" (with 2 questions) that addresses patients concern on side effects and risks, or doubts about drug efficacy; 3- the "recall screen" (with 2 questions) about difficulties remembering the doses.[11,12] The patients were classified as adherent if there was no positive response in all three screens; probable adherent if there was a positive response in one of the screens; probably low adherent if there was a positive response in the 2 screens and low adherent if there was positive response in the 3 screens.

B. Center for epidemiological studies- depression (CES-D). This is a 20 items survey with answers classified on a Likert scale that ranges from 0-60 and it is used to assess depressive symptoms. Values < 15 are normal, from 15 to 21 suggest mild to moderate depression and > 21, the likelihood of major depression.[13]

C. Beck anxiety inventory (BAI). It has 21 questions graded on a Likert scale and ranges from 0-63. Scores from 0-7 suggests minimal anxiety; from 8-25 mild anxiety; from 16-25 moderated anxiety and from 26-63 severe anxiety.[14]

- **Data Analysis:** Data was collected in frequency and contingency tables. Normality was accessed by Shapiro Wilks test. Nominal data were compared by chi-squared test and comparison of numerical data (CES-D values and BAI scores) in patients grouped according to treatment adherence was analyzed through Kruskal Wallis test. The adopted significance was 5%.

RESULTS

The sample had 69 patients with LN in all but one biopsy proven. The characteristics of this sample is on **Table 1** that shows that all of them were females. About 30. 4%[21/69] have had more than one nephritis flare.

Table 1. Main characteristics of studied sample: 69 patients with lupus nephritis.

	N (%)
Female sex	69 (100)
Median age – years (IQR)	41 (27-50)
Years of formal study	
≤ 8 years	17 (24.5)
9-12 years	38 (55.0)
>13 years	14 (20.1)
Mean disease duration- years (SD)	12.9±7.8
Number of nephritis flares	1 to 3; median 1 (1-2)
Glomerulonephritis class (first biopsy)	
Class II	17 (24.6)
Class III and III+IV	11 (15.9)
Class IV and IV + V	23 (33.3)
Class V	16 (23.1)
Class VI	1 (1.4)
No biopsy	1 (1.4)
Used drugs	Antimalarials – 54 (78.2)
	Mofetil mycophenolate -25 (36.3)
	Cyclophosphamide – 1 (1.4)
	Azathioprine – 8 (11.5)
	Tacrolimus – 9 (13.0)
	Cyclosporine – 1(1.4)
	Glucocorticoid – 13 (18.8)
	Rituximab -2 (2.8)
CES-D	0-48; median 18 (6-28.5)
	Normal – 32 (46.3)
	Mild to moderate depression - 9 (13.0)
	Major depression -28 (40.5)
Beck anxiety inventory	0-52; median 15 (6-27)
	Minimal anxiety – 14 (20.2)
	Light anxiety – 30 (43.3)
	Moderate anxiety -12 (17.3)
	Severe anxiety -13 (18.8)

The distribution of the studied sample according to treatment adherence was: low adherence in 13/69 (18.8%); probably low adherence in 30/69 (43.3%); probable adhesion in 23/69 (33,3%) and adhesion in 3/69 (4.3%).

The analysis of the BMQ results showed that 32/69 (46.3%) individuals scored positive in the "regimen screen"; 26/69 (37.6%) scored positive in the "belief screen" and 64/69 (92.7%) scored positive in the "recall screen".

The patients age (p=0.35), ethnic background (p=0.34), years of formal education (p=0,35) and number of glomerulonephritis flares (p=0.84) did not affect medication's adherence.

Studying the results of CES-D and BAI according to BMQ the results from **Table 2** were found.

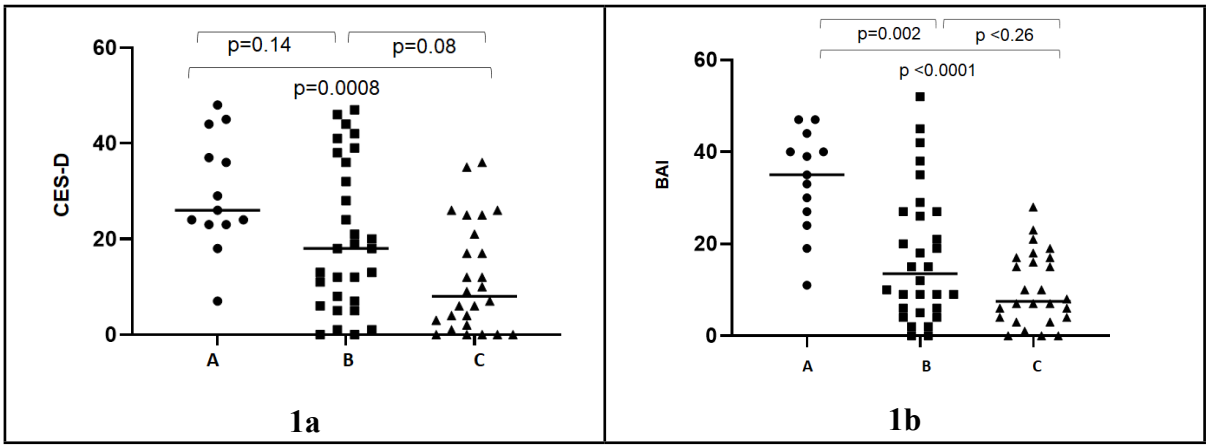
When the BMQ domains were studied according to depression and anxiety the results on **Table 2** were found showing that these two variables affected the regimen screen and belief screen but not the recall screen.

DISCUSSION

The rate of treatment adherence is frequently described as the percentage of the prescribed doses of the medication that are really taken by the patient over a computed period. There is not a definition for an acceptable adherence; most of the authors consider that rates over 80% are acceptable, but in some situations such as human immunodeficiency virus infection, rates greater than 95% may be essential. Rates under 20% are considered as non-adherence.[9]

The results obtained presently showed that the rate of adherence in the studied sample was quite low with 37.6% of adhesion and probable adhesion. Anxiety and depression affected the regimen and the belief screen, but not the recall screen that was the one with

Figure 1a. Values of CES-D in the different levels of treatment adherence. A- median value of 26.0 (23.0-40.5); B= median value of 18 .0 (6,7- 36.5); C=median value of 8.0 (1,75-22,0); p= 0.001, Kruskal Wallis.
Figure 1b. Values of BAI according to the different levels of treatment adherence. A= median value of 35.0 (25.5-42.0); B= median value of 13.5 (5.7-27.0); C=median value of 7.5 (3.7-17.0); p<0.0001, Kruskal Wallis.



CES-D = Center for epidemiological studies- Depression; BAI= Berg anxiety inventory.
A= low adherence (n=13); B= Probable low adherence (n= 30); C= Probable adherence (n=23) + adherence (n=3).
Adherence was studied together with probable low adherence due to small number of the sample.

Table 2. BMQ treatment adherence domains according to depression and anxiety.

	Score 0	Score > ou=1	p
Regimen screen			
Median CES-D	12.0 (2.5-25.0)	23.5 (11.2-36.7)	0.007
Median BAI	8.0 (4.0-18.5)	20.0 (9.0-38.5)	0.003
Belief screen			
Median CES-D	11.0 (4.0-25.0)	24.0 (16.7-39.7)	0.001
Median BAI	9.0 (4.0-18.0)	28.0 (14.0-40.5)	<0.0001
Recall screen			
Median CES-D	21.0(6.5-34.0)	17.5 (6.0-28.7)	0.72
Median BAI	28.0 (5.5-40.50)	15.0 (6.0-26.7)	0.41

CES-D = center for epidemiological studies- depression;
BAI= Beck anxiety inventory.

highest score in the BMQ. In this sample, ethnic background, age, and education did not interfere in the results. Neter et al.[15] also could not link non-adherence in lupus patients with age, or degree of formal education corroborating our findings.

Currently, the recall domain of BMQ had highest score showing that an effort helping the patients to remember to take their medication is essential. None of the studied patients had lupus central nervous system involvement neither were old enough to have cognitive impairment associated to age to explain this kind of difficulty. Moreover, on this sample, age did not correlate with non-adherence. However, this difficulty was already noted by other researchers and that credited them to the lack of routine and low health knowledge.[16]

Contrary to recall problems that lead to non-voluntary non-adherence, the belief barrier, found in 37.6% of the presently studied patients, has a voluntary aspect and is due either to difficulty in believing the medications is really needed or to the fear of their side effects. In such situation the attending doctor should interfere educating the patient about the drugs that are used and the prognostic importance in achieving the best result possible in the nephritis remission.

Anxiety and expression associated to non-adherence. This is an association already noted in other situations such as dialysis treatment in chronic renal failure and, in cardiac and DPOC patients.[16-18] It is believed that this is one of the ways that depression associates to increase mortality in chronically ill patients.[17] Emotional distress has been associated to treatment demands and related stressors, to uncertainty about illness and the restrictions that it imposes.[17]

This work is limited by its cross-sectional design and low number of participants. Also, caregivers and family members were not accessed presently and they may have important role in such context. Nevertheless, it highlights the magnitude of the problem and its association with depression and anxiety showing that mood disorders should be valued when treatment non-adherence is evaluated.

Concluding, this work shows that there is a great deal of non-adherence among lupus patients with glomerulonephritis and that memory barrier is the most common cause of non-adherence. It also shows that anxiety and depression but not epidemiological factors are associated with non-adherence.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

A SMALL EXPERIENCE WITH MULTITARGET THERAPY FOR LUPUS NEPHRITIS TREATMENT

UMA PEQUENA EXPERIÊNCIA COM TERAPIA *MULTITARGET* PARA TRATAMENTO DE NEFRITE LÚPICA

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Resumo: A nefrite lupica (NL) é uma complicação comum e grave dos lúpus eritematoso sistêmico (SLE). Atualmente, o seu tratamento, é feito com ciclofosfamida e mofetil micofenolato, que são drogas com múltiplos efeitos colaterais e para as quais a resposta nem sempre é satisfatória. A terapia multitarget (associação de mofetil micofenolato em baixa dose com tacrolimus) é uma opção utilizada mais recentemente e que parece trazer resultados satisfatórios. **Objetivo:** Analisar a experiência de um centro de reumatologia local com uso de terapia multitarget para tratamento da NL. **Métodos**: Estudo retrospectivo de análise de pacientes com SLE e LN comprovada por biópsia em uso de terapia multitarget por, pelo menos, um ano. **Resultados**: Vinte e três pacientes foram identificados; todavia 10 deles foram excluídos por não terem completado 1 ano de tratamento. Nos treze restantes observou-se, após 1 ano de uso, uma diminuição de proteinúria (p=0.002) e da necessidade de uso de glicocorticoides (p=0.04). Nesta amostra considerou-se que 46,1% obtiveram remissão completa; 38,4 %, remissão parcial e em 15,3% houve falha de tratamento. **Conclusão**: A terapia multitarget é uma opção eficaz no tratamento da NL.

Palavras chave: Lúpus eritematoso sistêmico. Nefrite lúpica. Tratamento.

Abstract: Lupus nephritis (LN) is a common and serious complication of systemic lupus erythematosus (SLE). Currently, its treatment is done with cyclophosphamide and mycophenolate mofetil, which are drugs with multiple side effects and for which the response is not always satisfactory. Multitarget therapy (association of low-dose mycophenolate mofetil with tacrolimus) is a more recently used option that seems to bring satisfactory results. **Objective:** To analyze the experience of a local rheumatology center with the use of multitarget therapy for the treatment of LN. **Methods:** This was a retrospective study of patients with biopsy-proven SLE and LN using multitarget therapy for at least one year. **Results:** Twenty-three patients were identified; however, 10 of them were excluded because they had not completed 1 year of treatment. In the remaining thirteen, a decrease in proteinuria (p=0.002) and the need for glucocorticoids use (p=0.04) was observed after 1 year of use. In this sample, 46.1% were considered to have had complete remission; 38.4% had partial remission, and 15.3% had treatment failure. **Conclusion:** Multitarget therapy is an effective option in the treatment of LN.

Keywords: Systemic lupus erythematosus. Lupus nephritis. Treatment.

INTRODUCTION

Nephritis is a common complication in systemic lupus erythematosus (SLE) affecting almost 50% of the SLE patients and it is one of most feared manifestations of this disease as almost 10% of them will develop end stage renal disease[1,2]. Most of patients develop renal involvement in the first five initial years after SLE diagnosis. Afro-descendant or Hispanic ethnic background, male sex and young age are considered risk factors for this complication[3,4,5].

Lupus nephritis has five histological classes according to ISN/RPS classification and this classification helps to choose the treatment. It includes: glomerulonephritis class I and II, that are referred as minimal and proliferative mesangial glomerulonephritis; type respectively, 3 and 4 that are focal and diffuse proliferative nephritis and class 5, also called membranous glomerulonephritis. The correct diagnosis requires renal biopsy with immunofluorescence studies[6].

The treatment of lupus nephritis aims to preserve renal function and to control the inflammatory process avoiding future dialysis or renal transplantation. Nowadays, the most used treatment for lupus nephritis is high dose glucocorticoid associated with cyclophosphamide or mophetyl mycophenolate (MMF)[7].

However, while pursuing this goal, two problems are observed. One is that the used medications are far from the desired efficiency. The Euro Lupus Nephritis TRIAL showed one-year complete remission in 54% of individuals using low dose cyclophosphamide and 46% in those using the regular dose[3]. The ASPREVA Lupus Management study (ALMS) that analyzed a multiethnic cohort of lupus patients demonstrated remission (total+partial) in 53% in those using cyclophosphamide for 6 months with 8.6% of complete remission, and 56% for MMF with 8.1% of complete remission. In 3,5 years, cyclophosphamide showed complete remission in 59% and MMF in 62%[3].

The other problem with current treatment options is the severe side effects these medications may have. Cyclophosphamide is associated with infertility, hemorrhagic cystitis and bladder cancer, may be harmful for the bone marrow and predisposes to infections[8,9]. MMF is better tolerated but may also cause bone marrow suppression, infections and gastrointestinal intolerance[10].

Achieving remission is crucial for the patients' prognosis. In a study from Lupus Nephritis Collaborative Study Group, the 43 percent of patients who attained a complete response had much higher kidney survival rates at five years (94 versus 46 percent) and at 10 years (94 versus 31 percent) compared with those who did not attain a complete response. Improvement was also noted in the patient overall survival (95 versus 60 percent at 10 years)[11].

One of recent proposed treatment regime for lupus nephritis is the multitarget therapy combining the use of MMF at low dose (1 g/day) and tacrolimus. However, few studies address this form of treatment in Brazilian patients.

Herein, the experience of a local rheumatological center with multitarget therapy for lupus nephritis treatment is reported.

METHODS

This is a retrospective study, approved by the Institutional Committee of Ethics in Research (CAAE-62696322.3.0000.0103) under protocol 5.685.377. Charts from a single rheumatological center that cares for the Public Health System patients, with lupus nephritis patients using multitarget therapy from 2017, January to 2020, January, were reviewed. To be included patients must have had biopsy proven lupus nephritis and follow up of at least one year. Data extraction included: epidemiological data, classification of lupus nephritis, use of previous medication, treatment response to multitarget therapy and adverse events. Complete response was defined as proteinuria of less than 0.5 g per 24 hours, and stable kidney function (an increase of 15% from baseline was allowed). Partial response was defined as proteinuria of less than 3.5 g per 24 hours and decreased by more than 50% from baseline, and stable kidney function (an increase of 15% from baseline was allowed)[12]. Response rate was evaluated one year after use of multitarget therapy.

Obtained data was collected in frequency and contingency tables. Numerical data distribution was studied by Shapiro Wilk test and central tendency was expressed in median and interquartile range (IQR, when not normal) and mean and standard deviation (SD, when normal). Chi squared test and Fisher test were used to compare nominal data and Mann Whitney and unpaired t test to compare numerical data. The adopted significance was 5%.

RESULTS

Twenty-three patients using multitarget therapy were identified. The epidemiological data, number of nephritis flares prior to multitarget therapy, glomeru-

lonephritis class and previous treatment are on **Table 1**. All patients used the combination of tacrolimus initially at dose of 0.3 mg/kg/ day and adjusted according to tacrolimus serum levels plus 1 g of mophetyl mycophenolate (500mg, orally every 12h).

Medication withdrawal occurred in 10 patients: one patient died (death by COVID-19); one could not obtain the medication (tacrolimus), 4 of them had worsening of creatinine; one because of pregnancy, 2 could not achieve adequate serum level and another one due to patients' option.

The comparison results of renal parameters in the 13 patients that remained using the treatment is on **Table 2**. Reduction in proteinuria and number of glucocorticoid users can be noted.

When treatment remission was judged after 1 year, it was found that 6/13 (46.1%) had total remission; 5/13 (38.4%) had partial remission and 2 (15.3%) had failure.

DISCUSSION

The present study showed the local experience in a small sample of lupus nephritis patients using multitarget therapy. The obtained results display that proteinuria and glucocorticoid requirement was significantly reduced in this group, with almost half of them going into total remission.

Table 1. Twenty-three SLE patients at indication for multitarget therapy

Sex	Females	20/23 – 86.9%
	Males	3/23 – 13.0 %
Age (years)	23-57	Mean 41.7±8.8
Disease duration (years)	2-27	Mean 14.7± 7.1
Number of renal flares	1 flare	8/23 – 34.7%
	2 flares	10/23 – 43.4%
	3 or more	5/23 – 21.7%
Glomerulonephritis class	Class III	8/23 – 34.7%
	Class III+V	4/23 – 17.3%
	Class IV	8/23 – 34.7%
	Class IV + V	1/23 – 4.3%
	Class V	2/23 – 8.6%
Use of previous medications	Azathioprine	9/23 – 39.1%
	Cyclophosphamide	17/23 – 73.9%
	Rituximab	4/23 – 17.3%
	MMF only	20/23 – 86.9%
Use of glucocorticoid		10/23 – 43.4%

MMF= mophetyl mycophenolate.

Table 2. Data comparison in 14 patients prior and after multitarget therapy

	Prior to multitarget	After multitarget	p
Systolic blood pressure- median (IQR)	130 (110-145)	125 (117-130)	0.72
Diastolic blood pressure- median (IQR)	80 (70-85)	80 (70-80)	0.86
24h proteinuria (mg)- median (IQR)	2.2 (1.45-3.10)	0.4 (0.22-1.09)	**0.002**
C3 - mg/dL - mean ± SD	83.5±26.4	101.3±41.08	0.24
C4 – mg/dL – median (IQR)	12.0 (11.0-32.0)	17.0 (11.5-32.5)	0.71
Creatinine (mg/dL) – median (IQR)	0.86 (0.69-0.93)	1,04 (0.78-1.36)	0.08
Prednisone users (n)	10 (76.9%)	4 (30.7%)	**0.04**

SD= standard deviation; IQR= interquartile range; n= number.

Tacrolimus – a calcineurin inhibitor, hinders T-cell activation, and, in doing so, suppresses autoantibody production preventing long-term kidney injury[13]. In addition to reducing glomerular deposition of immune complexes, tacrolimus may have direct protective effects on podocytes, including stabilization of the actin cytoskeleton and inhibition of podocyte apoptosis[14], which could contribute to the observed reduction in proteinuria.

Also, the glucocorticoid sparing properties of this drug, already known in minimal change nephrotic syndrome[15] and renal transplantation[16] are very important for lupus patients as this medication is responsible for a great deal of cumulative damage seen on this disease[17]. However, it is important to observe that tacrolimus has a very narrow therapeutic window, requiring periodic measurement of serum levels and resulting in numerous drug-related side effects[18]. Nephrotoxicity, diabetes mellitus, leukopenia, anemia, dyslipidemia, mouth ulcers, hypertension, and viral reactivations (cytomegalovirus and BK virus) are some of the known side effects[18]. In the present study almost half of the sample did not finish one year of treatment and the most common cause of drug withdrawal was the increase in creatinine levels. Acute nephrotoxicity of calcineurin inhibitors is associated to acute arteriolopathy caused by vasoconstriction effects, toxic tubulopathy, and thrombotic microangiopathy, together with functional modifications such as that of intrarenal hemodynamics and a reduced glomerular filtration rate, that are reversible with dose reduction[19]. Chronic nephrotoxicity causes progressive, irreversible damage of kidney structures such as the arteriolar hyalinosis of vessels, tubular atrophy and interstitial fibrosis or glomerular sclerosis[19,20].

Tacrolimus can be administered by oral, sublingual, topical, or intravenous routes. Although oral intake is the standard route of administration, tacrolimus shows poor water solubility and poor oral bioavailability[18]. In this patient's series, two of them could not achieve adequate serum level despite progressive increased in the prescribed treatment; nevertheless, it seems that compliance problems were related to this finding.

This research is limited by the small number of studied patients and by its retrospective design. Future studies with prospective design and high number of patients are needed to stablish the role of multitarget therapy in our population with lupus.

Concluding, the multitarget therapy is an alternative treatment that can be used in lupus nephritis patients that are refractory to conventional therapy.

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CASE REPORT AND LITERATURE REVIEW *RELATO DE CASO: REVISÃO DE LITERATURA*

INTRAUTERINE DEVICE IN THE PERITONEAL CAVITY

DISPOSITIVO INTRAUTERINO NA CAVIDADE PERITONEAL

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A 33-year-old woman presented with persistent pelvic discomfort for the past three months. She reported the insertion of an intrauterine device (IUD) one year ago. Symptoms included pelvic pain, dyspareunia, and irregular vaginal bleeding. She denied fever, chills, abnormal vaginal discharge, or dysuria.
Keywords: Case report, Intrauterine device, Migration, Intraperitoneal.

Relato de caso
Uma mulher de 33 anos apresentou desconforto pélvico persistente nos últimos três meses. Relatou a inserção de um dispositivo intrauterino (DIU) há um ano. Os sintomas incluíam dor pélvica, dispareunia e sangramento vaginal irregular. Negava febre, calafrios, fluxo vaginal anormal ou disúria.
Descritores: Relato de caso, Dispositivo intrauterino, Migração, Intraperitoneal.

CASE REPORT

A 33-year-old woman presented with persistent pelvic discomfort for the past three months. She reported the insertion of an intrauterine device (IUD) one year ago. Symptoms included sharp pelvic pain, dyspareunia, and irregular vaginal bleeding. She denied fever, chills, abnormal vaginal discharge, or dysuria.

PAST MEDICAL HISTORY

The patient had no significant past medical history or surgeries. She was sexually active with one monogamous male partner and denied a history of sexually transmitted infections. She was not taking oral contraceptives in addition to the IUD.

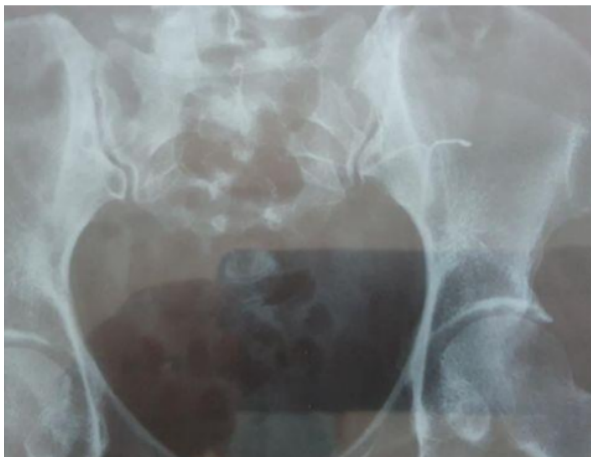
PHYSICAL EXAMINATION

Upon examination, the patient was afebrile with normal vital signs. Abdominal examination revealed tenderness to deep palpation in the left lower quadrant. Pelvic examination revealed a uterus in normal position, mobile, with tenderness on left adnexal palpation. There was no abnormal vaginal discharge and the IUD string was not palpable in the cervical canal.

ADDITIONAL INVESTIGATIONS

- Transvaginal ultrasound: Revealed the absence of the IUD within the uterine cavity.
- Beta-HCG level: Negative.
- Plain abdominal X-ray: Demonstrated an image compatible with the IUD in the left pelvis (**Figure 1**).

Figure 1. Plain abdominal X-ray: IUD in the left pelvis



DIAGNOSIS

Based on the clinical history, physical examination, and additional investigations, the patient was diagnosed with migration of the IUD into the peritoneal cavity.

TREATMENT

In this case, the laparoscopy was performed to locate and remove the IUD from the peritoneal cavity. The procedure was uncomplicated, and the patient recovered well, with resolution of her symptoms.

DISCUSSION

IUDs are a widely used form of contraception, employed by approximately 14% of women globally, with regional adoption rates reaching up to 27%.[1] However, IUD migration remains a rare but serious complication associated with their use.

Most IUD perforations occur during insertion (primary perforation), with a reported incidence of 0.4–1.1 per 1,000 procedures.[2,3] These perforations are often accompanied by severe abdominal pain, alerting the physician to a potential issue. Secondary perforations, occurring eight or more weeks after insertion, are more challenging to diagnose. The presumed mechanism involves progressive pressure and necrosis of the uterine wall.[4] This delayed migration of the IUD into the peritoneal cavity, a form of secondary perforation, can lead to damage of internal organs.[5]

Intramyometrial migration is another potential complication. It begins with the entrapment of an IUD arm within the myometrium (muscular layer of the uterus). Inflammatory processes and uterine contractions can then facilitate further migration of the IUD. Another study suggests that uterine forces may guide the IUD towards abnormal sites.[6]

Goldstuck and Wildemeersch categorized perforations as primary, secondary, or a combination of both.[7] They further proposed that myometrial contraction forces are the primary drivers of IUD expulsion from the uterine cavity. Additionally, they emphasized the critical role of force vector direction and the cumulative effect of these forces in determining the final migration path of the IUD.[7]

While IUDs are highly effective for contraception, a rare complication called IUD migration can occur. This happens when the IUD moves from its intended position within the uterus to another location in the body. Several factors can increase this risk, including technical errors during insertion, a hole in the uterus, an IUD size mismatch, or a prior childbirth with an IUD in place.[8]

Symptoms of IUD migration can vary widely, with some women experiencing none at all. However, the most common complaints include pelvic pain, painful sexual intercourse (dyspareunia), and irregular vaginal bleeding. In rare and severe cases, IUD migration can lead to serious complications such as intestinal perforation, obstruction, or pelvic adhesions.[7,9]

Diagnosis relies on a combination of a woman's medical history, a physical examination, and potentially various imaging tests.[10] Transvaginal ultrasound is the preferred method for detecting an extrauterine IUD, but other options like X-ray, CT scan, or MRI may be used in certain situations.[11,12] Additionally, beta-HCG levels can help rule out ectopic pregnancy, which can mimic some symptoms of IUD migration.[12]

When an IUD migrates from its intended position, removal is the primary treatment. The World Health Organization recommends minimally invasive surgical techniques like hysteroscopy, cystoscopy, colonoscopy, or laparoscopy, depending on the IUD's location.[14] Lap-

aroscopy is often preferred due to its minimally invasive nature and quicker recovery time.[15]

Fortunately, the prognosis for women with IUD migration is generally positive. Most women experience a full recovery without complications. However, it's important to note that a history of IUD migration may increase the risk of future occurrences.

CONCLUSION

In conclusion, while rare, IUD migration is a serious complication. Being aware of the risk factors, symptoms, and treatment options is crucial.

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CASE REPORT *RELATO DE CASO*

PRIMARY HYPERPARATHYROIDISM: LITERATURE REVIEW AND CASE REPORT

HIPERPARATIROIDISMO PRIMÁRIO: REVISÃO DA LITERATURA E RELATO DE CASO

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Introdução: O hiperparatireoidismo primário (HPP) é caracterizado pela elevação dos níveis de paratormônio (PTH) no sangue, que gera desequilíbrio na homeostase do cálcio devido a hiperfunção de uma ou mais glândulas paratireoides. Frequentemente os indivíduos apresentam-se de forma assintomática, mas podem manifestar quadros clínicos diversos decorrentes da hipercalcemia sérica como: náuseas, vômitos, fadiga intensa, constipação intestinal, confusão mental, dores musculares, astenia e outros. O diagnóstico do HPP é dado através do cálcio sérico ajustado para albumina elevado, na presença PTH intacto elevado ou inadequadamente normal. A paratireoidectomia é o tratamento padrão ouro, sendo considerado um procedimento seguro para o tratamento do HPP. **Objetivo**: Relatar um caso clínico de HPP associado a adenoma de paratireoide bem como revisar os principais aspectos clínicos de tal patologia. **Conclusão**: O HPP sempre deve ser investigado na presença de hipercalcemia sérica com PTH intacto elevado, especialmente em mulheres na menopausa, mesmo na vigência de sintomas inespecíficos.

Descritores: Adenoma de Paratireoide, Hiperparatiroidismo, Hipercalcemia, Hormônio da Paratireoide

Introduction: Primary hyperparathyroidism (PHPT) is characterized by elevated levels of parathyroid hormone (PTH) in the blood, leading to calcium homeostasis imbalance due to hyperfunction of one or more parathyroid glands. Individuals often present asymptomatically, but clinical manifestations can arise from serum hypercalcemia, such as nausea, vomiting, severe fatigue, constipation, mental confusion, muscle pain, asthenia, and others. The diagnosis of PHPT is made through elevated serum calcium adjusted for albumin, in the presence of elevated or inappropriately normal intact PTH. Parathyroidectomy is the gold standard treatment, considered a safe procedure for PHPT management. **Objective:** Report a clinical case of PHPT associated with parathyroid adenoma as well as review the main clinical aspects of this pathology. **Conclusion:** PHPT should always be investigated in the presence of elevated serum calcium with elevated intact PTH, especially in postmenopausal women, even in the presence of nonspecific symptoms.

Keywords: Parathyroid adenoma, hyperparathyroidism, hypercalcemia, parathyroid hormone (PTH).

CASE REPORT

V.M.B, female, 62 years old, Caucasian, admitted with complaints of weakness, diffuse myalgia, retrosternal pain, dyspepsia, dyspnea, and lower limb edema for the past two weeks. She has a previous comorbidity of type 2 diabetes mellitus, for which she was taking 2 grams of metformin per day.

On physical examination, vital signs were stable, she was oriented in time and space, albeit drowsy, scoring 15 on the Glasgow Coma Scale, with no neurological deficits. Cardiac and pulmonary auscultation were regular. Symmetric, mild bilateral lower limb edema was observed.

Admission complementary exams:

- Electrocardiogram: sinus rhythm, heart rate 75 bpm.
- Additional laboratory tests demonstrated: Ionized calcium: 6.5 mg/dL (reference range: 4.0 to 5.4 mg/dL); Serum calcium corrected for albumin: 13.1 mg/dL (reference range: 8.8 – 10.6 mg/dL)24-hour Urinary Calcium: 198.56 mg/24 hours (reference range: 280 mg/24h – revised); Intact PTH: 362 pg/mL (reference range: 294 pg/mL); Albumin: 2.79 g/dL (reference range: 3.5 – 5.2 g/dL); Magnesium: 2.26 mg/dL (reference range for women: 1.9 – 2.5 mg/dL); Vitamin D – 25 hydroxy: 12.3 ng/mL (reference range: 30.0 - 60.0 ng/mL); Phosphorus: 1.22 mg/dL (reference range: 2.5 - 4.5 mg/dL); Sodium: 137 mEq/L (reference range: 135 – 145 mEq/L); Potassium: 5.5 mEq/L (reference range: 3.5 – 5.5 mEq/L); Creatinine: 0.93 mg/dL (reference range: 0.7 -1.3 mg/dL).

Given the elevated serum calcium with suspicion of hyperparathyroidism, further investigation with imaging exams was requested, indicative of a functioning parathyroid gland. Additionally, long bone radiography showed incipient degenerative changes in the hip joint, primarily on the right side. Similarly, computed tomography of the lumbosacral spine and cervical region revealed degenerative disc changes.

Management and Case Evolution

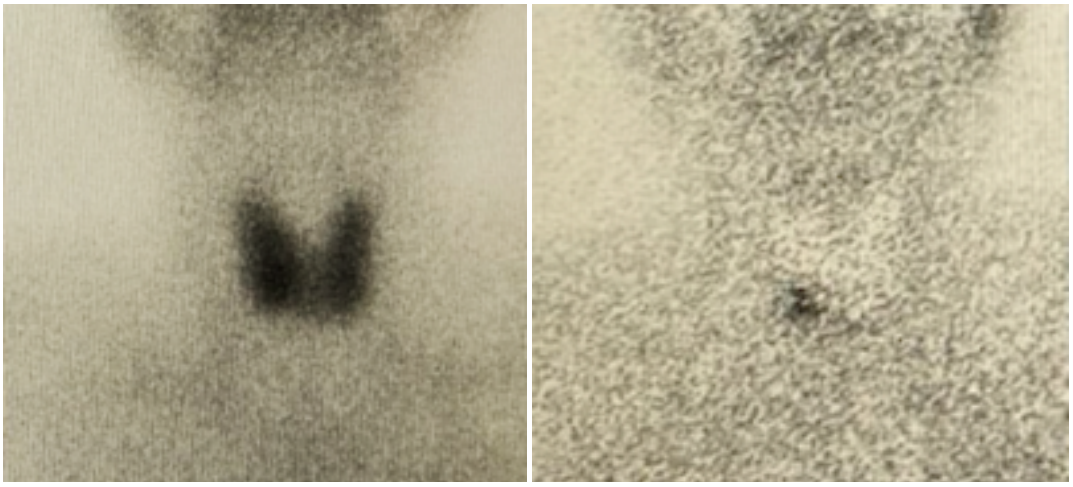
During hospitalization, the patient received intravenous furosemide at a dose of 80 mg/day for four days to reduce serum calcium levels, which remained high at 11.4 mg/dL. Subsequently, zoledronic acid 4mg/100ml was administered intravenously as an anti-reabsorptive medication. Three days after the medication administration, serum calcium levels corrected for albumin decreased to 10.9 mg/dL. Additionally, the patient received Calcitriol at a dose of 0.25 mg every 12 hours during the hospital stay.

With clinical and laboratory stability, the patient was discharged with outpatient follow-up instructions. With the primary diagnostic hypothesis of primary hyperparathyroidism, supported by cervical ultrasound, a more accurate examination for identifying parathyroid alterations, technetium pertecnetate scintigraphy, was requested. Additionally, bone densitometry was performed to aid in treatment decisions.

Upon outpatient follow-up, one month after discharge, the following complementary exams were presented:

- Serum calcium corrected for albumin: 12.1 mg/dL
- Intact PTH: 294
- Vitamin D: 35,5

Figure 1. Technetium-99m pertecnetate scintigraphy



Bone Densitometry: osteoporosis is detected.

Tipo exame	BMD	T-score	% de T-socre	Z-score	% do Z-socre
Coluna lombar	0,693	-3,2	66%	-1,6	79%
Coluna L. perfil					
Colo de Fêmur	0,554	-2,7	65%	-1,3	80%
Fêmur Total	0,626	-2,6	66%	-1,5	77%
Antebraço 1/3 radio					
Antebraço total radio					

Convenção OSM para laudos de Densiometria Ossea.
Coluna ou Colo femural - t-score 0,0 a 0,9 Dp - NORMAL
-1,0 q 2,4 DP - OSTEOPENIA
-2,5 e abaixo - OSTEOPOROSE

Technetium-99m pertecnetate scintigraphy: focal activity area of sestamibi-99mTc adjacent to the inferior pole of the right thyroid lobe, suggestive of hyperfunctioning parathyroid gland.

Thus, the diagnosis of primary hyperparathyroidism due to a hyperfunctioning parathyroid adenoma was concluded. As expected for such pathology, the patient continued to experience hypercalcemia and also presented with a bone complication: osteoporosis, indicating the need for surgical treatment. The patient was then referred to the head and neck surgery department for definitive treatment with parathyroidectomy.

DISCUSSION

Primary hyperparathyroidism (PHPT) is a metabolic disorder resulting from the autonomous overactivity of one or more of the parathyroid glands, leading to a progressive increase in serum parathyroid hormone (PTH) levels or inappropriately normal levels, along with calcium levels[1].

It predominantly affects females, with approximately 1 in every 500 women and 1 in every 2000 men affected annuallyls[2]. The prevalence is higher after the age of 50 due to menopauses[3]. With increased bone resorption revealing the hyperactivity of the parathyroid gland. Additionally, the incidence of hyperparathyroidism is higher among black individuals, followed by white, Asian, and Hispanic populations[3].

Single parathyroid adenoma is the primary etiology of primary hyperparathyroidism, accounting for up to 80 to 90 percent of cases. Other possible etiologies include parathyroid hyperplasia responsible for 5.74% of cases, double adenomas responsible for 4.14% of cases, and, finally, parathyroid carcinoma responsible for only 0.74% of cases[4]. In 90% of cases, primary hyperparathyroidism manifests as sporadic disease, while in only 10% of cases, it manifests as familial disease, with the most common germline genetic mutations being multiple endocrine neoplasia type 1 (MEN1), type 2A (MEN2A), type 4 (MEN4), and hyperparathyroidism-jaw tumor syndrome (HPT-JT)[5].

Parathyroid hormone (PTH) plays a crucial role in calcium regulation in the body. Parathyroid cells detect small variations in extracellular calcium levels through calcium-sensing receptors (CaSRs). Primary hyperparathyroidism (PHPT) is caused by the dysregulated growth of the parathyroid glands, along with reduced expression of CaSRs. About 10% of PHPT cases have a genetic basis, which may include syndromes such as multiple endocrine neoplasia (MEN1-MEN4) or isolated familial hyperparathyroidism. This condition can also be caused by non-syndromic isolated endocrinopathies, such as severe neonatal hyperparathyroidism[6].

Primary hyperparathyroidism (PHPT) is characterized by dysregulated growth of parathyroid tissue, resulting in excessive production of parathyroid hormone and reduced expression of the calcium-sensing receptor (CaSR). These abnormalities interfere with PTH's normal ability to maintain extracellular ionized calcium levels within the normal range. Parathyroid glands detect extracellular calcium levels through CaSR, which inhibits PTH secretion when activated. Additionally, PTH is regulated by 1,25(OH)2D and its receptor[7].

PTH defends against hypocalcemia by stimulating renal calcium reabsorption, suppressing renal phosphate reabsorption, increasing bone resorption, and stimulating intestinal calcium absorption, along with increasing the production of 1,25(OH)2D in the

proximal renal tubule. The renal CaSR promotes calcium excretion from the thick cortical ascending limb of the loop of Henle. Imbalance in these physiological processes leads to a series of consequences such as hypercalcemia, hypophosphatemia, and disorders in bone and renal resorption[7].

PHPT can be caused by solitary benign adenomas, multiglandular involvement, or rarely, parathyroid carcinoma. The genetic or hereditary basis is more common in cases of multiglandular disease, while parathyroid cancer is a rare cause of hypercalcemia[7].

PTH-secreting adenomas can also be found in the thymus. These tumors express a parathyroid-specific gene, GCM2, unlike normal human thymus, which does not express either PTH or GCM2, suggesting a origin from migrated parathyroid cells during embryogenesis[8].

Most patients are asymptomatic, but when hypercalcemia exceeds 14 mg/dL or when serum calcium increases rapidly, we have the so-called "calcemic crisis," which is characterized by anorexia, nausea, vomiting, severe fatigue, constipation, mental confusion, arrhythmias, stupor, and coma[9]. In less than half of the cases, PHPT may present with other clinical manifestations due to serum hypercalcemia such as kidney stones and nephrocalcinosis, systemic arterial hypertension, and various musculoskeletal symptoms like muscle weakness, myalgias, arthralgia, and chondrocalcinosis[9]. From an osteometabolic perspective, osteoporosis and, in advanced cases, cystic fibrous osteitis are observed. Epidemiological studies suggest that patients with PHPT have an increased incidence of fragility fractures in various skeletal sites, with this pathology associated with reduced bone mineral density (BMD), mainly in the cortical bone region, as found in the distal radius[1]. However, in the lumbar region, mainly composed of trabecular bone, and in the femoral region, composed of both cortical and trabecular bone, the decrease in BMD is less intense or even preserved[10].

The diagnosis of hyperparathyroidism is made by elevated serum calcium, adjusted for albumin, in the presence of elevated intact parathyroid hormone (80-90% of cases) or inappropriately normal (10-20% of cases). Tests should be collected on two occasions with at least a 2-week interval[7].

Normocalcemic primary hyperparathyroidism (NPHPT) is characterized by elevated levels of intact parathyroid hormone (PTH), detected by second or third-generation assays, on at least two occasions over 3 to 6 months. This occurs even with total adjusted calcium and ionized calcium levels within the normal range, after excluding other causes of secondary hyperparathyroidism. This type of NPHPT presents an additional diagnostic challenge due to the absence of hypercalcemia, requiring thorough evaluation to establish the diagnosis and determine appropriate management[7].

The differential diagnosis of Familial Hypocalciuric Hypercalcemia (FHH) may be suspected in younger individuals with a urinary calcium/creatinine clearance ratio <0.01 and/or in those with a family history of hypercalcemia, use of thiazide diuretics and lithium, or ectopic secretion of PTH[7].

Parathyroidectomy is the gold standard treatment and is considered a safe procedure for the treatment of PHPT, with high success rates and low incidence of complications. Normalization of PTH, calcium, and phosphorus levels, allowing for decreased osteoarticular and renal aggression, confirms the effectiveness of surgery in the treatment of primary hyperparathyroidism[11,12].

The criteria for surgical approach in PHPT include: (1) serum calcium concentration above 1 mg/dL of the upper limit of normal; (2) 24-hour urinary calcium >400 mg; (3) 30% reduction in age-adjusted endogenous creatinine clearance; (4) bone densitometry with T score below -2.5 standard deviations at any location; (5) age younger than 50 years. Surgical treatment should be indicated in the presence of at least one of these criteria[7,12].

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REVIEW *REVISÃO*

CARBOHYDRATE COUNTING: WHAT IS THE EVIDENCE AND HOW TO APPLY IT IN CLINICAL PRACTICE?

CONTAGEM DE CARBOIDRATOS: QUAIS SÃO AS EVIDÊNCIAS E COMO APLICÁ-LAS NA PRÁTICA CLÍNICA?

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Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease that increases morbimortality and reduces quality of life. Optimal glycemic control is the main objective to prevent its complications. Nutritional therapy plays a fundamental role in the multidisciplinary treatment, and within the strategies, carbohydrate counting is a method of nutritional planning for patients and should be part of nutritional strategies to prevent episodes of hypo and hyperglycemia. The "Projeto DOCE" –**D**iabetes **O**bjetivando **C**ontrole e **E**ducação -(FEMPAR, Brazil) is a multidisciplinary study that aims to offer quality service for patients with T1DM. Carbohydrate counting is part of the monitoring of these patients and is always done individually for each patient, associated with other nutritional intervention. The nutritional plan must always be carried out by the entire multidisciplinary team. The evidence shows that carbohydrate counting is an efficacious and safe technique to improve glycemic control and quality of life in T1DM patients. The technique of carbohydrate counting has been adopted by our service in a more structed way since July of 2023, when a registered dietitian has become part of the multidisciplinary team, and we are in the process of collecting data from our service to evaluate our results in order to publish soon.
Key-words: Type 1 Diabetes Mellitus, Nutrition Therapy, Carbohydrate Counting.

O Diabetes Mellitus tipo 1 (DM1) é uma doença autoimune que aumenta a morbimortalidade e reduz a qualidade de vida. O controle glicêmico adequado é o principal objetivo para prevenir as complicações. A terapia nutricional desempenha papel fundamental no tratamento multidisciplinar e, dentro das estratégias, a contagem de carboidratos é um método de planejamento nutricional dos pacientes e deve fazer parte das estratégias nutricionais para prevenção de episódios de hipo e hiperglicemia. O "Projeto DOCE" (**D**iabetes **O**bjetivando **C**ontrole e **E**ducação -FEMPAR, Brasil) é um serviço multidisciplinar que visa oferecer atendimento de qualidade aos pacientes com DM1. A contagem de carboidratos faz parte do acompanhamento desses pacientes e é sempre feita individualmente para cada paciente, associada a outras intervenções nutricionais. O fator insulina/carboidrato é sempre calculado de acordo com toda a equipe multidisciplinar. As evidências apontam que a contagem de carboidratos é uma técnica eficaz e segura para melhorar o controle glicêmico e a qualidade de vida em pacientes com DM1. A técnica de contagem de carboidratos tem sido adotada pelo nosso serviço de forma mais estruturada desde julho de 2023, quando uma nutricionista passou a fazer parte da equipe multidisciplinar, e estamos em processo de coleta de dados do nosso serviço para avaliar e mostrar nossos resultados em uma nova publicação em breve.
Descritores: Diabetes Mellitus tipo 1, Terapia Nutricional, Contagem de Carboidratos.

CARBOHYDRATE COUNTING

Type 1 Diabetes Mellitus (T1DM) is an autoimmune condition that results in insulin deficiency and loss of pancreatic function that requires lifelong insulin therapy[1]. The condition affected approximately 8.75 million individuals in 2022[2] worldwide. This disease results in a significant increase in morbidity and mortality due to both the acute and chronic complications it causes. The key to prevent/delay the complications is the achievement and maintenance of a good glycemic control. Insulin remains the only approved treatment for T1DM despite the advances in the area, and an intensive regimen with multiple daily insulin injections guided by self- monitoring of blood glucose and an appropriate diet have positive effects on postprandial hyperglycemia, improving glycated hemoglobin (HbA1c) control. Carbohydrate counting is considered the ideal way to calculate meal-related insulin doses as it allows greater flexibility in the diet, as it is a major determinant of postprandial blood glucose[1,3-6].

Carbohydrate is the nutrient with the highest impact on plasma glucose level, so monitoring the total amount of carbohydrates consumed in a meal is fundamental to control postprandial glucose fluctuations. Clinical guidelines in medical nutrition therapy recommend that patients with T1DM learn carbohydrate counting or similar experience-based methods to improve glycemic control[2,7,8].

Two levels of carbohydrate counting have been defined internationally with different learning objectives and increasing complexity: a basic and an advanced level. Basic carbohydrate counting includes understanding of the relationship between food, physical activity, and glucose levels with focus in the timing, type and amount distribution of carbohydrate-containing foods consumed. Advanced carbohydrate counting targets patients who ideally masters the basic counting, is on intensive insulin therapy and are prepared to learn how to adjust insulin according to carbohydrate intake[9].

The American Diabetes Association recommended in the 2024 Guideline to provide education on the glycemic impact of carbohydrate tailored to an individual's needs, insulin plan, and preferences to optimize mealtime insulin dosing. Although, when using fixed insulin doses, individuals should be provided with education about consistent patterns of carbohydrate intake with respect to time and amount consumed while considering the insulin action time, as it can result in improved glycemic levels and reduced the risk for hypoglycemia[10].

It is important to emphasize that carbohydrate-counting is complex, but worthwhile as it impacts on patient satisfaction among the disease treatment[11] and also the general wellbeing and treatment satisfaction are also significantly improved[12,13]. Carbohydrate counting is a planning strategy that may also reduce the burden of the disease[3,12-14]. The DAFNE study showed that skills training in insulin adjustment promotes dietary freedom, improves quality of life and glycaemic control in people with T1DM without worsening severe hypoglycaemia[12].

THE EVIDENCE

A lot of studies are available in the literature on carbohydrate counting. The pioneer DAFNE study[12], which included 169 adults with T1DM and moderate or poor glycaemic control, showed that after 6 months of a course providing the skills to enable patients to replace insulin matching it to desired carbohydrate intake on a meal-by-meal basis resulted in significantly better HbA1c in participants attending training immediately (immediate DAFNE patients, mean 8.4%), than in those acting as waiting list controls (delayed DAFNE patients, 9.4%) ($t=6.1$, $P < 0.0001$). The impact of diabetes on dietary freedom was significantly improved in immediate DAFNE patients compared with delayed DAFNE patients ($t= -5.4$, $P < 0.0001$), as well as the impact of diabetes on overall quality of life ($t=2.9$, $P < 0.01$)[12].

The pilot study of Scavone et al in 2010[4] with 256 T1DM randomized in a nutrition education programme or not, for 9 months, showed that the group on the program had a reduction in HbA1c (7.8±1.3–7.4±0.9%) compared to the group with no program (7.5±0.8–7.5±1.1%; $P < 0.01$), with less hypoglycemic events (4% vs. 7%; $p<0.05$) and a reduction in the dose of rapid insulin analogues (23.5±10.9 vs. 27.7±17.1 IU ⁄ 24 h; $p=0.03$).

Trento et al also evaluated the effects of adding a carbohydrate counting programme on metabolic control of 56 T1DM patients, with 27 randomized to receive 8 session on carbohydrate counting and 29 controls, with all patients on 4-daily insulin injections and practicing self-monitoring of blood glucose. At 30 months, HbA1c was lower in the carbohydrate counting patients as compared to controls (7.2±0.9 vs 7.9±1.4%, $p<0,05$)[9].

A recent systematic review and meta-analysis in patients with T1DM that included 11 randomized controlled clinical trials (n=899 patients) with a parallel-group design comparing any carbohydrate counting forms with standard care or other forms of dietary advice or insulin dose calculation in people with T1DM,

with a follow up period of at least 3 months. The first analysis showed that carbohydrate counting was not better in reducing HbA_{1c} levels (SMD-0.24%, 95% CI -0.68 to 0.21) than all dietary advice forms. However, this finding was highly heterogeneous, and a second analysis including only studies with low heterogeneity showed a meaningful reduction in HbA_{1c} levels (SMD-0.52%, 95% CI -0.82 to -0.23) as compared with usual diabetes education. The authors concluded that carbohydrate counting is an efficacious technique to safely reduce the levels of HbA_{1c} in adults and children compared with standard diabetes education, and its effect does not appear to change with prolonged time[3].

Those results were similar to what Bell et al described in a systematic review and meta-analysis that included 7 trials, comprising 599 adults and 104 children with T1DM with interventions longer than 3 months that compared carbohydrate counting with general or alternate dietary advice in adults and children with type 1 diabetes. The overall analysis showed no significant improvement in HbA1c concentration with carbohydrate counting versus the control or usual care (-0·35% [-3·9 mmol/mol], 95% CI -0·75 to 0·06; p=0·096), but in the five studies in adults with a parallel design there was a 0·64% point (7·0 mmol/mol) reduction in HbA1c with carbohydrate counting versus the control group (95% CI -0·91 to -0·37; p<0·0001)[14].

Schimidt et al[15] evaluated 27 studies, 6 randomized controlled trials and 21 observational studies, also with a large heterogeneity. The studies demonstrated a positive trend in reduction in HbA_{1C} after introduction of carbohydrate counting (0.0–1.2%), however, only a few improvements were considered clinically relevant. The majority of the studies assessing the incidence of hypoglycemic events found a significant reduction in the event rate and none reported an increase in the incidence. The authors concluded that the method appears preferable to other insulin dosing procedures.

HOW TO APPLY IT IN CLINICAL PRACTICE?

"Projeto Doce" is the outpatient clinic of the Faculdade Evangélica Mackenzie Paraná (FEMPAR, Curitiba, Brazil), that provides clinical treatment for T1DM patients since 2005. The project offers multidisciplinary care, with a diabetologist, residents in endocrinology, a dietitian and a psychologist, and is based on the DAFNE study principles, objecting carbohydrate counting strategy to provide the skills to enable patients to do insulin matching to the desired carbohydrate intake on a meal-by-meal, together with sliding scale charts to correct glycemia. Both levels of carbohydrate counting are instructed to the patients, the basic and the advanced level, depending on the patient's knowledge and preferences and the treatment phase the patient is on.

In patients with no previous contact with carb counting the dietitian usually initiates with the basic carbohydrate counting, focusing on teaching the relationship between food, physical activity, and glucose levels with focus in the timing, type and amount of carbohydrate-containing foods consumed, in accordance with the insulin plan. The patients are asked to practice and bring data information to the next appointment (about 4 to 12 weeks later).

After accomplishing the basic carbohydrate counting, and when well succeed, the dietitian usually starts the advanced carbohydrate counting teaching the patient to adjust insulin according to carbohydrate intake. The carbohydrate/insulin factor is calculated in accordance with the diabetologist and the residents in endocrinology and all the decisions on the strategy adopted are deeply discussed with the whole multidisciplinary group, based on the recommendation of 500 divided by total daily insulin dose (both basal and bolus doses)[16,17]. In July of 2023 a new multidisciplinary group has been created and the clinic, and since than carbohydrate counting has been better structured and all data has been collected for future publication to show its benefits.

Carbohydrate counting seems to be the best strategy for T1DM patients, but it should not be applied as the only strategy. It is recommended that those patients should get information for a better quality of the diet, adhering to diabetes-specific guidelines of daily energy intake of macronutrients, as well as intake of micronutrients and dietary fiber, in order to promote good glycemic control and to prevent long-term consequences. The adherence to nutrition guidelines is low, especially in teenagers (half of them follows the nutrition plan less than 75% of the time). A higher percentage of daily energy from fats have been associated with poorer HbA1C[15]. The dietitian of "Projeto DOCE" is compromised in providing improved nutrition education to promote better habits.

Also, obesity prevalence among people with T1DM has increased substantially, and data shows that over 50% of people with T1DM are overweight or present obesity[18]. So, it is important to emphases that in those patients weight management is recommended, so carbohydrate counting is those patients needs to be associated with eating plans that result in an energy deficit and that lower carbohydrate and glycemic

index, as well as high fiber content, adequate in fat (amount and type) and with lean protein[18].

Nutrition therapy must be individualized, and to achieve this individualization it is essential to analysis eating habits, to evaluate history of weight management and to consider physical activity, medications in use (doses and times), occurrence and times of hypo and hyperglycemia, as well as to understand patient preferences, access to food and the ability and availability to promote behavioral changes[6,9,8,16]. Our clinic is well compromised with this ideal, to promote education on diabetes, but always based on individual's needs.

CONCLUSIONS

The presence of a multidisciplinary team is essential for the successful treatment of patients with T1DM, and the dietitian has a fundamental role because nutritional therapy has a marked impact glycemic control. Within the possible nutritional strategies, carbohydrate counting is an efficacious and safe technique to improve glycemic control and quality of life in T1DM patients, and we are collecting data from our service to publish soon.

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REVIEW *REVISÃO*

EVALUATING THE DIAGNOSTIC ACCURACY OF ANTI-ZINC TRANSPORTER 8 ANTIBODIES IN TYPE 1 DIABETES: A SYSTEMATIC REVIEW AND META-ANALYSIS

AVALIADO A PRECISÃO DIAGNÓSTICA DOS ANTICORPOS ANTI-TRANSPORTE DE ZINCO 8 NO DIABETES TIPO 1: UMA REVISÃO SITEMÁTICA E META-ANÁLISE

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Introduction: The association between autoantibodies and the risk of type 1 diabetes mellitus (T1DM) is well established. However, there is still a lack of quantitative insight into the role of anti-zinc transporter 8 (anti-ZnT8) antibodies and their efficacy in T1DM diagnosis. **Objective:** To conduct a systematic review and meta-analysis on the association between anti-ZnT8 autoantibodies and the risk of T1DM. **Methods:** Relevant studies were retrieved from the PubMed database and analyzed using a fixed or random-effects model. **Results:** Out of 211 screened articles, 23 studies were selected and a total of 14,172 patients were included in the analysis. Our pooled analysis revealed that anti-ZnT8 autoantibody expression was significantly associated with an increased risk of T1DM development in both children and adults [OR 1.14 (95% CI: 1.12-1.16)]. **Conclusion:** Our systematic review and meta-analysis provides evidence supporting a significant association between anti-ZnT8 autoantibody positivity and an increased risk of T1DM development.
Keywords: Type 1 diabetes mellitus; Zinc transporter-8; Autoantibodies; Diagnosis.

Introdução: A associação entre autoanticorpos e o risco de diabetes mellitus tipo 1 (DM1) está bem estabelecida. No entanto, ainda há uma lacuna de conhecimento quantitativo sobre o papel dos autoanticorpos antitransportador de zinco 8 (anti-ZnT8) e sua eficácia no diagnóstico do DM1. **Objetivo**: Realizar uma revisão sistemática e metanálise sobre a associação entre autoanticorpos anti-ZnT8 e o risco de DM1. **Métodos**: Estudos relevantes foram obtidos da base de dados PubMed e analisados usando um modelo de efeitos fixos ou aleatórios. Resultados: De 211 artigos selecionados, 23 estudos foram incluídos e um total de 14.172 pacientes analisados. Nossa análise conjunta revelou que a expressão de autoanticorpos anti-ZnT8 estava significativamente associada a um risco aumentado de desenvolvimento de DM1 em crianças e adultos [OR 1,14 (IC 95% 1,12-1,16)]. **Conclusão**: Nossa revisão sistemática e metanálise fornecem evidências apoiando uma associação significativa entre a positividade para autoanticorpos anti-ZnT8 e um risco aumentado de desenvolvimento de DM1.
Descritores: Diabetes mellitus tipo 1; Transportador de zinco-8; Autoanticorpos; Diagnóstico.

INTRODUCTION

Zinc transporter 8 (ZnT8) antibodies have emerged as a promising biomarker for the diagnosis of type 1 diabetes mellitus (T1DM), a chronic autoimmune disease characterized by the destruction of insulin-producing beta cells in the pancreas, leading to insulin deficiency and hyperglycemia.[1] ZnT8 is a transmembrane protein predominantly expressed in pancreatic beta cells and plays a crucial role in regulating intracellular zinc homeostasis.[2] The presence of ZnT8 autoantibodies, specifically directed against intracellular ZnT8, is considered a hallmark of T1DM, often detectable years before clinical manifestation.[3]

T1DM remains a global health challenge, affecting millions of individuals worldwide, with a peak incidence in childhood and adolescence.[4] The disease is characterized by the progressive loss of beta cells, resulting in insulin deficiency and dysregulated blood glucose levels.[5] While insulin therapy serves as the mainstay of T1DM management, early diagnosis is crucial for timely intervention and potential disease prevention.[6] The identification of individuals at risk for T1DM holds immense promise for early intervention strategies, aiming to halt or delay beta cell destruction and preserve insulin secretion.

The association between ZnT8 antibodies and T1DM has been extensively studied, demonstrating a strong correlation between their presence and the development of the disease.[7] ZnT8 autoantibodies are detectable in the serum of individuals with T1DM, often preceding clinical symptoms by several years, making them valuable tools for early diagnosis and risk stratification.[8] The presence of these antibodies has been shown to have high specificity for T1DM, with minimal detection in other autoimmune conditions.[9] Additionally, ZnT8 autoantibody levels have been shown to correlate with the progression of beta cell destruction, providing a potential biomarker for monitoring disease activity.[10]

Despite the promising role of ZnT8 antibodies in T1DM diagnosis, there is still a need for a comprehensive assessment of their diagnostic accuracy.[11] The existing literature on ZnT8 antibodies is characterized by a substantial heterogeneity in study design, methodology, and patient populations, making it challenging to draw definitive conclusions about their overall performance.[12] A systematic review and meta-analysis are warranted to synthesize the available evidence and provide a robust evaluation of the diagnostic accuracy of ZnT8 antibodies for T1DM.

Therefore, the objective of this manuscript is to conduct a systematic review and meta-analysis of studies that have investigated the diagnostic accuracy of ZnT8 antibodies for T1DM. We will systematically identify, appraise, and synthesize relevant studies to estimate the pooled sensitivity, specificity, and positive and negative predictive values of ZnT8 antibodies for T1DM diagnosis. Additionally, we will explore potential sources of heterogeneity between studies and investigate factors that may influence the performance of ZnT8 antibodies. The findings of this review will provide valuable insights for clinicians and researchers seeking to utilize ZnT8 antibodies in the diagnosis and management of T1DM.

METHODOLOGY

Study identification and selection

A comprehensive search of the PubMed database will be conducted to identify relevant studies for this systematic review and meta-analysis. The search strategy will be developed using a combination of keywords and MeSH (Medical Subject Headings) terms related to ZnT8 antibodies, T1DM, diagnosis. The following search terms will be used: (znt8[All Fields] AND ("antibodies"[MeSH Terms] OR "antibodies"[All Fields])) AND ("diabetes mellitus, type 1"[MeSH Terms] OR "type 1 diabetes mellitus"[All Fields] OR "type 1 diabetes"[All Fields]).

The research strategy involved key electronic databases including PubMed from their 2007 Jan - 2024 June[13].

The search will be restricted to studies published in English, and no date limits will be applied. Additional studies will be identified by screening the reference lists of retrieved articles and relevant reviews.

Eligibility criteria

Studies will be included in the review if they meet the following eligibility criteria: The study is a primary or secondary study that investigates the diagnostic accuracy of ZnT8 antibodies for T1DM. The study reports data on sensitivity, specificity, positive and negative predictive values, or other relevant diagnostic measures for ZnT8 antibodies. The study is available in full text.

Studies will be excluded if they meet any of the following criteria: The study is a case report, review, editorial, letter to the editor, or other non-research article. The study does not report data on the diag-

nostic accuracy of ZnT8 antibodies. The study is not available in full text.

Data extraction

Two reviewers will independently extract data from the included studies using a standardized data extraction form. The data extraction form will include information on study characteristics, patient characteristics, study design, methodology, and diagnostic outcomes. Any discrepancies in data extraction will be resolved through discussion or by consulting a third reviewer.

Quality assessment

The quality of the included studies will be assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.[14] The QUADAS-2 tool is a checklist of 14 items that assesses the risk of bias in studies of diagnostic accuracy. Each item will be scored as "low risk," "high risk," or "unclear risk." The overall quality of each study will be judged as "good," "fair," or "poor" based on the number of high-risk and unclear risk items.

Data synthesis

Pooled estimates of sensitivity, specificity, positive and negative predictive values, and diagnostic odds ratios (OR) will be calculated for ZnT8 antibodies using a random-effects meta-analysis model. The I^2 statistic will be used to assess heterogeneity between studies. If significant heterogeneity is present, subgroup analyses will be conducted to explore potential sources of heterogeneity.

Assessment of the quality of evidence using the metaHUN: a web tool (http://softmed.hacettepe.edu.tr/metaHUN).[15]

Sensitivity analysis

A sensitivity analysis will be conducted to assess the robustness of the meta-analysis findings by excluding studies with high or unclear risk of bias.

Publication bias

Publication bias will be assessed using funnel plots and Egger's test.

Additional analyses

Additional analyses will be conducted to investigate the impact of study characteristics, such as patient age, disease duration, and assay type, on the diagnostic accuracy of ZnT8 antibodies.

CONSIDERATIONS REGARDING RESEARCH ETHICS

Since this investigation constitutes a secondary analysis of existing data, formal ethical approval was deemed unnecessary. This stems from the fact that secondary analyses, by their very nature, do not entail direct engagement with human participants. Consequently, they introduce no new ethical concerns beyond those inherent to the original data collection process.

RESULTS

To ensure transparency and methodological rigor in our study selection, we implemented a comprehensive and structured literature search strategy aligned with the PRISMA[16] framework (**Figure 1**). This process commenced with the identification of 211 potentially relevant articles using a meticulous search strategy that incorporated well-defined keywords and Boolean operators within relevant databases. Following an initial screening based on titles and abstracts, 82 articles were deemed worthy of a more in-depth evaluation. This subsequent phase involved a thorough examination of both abstracts and full-text manuscripts to meticulously assess their alignment with our research focus: the safety and therapeutic potential of topical insulin treatments in ophthalmology. Ultimately, the rigorous application of our predefined eligibility criteria yielded a final selection of 23 pivotal studies for data extraction and synthesis.[17-39]

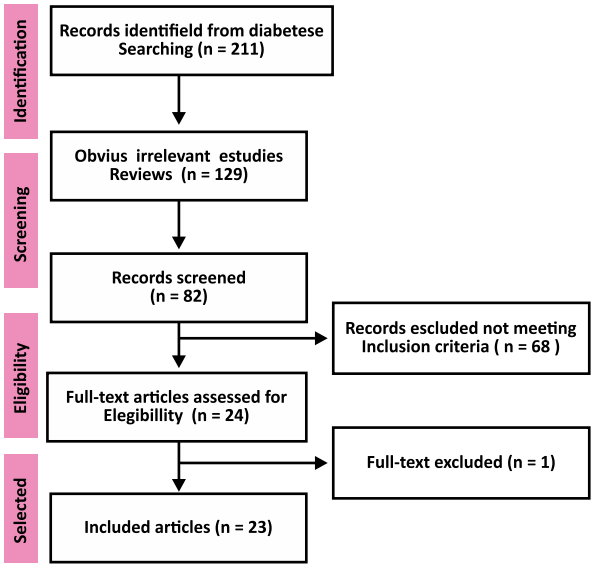

Figure 1. Flowchart of the selection process for the 23 studies included.

This systematic literature review aimed to comprehensively evaluate investigated the diagnostic accuracy of ZnT8 antibodies for T1DM. The twenty-two studies ultimately selected encompassed a diverse range of methodological approaches. These included case-control (n=2), retrospective (n=3), and cross-sectional (n=18) designs. The research originated from a variety of geographical locations and included studies with sample sizes ranging from 16 to 3,165 participants. After general evaluation of the studies, a combined total of 23 studies fulfilled our inclusion criteria for quantitative analysis within the systematic review.

Primary features of the chosen research works

Source 1: Basu M, et al.[17]; Objective: Assessing the autoantibody profile in children with T1DM; Type of study: cross-sectional study; Participants: 92 subjects with T1DM (33 males, 59 females); Results: ZnT8 antibody were present in 20.65% subjects; Conclusion: "T1DM is associated with a high prevalence of autoantibodies and antibody negative T1DM is rare".

Source 2: Lounici Boudiaf A, et al.[18]; Objective: Evaluate the prevalence of islets cells antibodies, glutamic acid decarboxylase, islet antigen type 2, insulin autoantibodies, and ZnT8 antibodies in young Algerian patients with T1DM; Type of study: cross-sectional study; Participants: 160 patients (74 males and 86 females) between 1 and 35 years old, diagnosed with type 1 diabetes; Results: ZnT8ab was positive in 70.3% females and 10.7% in males; Conclusion: "ZnT8ab is a good tool for differential diagnosis of T1DM".

Source 3: Reference: Zecevic-Pasic L, et al.[19]; Objective: to analyze presence of T1DM-related autoantibodies; Type of study: cross-sectional study; Participants: 67 patients with T1DN (40 male and 27 female); Results: 36 (53,7%) cases were positive for Zn-T8; Conclusion: "Zn-T8 antibodies is the most frequently detected and is an important serological marker of T1DM"

Source 4: Reference: Elmaoğulları S, et al.[20]; Objective: to investigate the prevalence of ZnT8A in Turkish children with new onset T1DM; Type of study: cross-sectional study; Participants: 84 patients diagnosed with T1DM and 50 healthy children without any autoimmune diseases; Results: ZnT8A positivity was detected in 58% of the patients with new onset T1DM and 8% of the control group; Conclusion: "ZnT8 measurement should be more widespread for clarifying the etiology in T1DM".

Source 5: Reference: Bhola S, et al.[21]; Objective: To establish the frequency of ZnT8 autoantibodies in black South Africans diagnosed with T1DM, and investigate potential correlations between ZnT8 autoantibody positivity, age at diagnosis, and duration of the disease; Type of study: cross-sectional study; Participants: Patients with T1DM (n = 183) and controls (n = 49) Results: The prevalence of ZnT8 autoantibody positivity was 17.5 % (32 of 183) in participants with T1DM and 27.3 % (6 of 22) in newly diagnosed participants; Conclusion: "The greater the numbers of autoantibodies present in an individual the earlier the age at diagnosis".

Source 6: Reference: Thewjitcharoen Y, et al.[22]; Objective: To describe the characteristics of long-standing T1DM in Thai patients and assess residual beta-cell function with status of pancreatic autoantibodies; Type of study: cross-sectional study; Participants: 20 patients (males 65%); Results: The prevalence rate of anti-ZnT8 was 10%; Conclusion: "Endogenous insulin secretion persists in some patients with long-standing T1DM".

Source 7: Reference: Andersson C, et al.[23]; Objective: To determine the diagnostic accuracy and associations among autoantibodies targeting the three different variants of ZnT8A at position 325, human leukocyte antigen-DQ, and autoantibodies against glutamic acid decarboxylase, insulinoma-associated protein 2, and insulin; Type of study: cross-sectional study; Participants: 3,165 patients with T1DM; Results: ZnT8A was found in 65% of the patients; Conclusion: "Analysis of ZnT8A increased the diagnostic sensitivity of islet autoantibodies for T1DM".

Source 8: Reference: Andersson C, et al.[24]; Objective: To evaluate the diagnostic sensitivity enhancement for T1DM through the combination testing of ZnT8RWQ autoantibodies, GAD65 autoantibodies, insulinoma-associated protein 2 autoantibodies, insulin autoantibodies, and islet cell cytoplasmic autoantibodies with human leukocyte antigen; Type of study: cross-sectional study; Participants: 3165 patients with T1DM; Results: ZnT8RWQA autoantibodies was found in 65% (449/686) of the patients; Conclusion: "The results suggest that ZnT8RWQA is a necessary complement to the classification and prediction of T1DM".

Source 9: Reference: Su YT, et al.[25]; Objective: to report the prevalence, diagnostic utility, and clinical characteristics of ZnT8A in children with T1DM; Type of study: cross-sectional study; Participants: 268 children (130 boys, 138 girls) newly diagnosed with T1DM; Results: ZnT8A was detected in 117 patients (43.7 %); Conclusion: "ZnT8A testing can diagnose up to 12 % more patients with T1DM along with three other antibodies".

Source 10: Reference: Fakhfakh R, et al.[26]; Objective: to evaluate the relationships between ZnT8-Ab, ZnT8 coding gene (SLC30A8) promoter polymorphism, and T1DM risk in newly diagnosed children; Type of study: cross-sectional study; Participants: ZnT8-Ab were measured in the serum of T1DM newly affected children (n = 156); Results: ZnT8-Ab was detected in 66/156 (42.3%); Conclusion: "ZnT8-Ab appears as a relevant diagnostic marker for T1DM in Tunisian children".

Source 11: Reference: Fuentes-Cantero S, et al.[27]; Objective: to evaluate the diagnostic efficiency of ZnT8 autoantibodies in diagnosis of T1DM in pediatric patients; Type of study: retrospective study; Participants: 80 patients under 16 years of age with suspected T1DM; Results: ZnT8A obtained the most significantly global diagnostic accuracy (0.75); Conclusion: "The results obtained indicate a higher efficiency of anti-ZnT8 autoantibodies for the diagnosis of T1DM in pediatric patients".

Source 12: Reference: Baumann K, et al.[28]; Objective: to evaluate autoantibodies anti-ZnT8 in schoolchildren from the general population and in people with autoimmune diabetes; Type of study: cross-sectional study; Participants: 137 schoolchildren with T1DM, 102 people at T1DM onset, 88 people with latent autoimmune diabetes in adults and 119 people with type 2 diabetes; Results: ZnT8 autoantibody positivity was found in 18% of autoantibody-positive schoolchildren, ZnT8 autoantibodies were found in 56% of people with T1DM, ZnT8 autoantibodies were detected in 10% of people with latent autoimmune diabetes in adults; Conclusion: "ZnT8 autoantibodies are useful markers for prediction of type 1 diabetes in a general population".

Source 13: Reference: Rogowicz-Frontczak A, et al.[29]; Objective: to assess the prevalence ZnT8 autoantibodies, other diabetes-related autoantibodies and clinical manifestation of type 1 diabetes in adults; Type of study: cross-sectional study; Participants: 119 patients with T1DM; Results: 45.4% T1DM < 35 and 34% T1DM ≥ 35 subjects were positive for ZnT8 autoantibodies; Conclusion: "ZnT8 autoantibodies positivity is related to higher title and more frequent occurrence of multiple diabetes-related".

Source 14: Reference: Gomes KF, et al.[30]; Objective: to evaluate the relevance of ZnT8A for T1DM diagnosis; Type of study: case-control study; Participants: 629 patients with T1DM and 651 controls; Results: Znt8A was detected in 68.7% of recent-onset T1DM patients and 48.9% of the entire patient cohort; Conclusion: "ZnT8A detection increases T1DM diagnosis rate even in mixed populations".

Source 15: Reference: Kawasaki E, et al.[31]; Objective: to determine the prevalence and role of autoantibodies to ZnT8A in fulminant form, acute-onset form, and slow-onset form of Japanese patients with T1DM; Type of study: cross-sectional; Participants: 196 new-onset patients with T1DM, 85 fulminant, 81 acute-onset, and 30 slow-onset T1DM type 1; Results: ZnT8A were detected in 58% patients with acute-onset and 20% with slow-onset type 1 diabetes; Conclusion: "ZnT8A are an additional useful marker for acute-onset T1DM".

Source 16: Reference: Garnier L, et al.[32]; Objective: Evaluate the added value of screening anti-ZnT8 antibodies for the diagnosis of T1DM within a large cohort of both children and adults; Type of study: Retrospective 2-year study; Participants: 516 patients (215 children, 301 adults); Results: 110 patients were ZnT8A-positive; Conclusion: "ZnT8A should be included in routine evaluation at diabetes onset and is a valuable biological marker to classify newly-diagnosed diabetics".

Source 17: Reference: Andersen MK, et al.[33]; Objective: to assess the prevalence of ZnT8 autoantibodies in patients with adult-onset diabetes; Type of study: cross-sectional; Participants: 264 T1DM and 294 latent autoimmune diabetes in adults; Results: ZnT8A were significantly more prevalent in latent autoimmune diabetes in adults (34.3%) compared to adult-onset T1DM (18.7%). Conclusion: "ZnT8A were more common and more persistent in patients with latent autoimmune diabetes in adults compared to adult-onset T1DM".

Source 18: Reference: Salonen KM, et al.[34]; Objective: to define the characteristics of humoral autoimmunity against ZnT8 in children and adolescents with newly diagnosed T1DM; Type of study:

cross-sectional; Participants: 2,115 subjects <15 years of age; Results: ZnT8 antibodies were detected in 63% of the cases; Conclusion: "Antibodies for ZnT8 is related to age and metabolic status at T1DM diagnosis".

Source 19: Reference: Yang L, et al.[35]; Objective: to evaluate the utility of ZnT8A for diagnosis of autoimmune T1DM in Chinese relative to other autoantibody markers; Type of study: cross-sectional study; Participants: 539 T1DM; Results: ZnT8A were present in 24.1% (130 of 539) of patients with T1DM; Conclusion: "ZnT8A is an independent marker for T1DM in Chinese".

Source 20: Reference: Petruzelkova L, et al.[36]; Objective: to evaluate prevalence of autoantibodies to ZnT8 in Czech children at the onset of T1DM; Type of study: case-control study; Participants: 227 children with newly diagnosed Type 1 diabetes and from 101 control children without diabetes; Results: ZnT8 autoantibodies were detected in 163/227 (72%) of children at T1DM onset and in 1/101 (1%) of the control subjects.

Conclusion: "Measurements of ZnT8 autoantibodies are important for Type 1 diabetes diagnosis and should be included in the panel of autoantibodies tested at the onset of T1DM".

Source 21: Reference: Fabris M, et al.[37]; Objective: to investigate ZnT8A as a complement to the current T1DM; Type of study: retrospective multicentre study; Participants: 213 T1DM paediatric patients; Results: ZnT8A showed positive results in 106/213 (49.8 %) T1DM patients; Conclusion: "Study confirms ZnT8A as an important additional and independent diagnostic marker of T1DM".

Source 22: Reference: Araujo DB, et al.[38]; Objective: to investigate the prevalence of ZnT8 autoantibodies in patients with T1DM; Type of study: cross-sectional study; Participants: 72 T1DM patients; Results: The prevalence of ZnT8A was of 24%; Conclusion: "ZnT8 autoantibodies is observed in non-Caucasian patients with T1D, even years after the disease onset".

Source 23: Reference: Niechciał E, et al.[39]; Objective: To assess the prevalence of ZnT8 autoantibodies in children and adults with T1DM onset; Type of study: cross-sectional study; Participants: 367 patients (218 children; 149 adults) at the T1DM onset; Results: ZnT8 autoantibodies (81.1%) in youth; Conclusion: "ZnT8 autoantibodies are associated with more acute diabetes onset".

Prevalence of ZnT8 antibodies

The studies reported a variable prevalence of ZnT8 antibodies in individuals with T1DM, ranging from 10.00 % to 90.00 %. The prevalence appeared to be influenced by factors such as age, ethnicity, and disease duration.

Diagnostic accuracy of ZnT8 antibodies

Some of the studies evaluated the sensitivity, specificity, and positive and negative predictive values of ZnT8 antibodies for T1DM diagnosis. The overall diagnostic performance varied across studies.

Some studies explored the association between ZnT8 antibody positivity and specific T1DM subtypes or disease progression.

This heterogeneity may have limited the ability to draw definitive conclusions about the overall diagnostic accuracy of ZnT8 antibodies.

Methodological Evaluation and Risk of Bias

The quality of the included studies will be assessed using the QUADAS-2 tool (**Table 1**).

Meta-Analysis of ZnT8 Autoantibody Positivity and T1DM

This study conducted a comprehensive meta-analysis to evaluate the association between ZnT8 autoantibody positivity and T1DM. We employed a systematic search strategy, encompassing all relevant association studies published between February 2007 and May 2024, identified through a thorough PubMed search.

The analysis yielded a total of 23 original studies investigating the relationship between ZnT8 autoantibodies and T1DM. These studies collectively included a substantial sample size of 14,172 subjects. To quantify the strength of this association, we estimated the relative risk for T1DM using allelic OR.

Reassuringly, the analysis revealed no statistically significant heterogeneity in the genotypic distribution across the included studies. This was confirmed by both the Woolf test (v2 = 43.36, df = 31, P = 0.05) and the Higgins statistic (I^2 = 28.1%). Additionally, to assess for potential publication bias, we employed the conservative Egger's regression asymmetry test and found no evidence of bias (P = 0.41).

Given the absence of heterogeneity and publication bias, we opted for a Mantel-Haenszel procedure (fixed effects model) to generate a pooled OR for the association between ZnT8 autoantibody positivity and T1DM. This analysis yielded a statistically significant pooled OR of 1.14 (95% CI: 1.12-1.16 - P = 0.05) (**Figure 2**).

Tabela 1. General Characteristics of the Studies Included

Author	year	Study type	Participants	Results %	Risk of bias category NIH/ QUADAS)-2 risk
Basu M, et al.[16]	2020	cross-sectional	92	20.65	Good quality /Low
Lounici Boudiaf A, et al.[17]	2018	cross-sectional	16	90.00	Good quality /Low
Zecevic-Pasic L, et al.[18]	2023	cross-sectional	67	53.7	Good quality /Low
Elmaoğulları S, et al.[19]	2018	cross-sectional	134	64.00	Good quality /Low
Bhola S, et al.[20]	2021	cross-sectional	183	64.80	Good quality /Low
Thewjitcharoen Y, et al.	2020	cross-sectional	20	10.00	Good quality /Low
Andersson C, et al.[21]	2013	cross-sectional	3,165	65.00	Good quality /Low
Andersson C, et al.[22]	2011	cross-sectional	3,165	65.00	Good quality /Low
Su YT, et al.[23]	2024	cross-sectional	268	43.70	Good quality /Low
Fakhfakh R, et al.[24]	2022	cross-sectional	156	42.30	Good quality /Low
Fuentes-Cantero S, et al.[25]	2024	Retrospective	80	75.00	Low quality /High
Baumann K, et al.[26]	2021	cross-sectional	327	84.00	Good quality /Low
Rogowicz-Frontczak A, et al.[27]	2018	cross-sectional	119	79.40	Good quality /Low
Gomes KF, et al.[28]	2017	case-control	1,280	68.70	High quality /High
Kawasaki E, et al.[29]	2011	cross-sectional	392	78.00	Good quality /Low
Garnier L, et al.[30]	2018	Retrospective	516	21.31	Low quality /High
Andersen MK, et al.[31]	2013	cross-sectional	558	53.00	Good quality /Low
Salonen KM, et al.[32]	2013	cross-sectional	2,115	63.00	Good quality /Low
Yang L, et al.[33]	2010	cross-sectional	539	24.1	Good quality /Low
Petruzelkova L, et al.[34]	2014	case-control	328	73.0	High quality /High
Fabris M, et al.[35]	2015	Retrospective	213	49.8	Low quality /High
Araujo DB, et al.[36]	2014	cross-sectional	72	24.00	Good quality /Low
Niechciał E, et al.[37]	2018	cross-sectional	367	81.10	Good quality /Low

Limitations

The studies included in this review exhibited heterogeneity in terms of study design, participant characteristics, and assay methods used for ZnT8 antibody detection.

DISCUSSION

Our systematic review and meta-analysis provides robust evidence supporting a significant association between anti-ZnT8 autoantibody positivity and an increased risk of T1DM development. In this study, we investigate the potential of ZnT8 autoantibody positivity as a diagnostic marker for T1DM, a chronic autoimmune condition characterized by pancreatic β-cell destruction. Given the established link between autoimmunity and increased susceptibility to other chronic immune disorders, a comprehensive evaluation of ZnT8's diagnostic accuracy in T1DM is warranted. This knowledge may prove instrumental in the development of future prophylactic and therapeutic strategies. To this end, we conducted a meticulous systematic review and meta-analysis, employing a rigorously defined search strategy across pertinent databases. By leveraging well-established Boolean operators and a curated selection of keywords, we aimed to extract the most relevant data on ZnT8's diagnostic efficacy in T1DM.

Prior investigations exploring the link between ZnT8 autoantibody positivity and T1DM yielded conflicting results.[40] To address this heterogeneity, we conducted a comprehensive meta-analysis encompassing 22 studies. This analysis revealed a statistical-

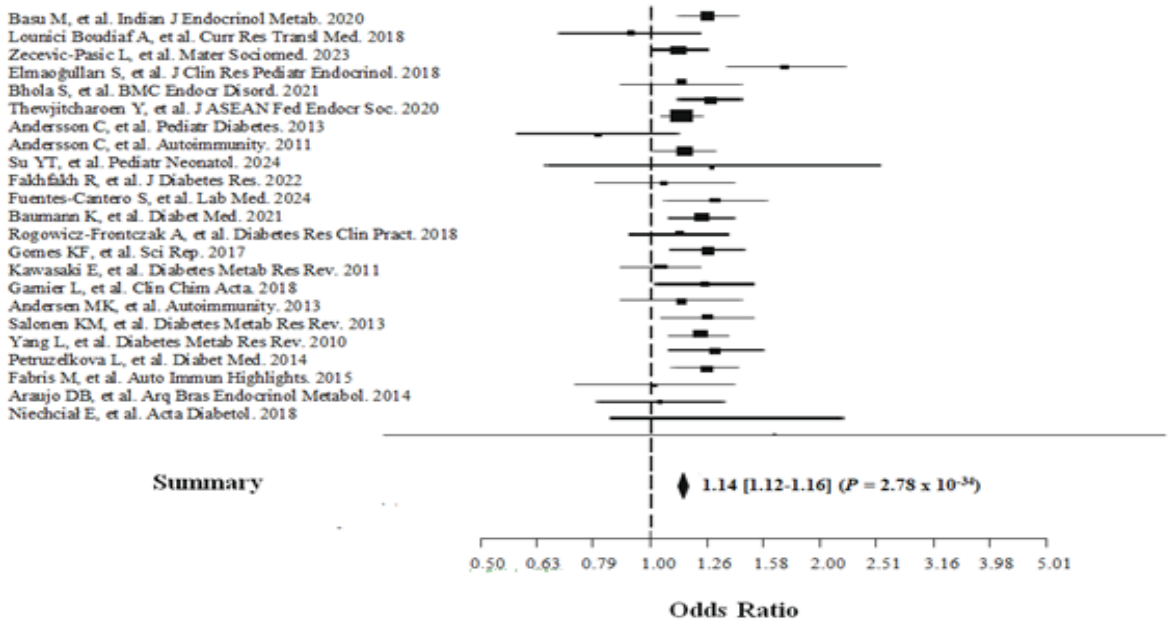

Figure 2. This forest plot depicts the association between the Znt8 gene and T1DM across various studies. Each study is represented by a square. The area of the square reflects the precision of the estimated effect size (OR). A larger square indicates a more precise estimate, stemming from a lower standard error. The horizontal line extending from the square represents the 95% confidence interval (CI) for the effect size. The overall association across studies is summarized by a diamond. The width of the diamond is inversely proportional to its standard error, again conveying the level of precision. The horizontal edges of the diamond represent the lower and upper limits of the pooled 95% CI for the summary OR.

ly significant elevation in ZnT8 positivity rates within T1DM cohorts compared to control groups, particularly within case-control studies. Notably, despite inherent variations amongst the included studies, analyses demonstrated a lack of undue influence from any single study on the overall meta-analysis OR. Collectively, these findings underscore the robustness and reliability of the conclusions drawn from this meta-analysis, strengthening the evidence for ZnT8 as a potential diagnostic biomarker in T1DM.

This systematic review underscores the value of routine islet autoantibody screening, particularly anti-ZnT8 antibodies, in identifying individuals at risk of developing T1DM. While a previous meta-analysis advocated for a similar approach,[41] the present study further strengthens the body of evidence supporting the role of ZnT8 antibodies in T1DM diagnosis. This, in turn, enhances our understanding of the contribution of autoimmunity to T1DM development in susceptible populations.

Early identification of T1DM cases enables prompt intervention to mitigate the risk of diabetic ketoacidosis and other T1DM-related complications,[20,42] as corroborated by a systematic review highlighting the increased risk of autoimmune comorbidities among T1DM patients.[17] Additionally, these strategies can also improve metabolic control.[41]

This systematic review and meta-analysis are not without limitations. Firstly, the observed heterogeneity among the included studies, potentially arising from variations in sample size, warrants cautious interpretation of the findings. Additionally, despite the substantial sample size, pooled estimates adjusted for covariates could not be extracted. To our knowledge, this represents the first systematic review and meta-analysis investigating the association between ZnT8 autoantibodies and T1DM in a diabetic population.

Despite these limitations, our comprehensive systematic review and meta-analysis provide compelling evidence supporting the robust association between anti-ZnT8 autoantibody positivity and an increased risk of T1DM development. The findings from this meta-analysis underscore the value of anti-ZnT8 autoantibodies as a diagnostic biomarker for T1DM, offering a promising tool for early identification and intervention strategies.

CONCLUSION

Our systematic review and meta-analysis provides robust evidence supporting a significant association between anti-ZnT8 autoantibody positivity and an increased risk of T1DM development. Additionally, studies investigating the cost-effectiveness of routine islet autoantibody screening, including anti-ZnT8 antibodies, are needed to inform clinical practice guidelines.

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WELCOMING AND GUIDANCE FROM A MULTIDISCIPLINARY TEAM TO CAREGIVERS OF PATIENTS WITH TYPE 1 DIABETES MELLITUS

ACOLHIMENTO E ORIENTAÇÃO DE UMA EQUIPE MULTIPROFISSIONAL A CUIDADORES DE PACIENTES COM DIABETES MELLITUS TIPO 1

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This article aims to describe an experience of listening and providing care in a multidisciplinary team to children with Type 1 Diabetes Mellitus and their caregivers, carried out at the medical specialty outpatient clinic at Faculdade Evangélica Mackenzie do Paraná. The importance of welcoming and guiding caregivers and patients was realized, promoting self-care.
Keywords: Diagnosis; Type 1 Diabetes Mellitus; Child caregivers

Este artigo tem como objetivo descrever uma experiência de escuta e atendimento em equipe multiprofissional a crianças com Diabetes Mellitus tipo 1 e seus cuidadores, realizada no ambulatório de especialidades médicas da Faculdade Evangélica Mackenzie do Paraná. Percebeu-se a importância de acolher e orientar cuidadores e pacientes, promovendo o autocuidado.
Palavras-chave: Diagnóstico; Diabetes Mellitus Tipo 1; Cuidadores de crianças

INTRODUCTION

According to the International Diabetes Federation (IDF) website, Type 1 Diabetes Mellitus (T1DM) is a condition that occurs when the pancreas can no longer produce insulin, which results in hyperglycemia. This disease requires insulin therapy for the entire life to avoid damage to several organs[1]. The incidence of T1DM is increasing, what is concerns public heath since patients will have T1DM longer, enduring mote exposure to risks and long-term complications[2,3]

According to the Brazilian Diabetes Society (SBD), T1DM affects the immune system of some people, mistakenly attacking the body's beta cells, thus little or no insulin is released into the body[4]. According to the Ministry of Health website, the main signs of T1DM are the urge to urinate several times, constant thirst, weight loss, fatigue, nervousness, frequent hunger, mood changes, among others. Two consequences that affect patients with T1DM are hyperglycemia, which occurs due to an increase in blood glucose. In the long term, if left untreated, this high level of blood glucose can cause damage to the body and lead to the failure of several organs. The other consequence/complication is the hypoglycemia, characterized by low blood glucose, which is accompanied by some symptoms, such as sweating, tremors, dizziness, tachycardia, drowsiness, among others, and in more serious cases can lead to convulsions and loss of consciousness[5]. According to the SBD hypoglycemia is considered a serious and frequent complication in patients with T1DM[4]. For this reason, it is important to monitor deal with the disease by patients, in order to improve quality and avoid serious consequences.

BUT WHAT ABOUT WHEN PATIENTS ARE CHILDREN?

How is the treatment given, or how is it possible to treat a child diagnosed with T1DM? As we will see in this text, the participation of caregivers is important, as children often do not have the age, understanding and necessary dexterity regarding treatment, such as the application of insulin.

The study was carried out in a qualitative evaluation after listening to caregivers of children with DM1 treated in the DOCE Extension Project, enrolled in the medical specialties outpatient clinic of Faculdade Evangélica Mackenzie do Paraná [FEMPAR], coordinated by professor Dr. Mirnaluci Paulino Ribeiro Gama.

The service experience has been ongoing since July 2023, when an innovative multidisciplinary group was implemented in the sector, including a licensed psychologist and nutritionist.

Some children aged between 8 (eight) and 12 (twelve) years old, and their caregivers were interviewed, among some who had successful treatment and others who did not. It was observed that, in some of the cases where there was no success in treatment, one of the possible reasons is the denial or rejection of treatment by the caregiver.

This article aims to address the experience of caregivers of patients with Type 1 Diabetes Mellitus (DM1) regarding treatment, based on psychological care, together with the multidisciplinary team.

Being a T1DM caregiver is definitely not easy. Parental ability to manage T1DM may impact their ability to cope with the unique demands that they experience daily. At the same time, these parents need to have the ability to promote age-appropriate T1DM self-management behaviors in their children to develop positive outcome expectancies[3].

It's also very difficult to understand the ideal glucose control manner. A very interesting review researched the link between parenting styles and glycemic control and adherence and concluded that higher family cohesion, parental warmth, and an 'authoritative' style of parenting are related to better outcomes in T1DM, whereas higher general family conflict, parental restrictiveness, criticism and an 'authoritarian' style of parenting predicted worse outcomes in those patients[6].

These patients and their caregivers reported their experience and trajectory with the diagnosis, disease and treatment related to DM1. Reports that range from everything being fine with the treatment and diagnosis, where the child also understands and helps, to the opposite, where some caregivers report not administered insulin [normally at night] for fear that the child will have hypoglycemia.

Debray (1995)[7], writes that:

> Despite the quality of the medical treatment to be carried out on your own, there will never be a guarantee of protection against hypo- or hyperglycemic illnesses, which can, depending on the case, lead to a coma. This is to say that the disease in itself constitutes a very heavy burden for its sufferer; burden that can have a secondary traumatic aspect for the patient and their environment. (p.30)

As a multidisciplinary team composed of diabetologist, residents in endocrinology, psychologist and nutritionist, we noticed the presence of caregivers of patients with T1DM, in health consultations, regardless of the patient's age, however in this text we will approach the caregivers of patients aged between eight (8) and twelve (12) years old, regardless of gender.

After the diagnosis of T1DM, several changes occur in the patient's life and perhaps even more so when the patient is a child, as it also brings about a change in the lives of their caregivers. For exemple, it is necessary to adjust the diet [which ends up influencing the diet of all family members who live with the patient with DM1], in the routine, in addition to the administration of insulin, which is often carried out by the child's caregiver. with T1DM. The parents understand, since the diagnosis that they must take on the burden of all T1DM challenges and also negotiate the many developmental challenges that their children encounter[3].

We observed in case discussions, as a multidisciplinary team, that some of these caregivers present difficulties and/or resistance, as well as doubts and anxieties, regarding the treatment of T1DM in their children, in most reports by I am afraid that the child will have hypoglycemia, which, as already mentioned, is a serious complication of T1DM , so, on their own, some caregivers avoid administering insulin according to medical advice, especially when their children will not be under their watchfulness.

Patton *et al* evaluated the fear of hypoglycemia in 24 parents of T1DM children and showed that 63% of them reported significant worry about their kids having hypoglycemia during sleep and 46% when away from a parent. Also, those parents reported that 38% were worry about their child having a seizure and that no one would be able to help their child during hypoglycemia[8].

Regarding the trajectory of T1DM that patients and their caregivers deal with let's start with the diagnosis of DM1. This often comes with a variety of negative feelings, such as uncertainty, doubts, fear, rejection, anxiety, among others, often because it is not an expected diagnosis, or because the patient or caregiver does not have much information about the disease. According to Pera (2012)[9],

> [...] the diagnosis of DM1 is accompanied by an intense emotional burden for all family members, because it presents itself unexpectedly, abruptly and with significant severity. Furthermore, aspects of the treatment such as insulin dependence and risks of hypoglycemia can cause rejection and fear.

But even after years of diagnosis we can observe through the speech of these caregivers that they still present a lot of doubts and insecurities about the amount of insulin they should administrate according to what their child consumed [the dietitian does a works with counting carbohydrates] and according to the glycemia at the mealtime. What if blood sugar drops too low? What if blood sugar rises too high? Questions that are repeated in different health care appointments, as well as several attempts to address these issues, seeking other sources and ways of understanding T1DM.

Vidotti (2019)[10], writes that [...]

> when the diagnosis of a chronic disease is carried out early in life, there are effects on both subjectivity of the parents and the baby". [...]. (p.17).

When they become clearer about the disease and what is happening to the patient, many caregivers express different ways of reacting. We have reports of caregivers who ask more questions during consultations, and others who say they seek more information on the internet.

On the one hand, this movement by caregivers may be good, but dangerous on the other, as each patient reacts differently to T1DM treatment, what works for one does not necessarily work for everyone and this cannot be denied, caregivers They need to be aware of this information, because in an attempt to help their children, they may not do so, causing consequences for the treatment and consequently for the patient.

Ferreira writes that[11],

> Dealing with a chronic illness requires the patient to make use of psychic resources, so that they can use strategies to face the illness and minimize its effects. These effects are not similar and the form of expression is linked to each person's personal history. From this point of view, coping with the disease can occur by accepting the limitations imposed and the disease having a limited and controllable place. Not accepting the disease or its treatment, the diabetes then begins to be experienced as a loss of one's own SELF, generating decompensation of the disease. (2009, p.30)

Therefore, it is important that the multidisciplinary team can welcome these caregivers and patients, listening to the anguish that appears, trying to reduce it and try to resolve their doubts, in addition to explaining about diabetes education, which, as mentioned by Vidotti (2019)[10]

> "[...] it is considered one of the important pillars of treatment management, as it offers information and knowledge about DM1. It brings together a range of activities that range from learning techniques and individual adjustments (goals, amount of insulin and diets) to contributing to coping with the disease and adapting to the new condition, with the participation of the patient and family as its horizon. in long-term treatment." (p.20)

Fingermann (2022, p.27)[12], states that "The body shows itself in these complaints with its limits or excesses that hinder each person's subjective performances, their capacity for bonds and their propensity to enjoy". Thus, the attempt by caregivers to "do the treatment" of DM1 on their own can be understood in two ways, initially.

One of them is excessive care, also understood as overprotection, an extra way of doing something for the patient's care and treatment, but this excess can generate negative feelings in the patient, such as feeling suffocated, in addition to a possible lack of autonomy in self-care and treatment.

For the caregiver, it can be seen as care for their child, a way to protect them, help them and alleviate the caregiver's possible feeling of guilt, as Vidotti (2019)[10] writes, that when referring to the parents of the patient with chronic illness,

> [...] highlights that attitudes of overprotection towards children, feelings of guilt, rejection or denial of the diagnosis, shock and disbelief are common, while on the children's side, one can find fixation in a position of dependence in relation to their parents , rebellion or opposition to medical interventions. (p.18)

The second possible way to understand this attitude of caregivers is through the possible deprivation of care, when the caregiver says that they do not administer the amount of insulin recommended by doctors, in a logic similar to overprotection, believing that they are doing the best for the patient, out of fear, that causes hypoglycemia, for example.

However, this attitude can have a negative effect on the patient, in the physical field, such as the possibility of hospitalization, and also in the emotional sphere, with no space for the patient's subjectivity, preventing him from possible autonomy, which can generate feelings of incapacity and worthlessness, and can be understood as denial or rejection of treatment by the caregiver[11].

Soler (2022, p.64-5)[13] writes that the mother "[..] like all love, she would sin by "partiality". And he continues, "[...]

things were limping, on the one hand, due to lack – castration – and, on the other, due to excess: the imperialism of drives, always partial, that never give up, not even at the price of displeasure. [...]".

Children with T1DM, according to Vidotti (2019), noticing emotional changes in their caregivers due to the repercussions that T1DM brings, they may feel guilty for their parents' suffering and in this way they may also interpret the treatment as a punishment. And in parents, the possible feeling of guilt for having caused the illness in their child can produce feelings of guilt, "[...]despair, impotence, aggressiveness and emotional reactions such as anxiety and depression".[10]

The Multicenter Study on T1DM in Brazil[14], "[...]evaluated, from the parents' perspective, the impact of T1DM on family functioning and children's educational practices, as well as the parents' health-related quality of life and the relationships between psychosocial variables and care with diabetes." This research was carried out with 1079 parents of patients with DM aged up to 18 years.

One piece of information that appears in the research regarding parents is that "[...] 36% reported discomfort and 51.2% anxiety and depression, with prevalence in mothers' reports"[14].

Other research cited was carried out in Tajikistan (2017)[15] and one thing that emerged was that parents of children and young people with T1DM and patients recognize the importance of using insulin, "[...], but still reported that They stopped making some injections, especially when there was a complaint from the child [...]".

Another search was carried out by Sand, Blom, Forsander and Lundin in 2017, who were interested in family dynamics when a child has a chronic illness. In this research, the main category appears as the "[...]sudden loss of the child's health, which produced a rupture in the bond between parents and children, as well as the feeling of guilt in parents for having failed to protect their children. (...)"[16].

Also, parents have to deal with other demands on T1DM children, which includes painful and anxiety-inducing procedures (such as injections and finger sticks), self-regulation of eating, prevention, maintaining physical activity habits and recognition and remediation of hypoglycemia.[3]

To conclude, it is necessary to understand that, for both the patient and their caregivers, receiving the diagnosis of T1DM requires a long process of elaboration as a way of overcoming the various stages of grief, as it is a diagnosis that generates a feeling of profound loss.[17]

CONCLUSION

We can then see that the diagnosis of a chronic disease, such as T1DM, affects not only the patient, but also those around them. We understand the importance of guiding caregivers and patients and welcoming them whenever necessary, helping them with care and treatment autonomy, strengthening them to deal with the disease, the changes it brings and the necessary treatment, in the best possible way.

The conclusions were based on a qualitative assessment of care for a small number of pediatric patients being treating diabetes. This article does not aim to close this topic, new studies must continue to be carried out.

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