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ORIGINAL ARTICLE

Prevalence of other autoimmune diseases in children and adolescents with type 1 *diabetes mellitus*

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Abstract

Objective: To evaluate the prevalence of autoimmune markers associated with beta-pancreatic cells, autoimmune thyroid diseases and celiac disease through research of anti-glutamic acid decarboxylase antibodies (GADA), antiislet cell antibodies (ICA), anti-insulin antibodies (IAA), anti-tyrosine phosphatase (IA-2A), anti-transglutaminase antibodies (anti-tTG), anti-endomysium anti-transglutaminase antibodies (EMA), immunoglobulin A (IgA), thyroperoxidase antibodies (anti-TPO) and thyroglobulin antibodies (anti-TG), in addition to related hormonal measurements in children and adolescents with type 1 diabetes mellitus. **Methods:** Observational, longitudinal study, with retrospective data collection by reviewing the medical records of 92 children and adolescents, diagnosed with DM1 between January 2014 and December 2016, seen at a Pediatric Endocrinology service of a tertiary hospital in Curitiba- PR. **Results:** At the diagnosis of DM1, the prevalence of anti-GAD antibody positivity was 69.6% (n = 79), ICA 42.9% (n = 70), IAA 32.8% (n = 64), IA2 of 60.6% (n = 33). Positivity for markers of other associated autoimmune diseases was 27.4%, EMA 6.0% (n = 50), anti t-TG IgA 12.5% (n = 8), anti-TPO 13, 1% (n = 84) and 22.9% anti-TG (n = 83). Autoimmune thyroid diseases were the most frequently found comorbidity at diagnosis (22.1%). Among patients with positivity for antithyroid antibodies, 19% developed clinical hypothyroidism. **Conclusions:** Patients with DM1 have a high prevalence of autoimmunity. The importance of screening for such diseases to diagnosis and clinical follow-up is reinforced, in order to assist in predicting the development of associated diseases and their complications, as well as providing adequate treatment.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is the most common chronic endocrine and metabolic disorder in children and adolescents and the third most prevalent chronic disease in children.[1,2] It is characterized by obliteration of beta-pancreatic cells and consequent insulin production decrease.[3] T1DM is subdivided into types 1A (immune-mediated) and 1B (idiopathic); the first has been associated with the presence of islet cell antibodies (ICA), anti-glutamic acid decarboxylase (GAD) autoantibodies, islet antigen-2 autoantibodies (IA-2A), anti-insulin antibodies (AIA), and anti-Zinc transporter 8 protein antibodies (ZnT8A).[4]

The immune process that gradually develops in the beta-pancreatic cells of individuals with the disease may affect other organs and cause autoimmune disorders, which are more frequent among individuals with diabetes when compared to the general pediatric population.[5] The disorders with the highest prevalence are Hashimoto's disease and Graves' disease (15-30%), jointly referred to in this study as autoimmune thyroid diseases (AITD), followed by celiac disease (1-16%), autoimmune gastritis/pernicious anemia (5-10%), vitiligo (2-10%), and Addison's disease (0.5%)[6]

Considering the pathophysiological of autoimmunity and the impairment in glycemic and lipid control that may result from the coexistence of T1DM and other autoimmune disorders, this study aimed to assess the prevalence of autoimmune markers associated with pancreatic beta cells, thyroiditis, and celiac disease in children and adolescents with T1DM at the time of diagnosis and during clinical follow-up. Secondarily, it aimed to establish associations between clinical and demographic characteristics of patients and autoimmunity, as well as predisposition associations between diseases based on the presence of certain antibodies.

MATERIALS AND METHOD

This longitudinal observational study included retrospective data from the medical records of children and adolescents seen at the Pediatric Endocrinology Outpatient Clinic or admitted to a tertiary referral hospital in Curitiba, Brazil.

The study included patients under 16 years of age seen in the first visit to the diabetes outpatient clinic of the Pediatric Endocrinology Unit or admitted to the hospital's pediatric care units from January 2014 to December 2016. Patients with incomplete or inconclusive medical records were excluded.

The following variables were analyzed: (a) sociodemographic characteristics (current age and at the time of diagnosis of T1DM; sex; place of birth); (b) clinical characteristics (emotional stress factors related to the diagnosis of T1DM; associated autoimmune disorders at the time of diagnosis of T1DM and progress during clinical follow-up; patient and family history of comorbid conditions); workup data (blood gas pH and bicarbonate levels; ketoacidosis and severity at diagnosis; thyroid function – TSH and free T4; pancreatic autoantibodies – anti-GAD, ICA, IA-2A and AIA, anti-thyroid autoantibodies – thyroid peroxidase (TPO) antibodies, and anti-thyroglobulin (TG) antibodies; autoantibodies related to celiac disease - anti-endomysial antibodies (EMA) and anti-tissue transglutaminase (anti-tTG) antibodies; serum levels of total immunoglobulin A (IgA).

Family history of autoimmune disease was considered positive only when known cases were reported in first- or second-degree relatives (parents, siblings, grandparents). Ketoacidosis at diagnosis and severity were defined according to the ISPAD[7] criteria: mild when pH < 7.3 or bicarbonate < 15 mmol/L; moderate when pH < 7.2 or bicarbonate < 10 mmol/L; and severe when pH < 7.1 or bicarbonate < 5 mmol/L. Serum TSH (reference value: 0.35 - 4.94μlU/mL), free T4 (0.7 - 1.48ng/dL) levels and levels of anti-thyroid autoantibodies TPO (RV < 5.61IU/mL) and TG (RV < 4.11IU/mL) were obtained with chemiluminescent assays.

Diagnosis of autoimmune thyroid disease was confirmed when one or more thyroid autoantibodies were present. Patients were diagnosed with clinical hypothyroidism when they had autoimmune thyroid disease along with low free T4 and/or high TSH levels and/or goiter on physical examination. A diagnosis of subclinical hypothyroidism was made when patients had normal T4 and slightly elevated TSH levels.

As for pancreatic autoantibodies, anti-GAD autoantibody levels were obtained via tests with different enzyme-linked immunosorbent assay (ELISA) kits, with positive reference values > 5UI/mL or > 10UI/mL; ICA levels were obtained via indirect immunofluorescence methods (RV < 1: 4) and ELISA ("non-reactive" RV); IA-2A levels were measured with radioimmunoassay (RV < 10UI/mL) and ELISA (RV < 8UI/mL) kits; and AIA levels were obtained with radioimmunoassay (RV < 0.4U/mL), (RV < 7.3%), and ELISA kits (RV < 8.6%).

ELISA kits were used to measure anti-tTG IgA (RV < 20U/mL) and IgG (RV < 20U/mL) levels; levels of anti-endomysial antibodies were measured by indirect immunofluorescence (RV "negative"). Total serum IgA was measured with turbidimetry, with specific reference values for each age group (RV 0 to 3 months: 1 - 34 mg/dL; > 3 months to 1 year: 8 - 91 mg/dL; > 1 year to 12 years: 21 - 282 mg/dL; > 12 years: 65 - 421 mg/dL). The tTG-IgG test for celiac disease was performed in patients with IgA deficiency at the time of T1DM diagnosis.

Pancreatic autoantibody levels were tested at an accredited laboratory. Testing was not possible during parts of the time period covered by the study; therefore, not all patients had pancreatic autoantibody levels measured. The same applied to anti-endomysial antibodies.

The data collected between July 2016 and August 2017 was stored on a Microsoft Excel® spreadsheet and exported for statistical analysis onto software package Statistica v 10.0-Statsoft®. Student's t-test was used to estimate the differences between continuous variables following a normal distribution. Variables with an asymmetric distribution were analyzed using the Mann-Whitney test, the Friedman test with Mann-Whitney

post-hoc test. Differences between categorical variables were estimated with Fisher›s exact test and Pearson›s chi-squared test. For all tests, a minimum significance level of 5% and a minimum test power of 90% were considered.

The Ethics Committee on Human Research of the institution approved the study (certificate no.: 32714314.8.0000.0096).

RESULTS

Ninety-two children and adolescents diagnosed with T1DM were included in the study. Forty-seven (51.1%) were females. The demographic characteristics of the included patients are shown in Table 1.

Table 1. Demographic characteristics of included patients.

Sex and place of birth	n (%)
Sex	
Female	47 (51.1%)
Male	45 (48.9%)
Place of birth	
Curitiba	42 (46.2%)
Metropolitan Area of Curitiba	24 (26.3%)
Paraná (PR)	21 (23.1%)
São Paulo (SP)	1 (1.1%)
Pernambuco (PE)	2 (2.2%)
Pará (PA)	1 (1.1%)

Median age at diagnosis was 8.5 years, ranging from 0.9 to 14.6 years. In twelve cases (13%), patients or their families reported the presence of emotional stressors at the time of T1DM diagnosis, the most prevalent of which was parents going through a divorce (50%). As illustrated in Table 2, about a quarter of the included patients (27.2%) had a positive family history of autoimmune disorders, with hypothyroidism (64%) ranking atop others.

At the time of T1DM diagnosis, 64.8% of the patients had diabetic ketoacidosis (DKA). Twenty-three (25%) had mild DKA, 16 (18.2%) had moderate involvement, and 19 (21.5%) had severe DKA. Arterial blood gas analysis showed a mean pH of 7.28±0.14 and a mean HCO3- of 11.2, ranging from 1.5 to 24.9.

Positive tests for autoantibodies associated with beta-pancreatic cells were as follows: 69.6% of the patients had positive tests for anti-GAD autoantibodies; 60.6% tested positive for IA-2A; 42.9% for ICA; and 32.8% for AIA; only 20% of the patients did not have positive tests for the mentioned markers. As for autoimmune thyroid disease, positive tests for anti-TPO and anti-TG antibodies were seen in 13.1% and 22.9% of the patients, respectively; 10.7% of the patients had positive tests for both. Considering the markers related to celiac disease, positive test results for anti-tTG IgA and anti-endomysial antibodies were the least common (12.5% and 6.0%, respectively). Table 3 shows the proportions of patients with positive autoantibody tests.

Table 2. Epidemiological characteristics of the included patients at the time of T1DM diagnosis.

Disease characteristics	n (%)
Stressors at the time of diagnosis	
Parents getting a divorce	6 (50.0%)
Physical abuse	2 (16.6%)
Moving to a different town/school	2 (16.6%)
Death in the family	1 (8.3%)
Diagnosis of disease in the family	1 (8.3%)
Family conflict	1 (8.3%)
Loss of a pet	1 (8.3%)
Family history of autoimmune disease	
Hypothyroidism	16 (64.0%)
Diabetes mellitus type 1	4 (16.0%)
Hyperthyroidism	3 (12.0%)
Celiac disease	3 (12.0%)
Vitiligo	1 (4.0%)
Myasthenia gravis	1 (4.0%)
Rheumatic disease	1 (4.0%)

More than a quarter (27.4%) of the study population had autoantibodies for other autoimmune disorders at the time of T1DM diagnosis. Autoimmune thyroid disease (AITD) was the most prevalent comorbid condition with 21 pediatric patients (22.1%), followed by celiac disease with three (3.2%), and vitiligo and alopecia, with one each (1.05%).

One of the patients with thyroid involvement had been previously diagnosed with clinical hypothyroidism. Three other patients developed the disease during follow-up and required levothyroxine replacement; they accounted for 19.0% of the patients who tested positive for TPO and/or TG antibodies. The first follow-up visit since the start of thyroid function monitoring was held after a median of 13.2 months (7.1-17.1) and the second after 25.8 months (19.3-30.1). On average, serum TSH and free T4 levels remained within the reference value range, with no statistically significant difference between patients with and without positive antibody tests (TPO and/or TG). During follow-up, one patient tested negative and five tested positive for TG antibodies after a median of 12.4 months, increasing the prevalence of AITD to 26.3%.

Regarding the prevalence of the autoantibodies according to patient age, no statistically significant difference

Table 3. Prevalence of autoantibodies in children with type 1 diabetes mellitus at the time of diagnosis.

Laboratory tests (number of patients tested)	n (%)
Antibodies associated with pancreatic beta cells	
Anti-GAD (n=79)	55 (69.6%)
ICA (n=70)	30 (42.9%)
IA-2A (n=33)	20 (60.6%)
IAA (n=64)	21 (32.8%)
Antibodies associated with thyroiditis	
TPO (n = 84)	11 (13.1%)
TG (n = 83)	19 (22.9%)
Antibodies associated with celiac disease	
Anti-endomysial antibodies (n = 50)	3 (6.0%)
tTG-IgA (n = 8)	1 (12.5%)
tTG-IgG (n = 4)	0 (0.0%)

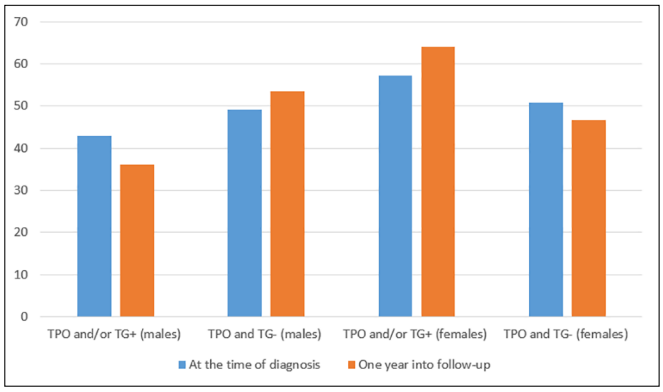
Note: anti-GAD = anti-glutamic acid decarboxylase autoantibodies; ICA = islet cell antibodies; IA-2A = islet antigen-2 autoantibodies; AIA = anti-insulin antibodies; TPO = thyroid peroxidase antibodies; anti-TG = antithyroglobulin antibodies; tTG = anti-tissue transglutaminase antibodies; IgA = immunoglobulin A; IgG = immunoglobulin G.

was seen between patients aged less than five years, patients aged between five and 9.9 years, and patients aged ten and older for any of the antibodies of interest (Fisher's exact test, $p > 0.05$).

Having a positive test for anti-GAD autoantibodies was not a predisposing factor to having positive tests for TPO (p = 0,16) or TG (p = 0.24) antibodies over time. Similarly, sex did not affect the prevalence of thyroid autoantibodies at the time of T1DM diagnosis (p = 0.40). However, after one year of follow-up, a trend towards a positive association was observed between female sex and testing positive for thyroid antibodies, with females testing positive more often than males (64% vs. 36%) (p = 0.11), as shown in Graph 1. Additionally, having a positive test for one thyroid antibody was linked to increased likelihood of testing positive for a second antibody (OR = 27.9 $p < 0.001$).

DISCUSSION

The present study provided an overview of the clinical presentations of pediatric patients at the time of T1DM diagnosis, with emphasis on the analysis of associated autoimmunity markers. In addition to causing morbidity, the coexistence of autoimmune thyroid disorders may negatively affect the metabolic control of T1DM by introducing dysfunctions in affected target organs.[8]



Graphic 1. Positive tests for autoantibodies at diagnosis and one year into follow-up. Note: TPO = thyroid peroxidase antibodies; TG = anti-thyroglobulin antibodies.

A factor causing a strong association between autoimmune thyroid diseases is the presence of a common genetic origin for them (HLA haplotypes), as well as defects in the regulation of the immune system of affected individuals, who end up developing a considerably higher risk of presenting autoantibodies against several organs and tissues.[9]

An important element in the pathogenesis of immune-mediated obliteration of insulin-producing beta cells, pancreatic autoantibodies have different levels of prevalence reported in the literature. Brazilian studies enrolling diabetic children have demonstrated widely distinct frequencies of anti-GAD autoantibodies, ranging from 5.9% to 80%.[4,10] In a study carried out in southern Brazil, Moreira et al.[11] reported the closest prevalence numbers to our study, with 63.8%. Reported frequencies of occurrence of other autoantibodies have been 55% for ICA, 35% for AIA, and more than 50% for IA-2A, all of which consistent with our study findings.[12,13,14] Variations between the results reported by different authors may be explained by the use of different tests, with different reference values for the same antibodies. Nevertheless, the literature has consistently reported presence of anti-GAD autoantibodies in the vast majority of cases, with the highest prevalence among autoimmune pancreatitis markers at the time of T1DM diagnosis.[6,9,12,15] This confirms the theory that glutamic acid decarboxylase (GAD) might be the first antigen to act as a target for autoimmune pancreatitis, still in the early and asymptomatic stages of disease.[16]

AITD followed by celiac disease (CD) were confirmed as the most common autoimmune disorders to occur in association with T1DM.[5,6,14] With regard to antithyroid autoantibodies, it is still unclear whether they are directly responsible for the pathogenesis of AITD or if they appear as a result of the destruction of the gland, mediated by T-cell infiltration. International studies have reported presence of these markers in 20% to 30% of study populations, which is in line with results observed in patients from the Brazilian Northeast and South.[4,17] Our study found proportions closer to the lower end of the prevalence spectrum described in the literature.

The inability to demonstrate a positive association between female sex and older age/longer time with disease *and* testing positive for antithyroid antibodies may be explained by the size of the study population and the short follow-up period after diagnosis of T1DM, which was only 12.4 months on average for analysis of thyroid autoimmunity.[15,17,18] In long-term follow-up, it is possible that the number of patients developing clinical hypothyroidism will grow by proportions consistent with literature reports.[15,19]

Another frequent association found in the literature between abnormal immune reactions directed concomitantly to the pancreas and the thyroid concerns the anti-GAD autoantibodies. Its relationship with thyroid autoantibodies may be explained by the fact that GABA, the product of the enzymatic reaction triggered by its antigen (GAD), is not only present inside the pancreatic islets, but also in several other tissues, such as the brain, stomach and thyroid.[5,15] Cross-sectional and longitudinal studies also demonstrated that patients with T1DM and AITD have higher serum levels of anti-GAD autoantibodies compared with patients without AITD, and that the presence of anti-GAD autoantibodies in patients with T1DM increases the risk of AITD by up to two times when compared with individuals tested negative for this marker.[12,18] Although not statistically significant, our results showed a tendency towards the confirmation of this positive association based on the analysis of the correlations between anti-GAD and TPO seroconversion.

Reported rates of occurrence of celiac disease are lower, ranging worldwide between 1.6% and 12.3%, although they are 20 times higher in diabetic patients than in the general population.[20] None of the patients in our study population had been diagnosed with CD at the time of T1DM diagnosis, contradicting the idea that the vast majority of cases (75-90%) are diagnosed only via screening tests, years after the development of diabetes.[21] Similarly to our findings, studies enrolling Brazilian children reported prevalence of anti-tissue transglutaminase antibodies ranging between 2.5% and 15.8% and 3.1% in individuals diagnosed with CD.[4,22]

Length of follow-up since a diagnosis of T1DM, study population size, differences in laboratory techniques and test reference values, and procedural difficulties in performing screening tests are among the limitations of the present study, which might explain the differences seen in relation to the literature.

CONCLUSION

Children and adolescents with T1DM are susceptible to the development of autoantibodies, directed not only to the pancreas, but also to other tissues and target organs. The high prevalence of association of T1DM with other autoimmune diseases, particularly autoimmune thyroid disease and celiac disease, indicates the need for routine screening tests for these comorbid conditions. Early detection of these diseases allows the introduction of measures to prevent negative repercussions on T1DM control and other complications.

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