Autoantibodies in Type 1 Diabetes: A Retrospective Study of Children Diagnosed Under Ten Years of Age

Autoanticorpos na Diabetes Tipo 1: Estudo Retrospetivo em Crianças com Diagnóstico Inaugural em Idade Inferior a 10 Anos

Cristiana Maximiano[1*], Alexandra Vilas Fabião[2], Diana Rita Oliveira[1], Ângela Dias[3], Filipa Correia[4], Sofia A. Martins[5]

***Autor Correspondente/Corresponding Author:**
Cristiana Maximiano [cristiana.maximiano@gmail.com]
ORCID iD: 0000-0001-9916-925X

RESUMO

INTRODUÇÃO: A diabetes *mellitus* tipo 1 é uma doença crónica caraterizada por uma perda seletiva de células β pancreáticas, com um aumento desproporcional recente em idades inferiores a 5 anos. A sua etiologia é multifatorial, para a qual contribuem fatores imunitários através da formação de autoanticorpos pancreáticos. O objetivo principal deste estudo é avaliar se o tipo de autoanticorpo pancreático influencia as manifestações clínicas, hemoglobina glicada e glicemia ao diagnóstico inaugural de diabetes *mellitus* tipo 1 em crianças até aos 10 anos.

MÉTODOS: Estudo observacional, retrospetivo e analítico com um total de 95 doentes incluídos. Foram comparados dois grupos etários (≤60 meses e >60 meses) e cada tipo de autoanticorpo (positivo/negativo) relativamente a caraterísticas demográficas, imunitárias, clínicas e laboratoriais. Os autoanticorpos estudados foram os anti-célula dos ilhéus, anti-descarboxilase do ácido glutâmico 65, anti-insulina e anti-transportador 8 do zinco. Foi estudado o impacto da autoimunidade, hemoglobina glicada, género e idade nos parâmetros clínico-laboratoriais destas crianças.

RESULTADOS: Crianças diagnosticadas acima dos 60 meses apresentaram maior valor de hemoglobina glicada (p=0,005), sendo este parâmetro laboratorial o único que evidenciou ter impacto na apresentação sob a forma de cetoacidose diabética (CI95%: OR=1,66;p=0,001). O valor da glicemia à admissão demonstrou ser influenciado negativamente pela idade (β=--0,25;p=0,022) e positivamente pela hemoglobina glicada (β=0,35;p=0,001). Nenhum dos autoanticorpos avaliados mostrou interferir nas manifestações clínico-laboratoriais da diabetes *mellitus* tipo 1.

CONCLUSÃO: As caraterísticas demográficas e clínico-laboratoriais não apresentaram diferenças estatisticamente significativas entre os grupos de autoanticorpos pancreáticos (positivo/negativo) analisados. É crucial desenvolver mais estudos no âmbito da autoimunidade que permitam estruturar potenciais fenótipos imunitários e auxiliar a descoberta de novos alvos terapêuticos.

PALAVRAS-CHAVE: Autoanticorpos; Autoimunidade; Criança; Diabetes Mellitus Tipo 1

1.Pediatric Department, Hospital de Braga, Braga, Portugal. 2. School of Medicine, Universidade do Minho, Braga, Portugal. 3. Pediatric Endocrinology Unit, Hospital Senhora da Oliveira, Guimarães, Portugal. 4. Pediatric Department, Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real, Portugal. 5.Pediatric Endocrinology Unit, Hospital de Braga, Braga, Portugal.

Recebido/Received: 2022/11/16 - Aceite/Accepted: 2023/06/20 - Publicado online/Published online: 2023/09/06 - Publicado/Published: 2023/12/31

ABSTRACT

INTRODUCTION: Type 1 diabetes is a chronic disease characterized by a selective loss of pancreatic β-cells with a disproportionate recent increase at ages under 5-years old. Its etiology is multifactorial, to which immune factors contribute through pancreatic autoantibodies. Our main aim is to assess whether the type of pancreatic autoantibody influence the clinical symptoms, glycated hemoglobin and glycemia at diagnosis of type 1 diabetes in children diagnosed under ten-years of age.

METHODS: Observational, retrospective and analytical study carried out with a total of 95 patients included. We compared two groups (aged ≤60 months and >60 months) and each type of autoantibody (positive/negative) regarding demographic, immune, clinical and laboratory characteristics. The autoantibodies studied were islet cell autoantibodies (against cytoplasmic proteins in the β-cell), antibodies to glutamic acid decarboxylase, anti-insulin and anti-zinc transporter 8. The impact of autoimmunity, glycated hemoglobin, gender and age on clinical and laboratory parameters of these children was analyzed.

RESULTS: Children diagnosed over 60 months had a higher glycated hemoglobin value (p=0.005) and this laboratory parameter was the only one that showed an impact on the initial presentation as diabetic ketoacidosis (CI95%: *OR*=1.66;p=0.001). The value of glycemia at admission showed to be influenced negatively by age (β=--0.25;p=0.022) and positively by glycated hemoglobin (β=0.35;p=0.001). None of the autoantibodies evaluated seemed to interfere in the clinical and laboratory manifestations of type 1diabetes.

CONCLUSION: Demographic, clinical and laboratory characteristics showed no statistically significant differences between two groups of positive/negative pancreatic autoantibodies analyzed. It is crucial to develop further studies in the scope of autoimmunity that allow to structure potential immune phenotypes and assist in the discovery of new therapeutic targets.

KEYWORDS: Autoantibodies; Autoimmunity; Child; Diabetes Mellitus, Type 1

INTRODUCTION

Type 1 diabetes (TID) is a chronic and immune-mediated disease, characterized by destruction of β-cells, the insulin-producing cells.[1] Consequently, there is a deficiency in secretion of this hormone, crucial in the metabolic regulation of carbohydrates, enhancing a permanent state of hyperglycemia.[2] About 90% of initial TID cases arise in children and adolescents, reflecting approximately 500 000 pediatric patients worldwide, 26% of whom are European.[3-6] The incidence rate of TID has been increasing in recent decades, by around 3%-4% per year, especially in younger children.[7] Some studies report a recent disproportionate increase in children under 5 years of age, with a predisposition to double in the future.[8-10] In 2015 in Portugal, children aged 0-14 years had a prevalence of TID of 0,13% and an incidence of 13.3 new cases per 100 000 children.[11]

The etiology of DM1 is multifactorial, to which genetic, environmental, and immune systems contributes. The potential trigger underlying pancreatic endocrine failure remains unknown, however, viral infections are currently one of the most consensual hypotheses.[12,13] The HLA-DR-DQ haplotype appears to be the most important genetic determinant, as it contributes more than 50% to the individual genetic risk for developing TID.[6-10]

About 70%-90% of patients had an abnormal activation of the immune system, which leads to autoantibodies whose target are β-cell proteins.[7] More than 90% of individuals newly diagnosed have at least two positive autoantibodies (AA+) and the order of appearance seems to be related to the HLA-DR-DQ haplotype.[14,15] Thus, autoimmunity arises months to years before diagnosis, with a mean time of 3.5 years from seroconversion to the manifestation of the disease.[6-16]

Autoantibodies are biological indicators of β-cells damage, although they are not directly responsible for tissue destruction. This chronic inflammatory process is triggered by mononuclear cells and TCD8+ lymphocytes.[1,10,17] When this destruction rises above 90%, the symptoms of hyperglycemia appear.[18] The autoantibodies involved in this process are islet cell antibodies (ICA, against cytoplasmic proteins in the β-cell), antibodies to glutamic acid decarboxylase (GAD-65), anti-insulin (IAA) and anti-zinc transporter 8 (ZnT8).[18-20] The risk of progression to a symptomatic stage is related to the number of existing autoantibodies and with the age of seroconversion. In case of two AA+, the risk of developing TID during childhood or adolescence is around 80%.[12,15-16]

The presentation of TID in young people may present with symptoms characteristic of hyperglycemia, such as polyuria, polydipsia, polyphagia, asthenia and unintentional weight loss.[5,19] In more severe cases, diabetic ketoacidosis may be the first sign of TID, secondary to an absolute or relative insulin deficiency combined with an excess of counter-regulatory hormones. At an early stage, as the bicarbonate ion is still found normal, it could neutralize ketone bodies, so ketosis may occur without acidosis.[19,21]

According to international consensus, if a child has glycated hemoglobin ≥6.5% or classic signs and symptoms with serum glucose ≥ 200 mg/dL, we are facing a diagnosis of diabetes.[5,19,22] In TID, it is expected that there will be positivity for at least one autoantibody against β-cells.[19] Glycated hemoglobin is an indicator of great clinical utility, as it translates average blood glucose over the past 4-12 weeks. Taking into account that the half-life of erythrocytes is approximately 120 days, glycated hemoglobin should not be used as a diagnostic criterion if we are facing clinical conditions that affect the normal turnover of erythrocytes.[22-24]

Nowadays there are few epidemiological data regarding the immune scenario underlying TID in the pediatric population worldwide, especially in Portugal.[10] Thus, it is highly relevant to carry out studies that allow us to understand which autoantibodies are affected, their association with clinical and laboratory parameters (glycemia and glycated hemoglobin) and with the age of disease presentation. Given the prevalence, chronicity and high burden of disease associated with TID, it is important to expand the knowledge of autoimmunity to allow us to understand the pathological mechanisms and further that to explore potential therapeutic targets in the future.

Our major aim is to assess the influence of autoantibody positivity in the clinical symptoms, HbA1c and glucose at diagnosis. A secondary aim is to clarify if gender and age at diagnosis have a role in the clinical symptoms and laboratory parameters in children with inaugural TID up to 10 years of age.

MATERIAL AND METHODS

An observational, retrospective and analytical study was performed in the Pediatric Endocrinology and Diabetology Unit at Hospital de Braga including all children with inaugural diagnosis of TID aged 10 years and below. The exclusion criteria were: children diagnosed with TID beyond 10 years of age; children with neonatal diabetes; children whose clinical record does not contain information relating to any of the four autoantibodies under study; children with other clinical conditions that may interfere with the real value of the glycated hemoglobin, either by reducing or increasing it, namely: hemoglobinopathies, anemia hemolytic, hematologic neoplasms, nutritional deficiency of folic acid, vitamins B12 or B6, hyperthyroidism, chronic kidney disease, nutritional iron deficiency or any other conditions that promote an increase in the number of erythrocytes and/or hematocrit.[22-25]

All data collection for the study was carried out based on clinical records, through Glintt® software, detained by Hospital de Braga and included:

- Demographic parameters: gender (female/male) and age of child at diagnosis (in months);
- Clinical parameters presented at diagnosis: signs/symptoms of hyperglycemia, ketosis without acidosis and ketoacidosis [defined as: glycemia >11 mmol/L (200 mg/dL), venous pH <7.3 or serum bicarbonate <15 mmol/L and ketonemia (***β***-hydroxybutyrate ≥3 mmol/L) or ketonuria moderate to severe][21];
- Laboratory parameters: blood glucose value on admission (mg/dL); and glycated hemoglobin value (in %; analysis performed by the ion exchange chromatography technique and agarose gel electrophoresis);
- Autoimmunity: serology (positive/negative) of the pancreatic autoantibody type (ICA, GAD-65, IAA and ZnT8; assay performed by immunofluorescence method).

The antibodies and laboratory parameters were measured in the same laboratory.

For the statistical study, the Statistical Package for the Social Sciences program in version 26.0 was used for the Window operating system (SPSS, Inc).[26]

Primarily, the sample was characterized using descriptive statistics. At continuous quantitative variables that fulfilled the normality assumptions were represented through the mean±standard deviation (M±SD), while the rest were presented using the median (Mdn) and interquartile range (IQR). Regarding the categorical variables, these were described using absolute frequencies and percentages. The normality of the quantitative variables was tested using the Shapiro-Wilk test, from histogram and asymmetry and kurtosis values. When quantitative variables did not show a normal distribution, non-parametric tests were performed. For each individual type of autoantibody (ICA, GAD-65, IAA and ZnT8), patients were divided into two groups (AA+ vs AA-), making a total of 4 pairs of different groups.

To assess the existence of differences between each two groups of autoantibodies (positive/negative), in concern age, gender and clinical and laboratory characteristics of the patients (glycated hemoglobin values, glycemia at admission and symptoms), comparisons were made between these groups. Additionally, patients were divided into two age groups: age ≤60 months and age >60 months. To assess differences between the two groups, these were compared regarding the type and number of AA+, gender and clinical and laboratory characteristics. The type of statistical test applied was suitable for the type of variable dependent (VD).

Thus, for continuous quantitative variables with a normal distribution, the test was performed T-Student for independent samples (t), the Levene's test was applied to assess homogeneity of variances and the d-Cohen (d) was calculated as a measure of effect size. For the quantitative variables that did not meet the normality assumptions, the Mann-Whitney (U) test was used and r as measure of effect size. Additionally, the Chi-Square (X) test was performed for categorical VDs. As a measure of effect size, the Phi (φ) was presented when the variables were dichotomous and Cramer's V (φc) when the variables had more than two categories.

The magnitude of the effect was classified as "weak", "intermediate", "strong" or "very strong", according to what is established for the effect size of each test.[27,28] In order to determine the impact of the autoantibody(s), age and gender in the presence of ketoacidosis at diagnosis, glycemia and glycated hemoglobin values at admission, regressions were performed. Considering the VD ketoacidosis (present/absent), the adjusted odds ratio (OR) was calculated through the realization of a binary logistic regression (BLR). To predict quantitative outcomes (glycemia and glycated hemoglobin), the β (standardized coefficient) was presented through the execution of a multiple linear regression (MLR).

Ethical Committee of Hospital de Braga and Ethical Committee of School of Medicine of University of Minho approved this study.

RESULTS

This study included 95 patients whose demographic, immune, clinical characteristics and laboratory tests are represented in Table 1. There was a 51.6% of gender male, with a median age of 71 months. Regarding the autoantibodies, the majority were negative; related to the minority with positive antibodies, there was a predominance of ICA (82.1%) and GAD-65 (32.6%). At diagnosis, 41.4% of the children presented with ketosis without acidosis. The median glycemia on admission was 484 mg/dL and the mean glycated hemoglobin was 11%.

TABLE 1. Sample characterization of patients with inaugural diagnosis of TID aged under 10 years

Variable	n sample	Missing data n (%)	Variable Description
Demographic characteristics			
Age (months)	95	0 (0.00%)	Mdn (IQR) 71.0 (49.0)
Age group		0 (0.00%)	(%)
≤ 60 months	40		42.1%
>60 months	55		57.9%
Gender		0 (0.00%)	(%)
Male	49		51.6%
Female	46		48.4%
Immune characteristics			
Autoantibodies			
ICA		4 (4.20%)	(%)
Positive	13		13.7%
Negative	78		82.1%
GAD		29 (30.5%)	(%)
Positive	31		32.6%
Negative	35		36.8%
IAA		41 (43.2%)	(%)
Positive	22		23.2%
Negative	32		33.7%
ZnT8		72 (75.8%)	(%)
Positive	10		10.5%
Negative	13		13.7%
Number of positive autoantibodies		0 (0.00%)	(%)
Nº = 0	49		51.6%
Nº = 1	22		23.2%
Nº = 2	18		18.9%
Nº = 3	6		6.30%
Clinical characteristics			
Clinical presentation		0 (0.00%)	(%)
signs/symptoms of hyperglycemia	21		22.1%
ketosis without acidosis	39		41.1%
ketoacidosis	35		36.8%
Laboratory parameters			
Glycemia value at admission (mg/dL)	93	2 (2.10%)	Mdn (IQR) 484 (207)
Glycated hemoglobin (%)	92	3 (3.20%)	M±SD 11.0±1.79

TABLE 2. Evaluation of the differences between the GAD-65+/GAD-65- groups regarding the demographic, clinical and laboratory characteristics of the patients.

Variable	GAD-65 + (n=31)			GAD-65 -- (n=35)			Statistical test value
	n	%	M±SD or Mdn (IQR)	n	%	M±SD or Mdn (IQR)	
Demographic Characteristics							
Age (months)							
			68.0			65.0	U = 531 p = 0.88 r = <0.001
			(55.0)			(41.0)	
Gender							
Male	16	51.6		21	60.0		$X^2(1)$ = 0.47 p = 0.49 φ = 0.084
Female	15	48.4		14	40.0		
Clinical characteristics							
Presentation							
signs/symptoms of hyperglycemia	7	22.6		9	25.7		$X^2(2)$ = 0.41 p = 0.82 φc = 0.079
ketosis without acidosis	11	35.5		14	40.0		
ketoacidosis	13	41.9		12	34.3		
Laboratory parameters							
Glycemia value at admission (mg/dL)			500±152			497±164	t(62) = -0.083 p = 0.93 d = 0.021
Glycated hemoglobin (%)			11.0			11.0	U = 491 p = 0.63 r = .050
			(2.40)			(2.80)	

M±SD - mean±standard deviation; Mdn (IQR) - median (interquartile range); n - number of cases (absolute frequency); % - relative frequency; GAD-65 -glutamic acid decarboxylase 65-kilodalton autoantibody; t - T-Student test independent samples; d - d Cohen; U - Mann-Whitney test; X^2 - Chi-Square test; r - effect size; φ - *Phi*; φc - V Cramer.

TYPE OF ANTIBODY PRESENTED AT INAUGURAL DIAGNOSIS (POSITIVE/ NEGATIVE)

In both genders, there was a predominance of zero AA+ at diagnosis. However, the second most common frequency was two AA+ in females and one AA+ in males.

Table 2 represents the differences between the GAD-65+/GAD-65- groups regarding the characteristics of the patients, the autoantibody with the highest number of positive results of this sample (n=31). There are no statistically significant differences demonstrated. The two groups were similar, although GAD-65 positive is more frequent in males (51.6%) and in older children (median age of 68 months).

Table 3 characterizes the comparison referring to the ICA. This autoantibody had the highest number of negatives (n=78), with an evident imbalance between the two groups. There were no statistically significant differences between the ICA+/- in none of the considered parameters.

Tables 4 and 5 represents the detailed comparison of IAA and ZnT8 antibodies, respectively. In both cases, there were no statistically significant differences between the groups of AA +/-.

AGE GROUPS

Table 6 shows the comparison between the two age groups regarding gender and clinical, laboratory and immune characteristics. Regarding the percentage of glycated hemoglobin, with an intermediate effect association, the group aged >60 months had a higher value (median 11.4%) than other group (median 10.0%) (p=0.005;r=0.33).

From an immune point of view, the group aged >60 months had the highest number of AA+ per classes.

THE IMPACT OF AUTOIMMUNITY ON CLINICAL AND LABORATORY PARAMETERS AT DIAGNOSIS

It were created different models of potential predictors with a combination of autoantibodies (individuals and pairs) along with age and gender factors and with other relevant factors evidenced in the literature, namely glycated hemoglobin. The aim of this grouping was to minimize the effects of missing data on regression results, although there is always a model that contains all autoantibodies simultaneously as predictors.

TABLE 3. Evaluation of the differences between the ICA+/ICA- groups regarding the demographic, clinical and laboratory characteristics of the patients.

Variable	ICA + (n=13)			ICA -- (n=78)			Statistical test value
	n	%	M±SD or Mdn (IQR)	n	%	M±SD or Mdn (IQR)	
Demographic characteristics							
Age (months)							
			90.0			68.5	$U = 352$ $p = 0.078$ $r = 0.20$
			(45.0)			(48.0)	
Gender							
Male	9	69.2		37	47.4		$X^2(1) = 2.12$ $p = 0.15$ $\varphi = 0.15$
Female	4	30.8		41	52.6		
Clinical characteristics							
Presentation							
signs/symptoms of hyperglycemia	2	15.4		18	23.1		Fisher test $p = 0.80$ $\varphi c = 0.083$
ketosis without acidosis	5	38.5		32	41.0		
ketoacidosis	6	46.2		28	35.9		
Laboratory parameters							
Glycemia value at admission (mg/dL)			538			483	$U = 470$ $p = 0.78$ $r = <0.001$
			(131)			(223)	
Glycated hemoglobin (%)			11.3±1.51			11.0±1.84	$t(86) = -0.55$ $p = 0.59$ $d = -0.17$

M±SD - mean±standard deviation; Mdn (IQR) - median (interquartile range); n - number of cases (absolute frequency); % - relative frequency; ICA - islet cell cytoplasmic autoantibody; t - T-Student test independent samples; d - d Cohen; U - Mann-Whitney test; X^2 - Chi-Square test; r - effect size; φ - Phi; φc - V Cramer.

TABLE 4. Evaluation of the differences between the IAA+/IAA- groups regarding the demographic, clinical and laboratory characteristics of the patients.

Variable	IAA + (n=22)			IAA -- (n=32)			Statistical test value
	n	%	M±SD or Mdn (IQR)	n	%	M±SD or Mdn (IQR)	
Demographic characteristics							
Age (months)							
			67.0			82.5	$U = 322$ $p = 0.59$ $r = 0.050$
			(49.0)			(44.0)	
Gender							
Male	12	54.5		18	56.3		$X^2(1) = 0.015$ $p = 0.90$ $\varphi = -0.017$
Female	10	45.5		14	43.8		
Clinical characteristics							
Presentation							
signs/symptoms of hyperglycemia	4	18.2		6	18.8		$X^2(2) = 0.53$ $p = 0.77$ $\varphi c = 0.099$
ketosis without acidosis	11	50.0		13	40.6		
ketoacidosis	7	31.8		13	40.6		
Laboratory parameters							
Glycemia value at admission (mg/dL)			508			482	$U = 315$ $p = 0.63$ $r = 0.10$
			(174)			(225)	
Glycated hemoglobin (%)			11.3			11.1	$U = 323$ $p = 0.74$ $r = 0.050$
			(2.50)			(2.30)	

M±SD - mean±standard deviation; Mdn (IQR) - median (interquartile range); n - number of cases (absolute frequency); % - relative frequency; IAA - autoantibodies against insulin; t - T-Student test independent samples; d - d Cohen; U - Mann-Whitney test; X^2 - Chi-Square test; r - effect size; φ - Phi; φc - V Cramer.

TABLE 5. Evaluation of differences between the ≤60 months and >60 months age groups regarding the patients' immune, demographic, clinical and laboratory characteristics.

Variable	Age ≤60 months (n=40)			Age >60 months (n=55)			Statistical test value
	n	%	M±SD or Mdn (IQR)	n	%	M±SD or Mdn (IQR)	
Gender							
Male	22	55.0		27	49.1		$X^2(1) = 0.32$ $p = 0.57$ φ= -0.058
Female	18	45.0		28	50.9		
Clinical characteristics							
Presentation							
signs/symptoms of hyperglycemia	11	27.5		10	18.2		$X^2(2) = 1.19$ $p = 0.55$ φc = 0.11
ketosis without acidosis	15	37.5		24	43.6		
ketoacidosis	14	35.0		21	38.2		
Laboratory parameters							
Glycemia value at admission (mg/dL)			486 (155)			479 (244)	$U = 947$ $p = 0.44$ $r = 0.10$
Glycated hemoglobin (%)			10.0 (2.70)			11.4 (2.10)	$U = 685$ **$p = 0.005$** $r = 0.33$
Immune characteristics							
Autoantibodies							
ICA (%)							
Positive	3	7.90		10	18.9		$X^2(1) = 2.18$ $p = 0.14$ φ = 0.16
Negative	35	92.1		43	81.1		
GAD-65 (%)							
Positive	13	43.3		18	50.0		$X^2(1) = 0.29$ $p = 0.59$ φ = 0.067
Negative	17	56.7		18	50.0		
IAA (%)							
Positive	10	50.0		12	35.3		$X^2(1) = 1.13$ $p = 0.29$ φ = -0.15
Negative	10	50.0		22	64.7		
ZnT8 (%)							
Positive	4	33.3		6	45.5		Fisher test $p = 0.41$ φ = 0.21
Negative	8	66.7		5	54.5		
Number of positive autoantibodies							
Nº = 0	21	52.5		28	50.9		Fisher test $p = 0.51$ φc = 0.15
Nº = 1	11	27.5		11	20.0		
Nº = 2	5	12.5		13	23.6		
Nº = 3	3	7.50		3	5.50		

NOTE: Statistically significant *p*-values are highlighted in bold.

M±SD - mean±standard deviation; Mdn (IQR) - median (interquartile range); n - number of cases (absolute frequency); % - relative frequency; GAD-65 - glutamic acid decarboxylase 65-kilodalton autoantibody; ICA - Islet Cell Cytoplasmic autoantibody; IAA - autoantibodies against insulin; ZnT8 - zinc transporter 8 autoantibody; t - T-Student test independent samples; d - d Cohen; U - Mann-Whitney test; X^2 - Chi-Square test; r - effect size; φ - Phi; φc - V Cramer.

Since the ZnT8 autoantibody had the largest number of data omissions (75.8%), this was eliminated from the regressions, as it limits the number of patients included and, therefore, the respective results.

OCCURRENCE OF KETOACIDOSIS

It was analyzed the individual influence of each predictor on the outcome, evidenced through *OR*, where glycated hemoglobin value was the only statistically significant variable (*OR*=1.59;*p*=0.001).

On the other hand, it was detailed the analyses of different regression models, considering the contribution of each autoantibody. Glycated hemoglobin was the only significant independent predictor of ketoacidosis at diagnosis (*OR*=1.64;*p*=0.001) (*OR*=1.66;*p*=0.001), the only two that showed statistical significance.

TABLE 6. Assessment of the individual impact of predictors age, gender, ICA, GAD-65, IAA and glycated hemoglobin in the presence of ketoacidosis at diagnosis.

Predictors	*OR* (IC 95%)	*p*
Age (months)	1.00 (0.99-1.01)	0.91
Gender (female/male)	1.19 (0.52-2.74)	0.69
ICA (positive/negative)	1.53 (0.47-5.00)	0.48
GAD-65 (positive/negative)	1.38 (0.51-3.76)	0.52
IAA (positive/negative)	0.68 (0.22-2.14)	0.51
Glycated hemoglobin (%)	1.59 (1.20-2.11)	**0.001**

Note: Ketoacidosis is the referential category. Statistically significant *p*-values are highlighted in bold.

OR - odds ratio; IC - confidence interval; GAD-65 - glutamic acid decarboxylase 65-kilodalton autoantibody; ICA - Islet Cell Cytoplasmic autoantibody; IAA - autoantibodies against insulin; ZnT8 - zinc transporter 8 autoantibody.

Through a predictive model, we concluded that there is a 66% increase in the child's chance of developing ketoacidosis, considering the same gender, age and ICA serology.

GLYCEMIA VALUE

Considering the laboratory parameters at diagnosis (glycemia and glycated hemoglobin) as an outcome of study, different models of MLR were performed.

Each type of autoantibody was individually combined to determine the respective impact on admission glycemia. On MLR performed, glycated hemoglobin was the predictor with the greatest influence on glycemia. Considering the same age, gender and ICA serology, for each increase percentage in glycated hemoglobin, admission glycemia increases, on average, by 38.1 mg/dL. The male gender has a higher glycemia at admission, on average, 106.6 mg/dL compared to the gender feminine.

It was also performed regression models, in which the autoantibodies were combined to determine their impact on admission glycemia. Considering the same age, gender, GAD-65 and IAA serology, for each percentage increase in glycated hemoglobin value, on average, occurs a 38.6 mg/dL increase in glycemia at admission. A male child has a glycemia, on average, 87.9 mg/dL higher than the opposite sex, taking count the same age, glycated hemoglobin value, GAD-65 and IAA serologies.

GLYCATED HEMOGLOBIN VALUE

We performed the different regression models, in which each type of autoantibody was individually combined to measure its impact on glycated hemoglobin. Age was the only predictor significant independent (β=0.30; p=0.004;β=0.30;p=0.006). Thus, for every month increase in the child's age, an average increase of 0.018% in glycated hemoglobin is expected, considering the same gender and ICA serology.

DISCUSSION AND CONCLUSION

In our study, 48.4% of the children had at least one AA+ and the male gender was the one that expressed the greatest global positivity, findings not corroborated by the literature, which reports approximately twice as many patients with AA+ at diagnosis,[1,29-31] with female preference.[29]

Of the four types of autoantibodies analyzed, GAD-65 was the most prevalent (32.6%), followed by the IAA (23.2%). On the other hand, the largest number of negatives was verified in the ICA autoantibody (82.1%). The literature classifies the GAD-65 as the most prevalent autoantibody in TID patients, followed by IAA.[20,30,32-33] The detection of autoantibodies tends to decrease with age, and ICA autoantibody appearing to be the fastest to become negative with the progression of TID. Its frequency drops to 25% at 5 years and <5% at 10 years of disease, so this may be a plausible justification for our majority negativity.[1,20,34] In this sample, the antibodies panel may not have been performed exactly at the time of inaugural diagnosis, which may have conditioning a decrease of its levels to undetectable values when harvesting, something expected to happen with the progression of the disease.[29,35] However, it can be speculated that in 51.6% of our patients, autoantibodies will develop after diagnosis, translating a TID currently with no humoral response or, alternatively, with humoral activity but against antigens not officially recognized as part of this pathology, as is the case of tetrasparin-7 recently identified.[29,36] Another hypothesis to be considered is the possibility that some of these children may have another subtype of diabetes where the destruction of β-cells does not appear to be humoral, but eventually related to another cellular pathway or genetics.[19,29,33]

As stated by Bravis V *et al*[29] and Perchard R *et al*,[31] our study also showed that having a certain type of autoantibody did not determine statistically significant differences regarding demographic, clinical parameters and laboratory tests of these children. According to the most recent international guidelines, ICA autoantibodies are no longer included as markers of autoimmunity for the diagnosis of TID, so its dosage is no longer in use.[19,22] The analysis of this autoantibody becomes more relevant in cases of negativity for the remaining autoantibodies.[37]

In the present study, no statistically significant differences were found between the GAD-65 groups regarding the presence of ketoacidosis, a result similar to that described by Sabbah E *et al.*[33] However, as it is stated in other studies, GAD-65 appears to be more common in older and female patients.[32,33,38,39] Additionally, according to the literature, the IAA seems to be the first autoantibody to emerge and is significantly positive at younger ages, something that was not verified in our study.[1,10,32-33,40] Despite the ZnT8 autoantibodies not showing significant differences between groups, a very important impact in the clinic at admission was detected (φc=0.42). Probably if there were more data regarding this autoantibody (78.6% omitted), statistically significant differences would be expected proven, as the size of the effect reflects an apparent relevance of ZnT8 in clinical parameters, as was evident in the study performed by Niechciał E *et al.*[41]

Regarding the clinical and laboratory characterization of this sample, there was a slight predominance of males (51.6%), an aspect consistent with the literature.[10-11,29,30,39] Between our 95 patients evaluated, 36.4% manifested ketoacidosis at admission. This represents one of the complications with the highest risk of morbidity and mortality from TID, although it has a highly variable frequency around the world, around 15% to 67%, possibly due to the heterogeneity of the criteria implemented in its definition.[20,30,40,42-43] In our sample, ketosis without acidosis was the main form of presentation of inaugural TID (41.1%), so it is assumed that there is a greater perception of hyperglycemic symptoms by the caregivers, preventing more severe metabolic decompensation in the form of ketoacidosis. Another important finding is the significant differences in the percentage of glycated hemoglobin value at diagnosis between age groups, as patients diagnosed over 60 months presenting a percentage superior of this laboratory parameter (association of intermediate effect), finding in agreement with the literature.[44-45] Thus, older patients probably have worse metabolic control in 4-12 weeks prior to diagnosis, translating a longer process of pancreatic failure and possibly, similarly to that described by Hanberger L *et al*,[45] with glycated hemoglobin values also higher during follow-up and facing higher risk of future complications.[46-47] A plausible explanation for these facts is that younger children, through a destructive autoimmune or cellular process, present a more rapid and extensive destruction of β-cells. This results into an abrupt insulin deficit, with a shorter duration of hyperglycemic symptoms and a tendency to severe and acute presentations in the form of ketoacidosis.[43-45,48] In contrast, no differences were reported between groups regarding glycemia at admission and immune characteristics, but most of the literature reports a significantly higher presence of the IAA autoantibody as an early marker of pancreatic destruction in younger children[6,10,32,40,44] and of GAD-65 in older children.[29,48] Similarly to our study, Chen YC *et al*[44] and Komulainen J *et al*[40] did not report age differences regarding ICA and GAD-65 autoantibodies.

The percentage of glycated hemoglobin proved to be the only independent predictor statistically significant in the occurrence of ketoacidosis at diagnosis, adjusting sample for age, gender and serology of the ICA. For every percentage increase in glycated hemoglobin value, there is a 66% increase chance of ketoacidosis. Cherubini V *et al*[43] concluded that children under 4 years are twice as likely to have ketoacidosis than older girls. Niechciał E *et al*[41] reported that AA ZnT8+ increases the risk of ketoacidosis, not corroborating by your study.

Considering children with the same age, gender and serology as the ICA, for each increase in 1 month of age, a significant increase in glycated hemoglobin value is expected and this finding is according to the most recent literature. In this sample, male sex and higher percentages of glycated hemoglobin values are related to higher glycemic values and older children have, in general, lower glycemia values. A higher incidence, reported in the literature, of metabolic decompensation in younger children may justify this last finding.[43-45,48]

As this is a retrospective study, it was carried out based on pre-existing data, incomplete in some cases, especially about the serology of autoantibodies, which exponentially limited the results underlying. The small size of this sample and the high amount of missing data regarding autoantibodies may justify the lack of documented statistical significance. However, it is important to emphasize that the AA ZnT8 only started to be routinely dosed recently, which may justify its majority percentage of missing data.

The recent increase in inaugural diagnoses in younger age groups encourages carrying out larger studies aimed at understanding the influence of the immune context in the clinical and laboratory characteristics of the pediatric population diagnosed with TID. As a conclusion, authors would like to emphasize the importance of standardized and protocoled studies in the context of autoimmunity that allow to stratify patients into potential immune phenotypes and promote the discovery of new therapeutic targets, encouraging a future based on Precision Medicine.

DECLARAÇÃO DE CONTRIBUIÇÃO/ CONTRIBUTORSHIP STATEMENT

CM e SM: Pesquisa, conceção do estudo, aquisição de dados, análise de dados, escrita do artigo e revisão final

AF: Pesquisa, conceção do estudo, análise de dados, escrita do artigo e revisão final

DO: Aquisição de dados, análise de dados, escrita do artigo e revisão final

AD e FC: Aquisição de dados e revisão do artigo

CM and SM: Research, study design, acquisition of data, data analysis, paper writing and final review

AF: Research, study design, data analysis, paper writing and final review

DO: Acquisition of data, data analysis, paper writing and final review

AD and FC: Acquisition of data and article revision

RESPONSABILIDADES ÉTICAS

CONFLITOS DE INTERESSE: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho.

FONTES DE FINANCIAMENTO: Não existiram fontes externas de financiamento para a realização deste artigo.

CONFIDENCIALIDADE DOS DADOS: Os autores declaram ter seguido os protocolos da sua instituição acerca da publicação dos dados de doentes.

PROTEÇÃO DE PESSOAS E ANIMAIS: Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pela Comissão de Ética responsável e de acordo com a Declaração de Helsínquia revista em 2013 e da Associação Médica Mundial.

PROVENIÊNCIA E REVISÃO POR PARES: Não comissionado; revisão externa por pares.

ETHICAL DISCLOSURES

CONFLICTS OF INTEREST: The authors have no conflicts of interest to declare.

FINANCING SUPPORT: This work has not received any contribution, grant or scholarship.

CONFIDENTIALITY OF DATA: The authors declare that they have followed the protocols of their work center on the publication of data from patients.

PROTECTION OF HUMAN AND ANIMAL SUBJECTS: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2013).

PROVENANCE AND PEER REVIEW: Not commissioned; externally peer reviewed.

REFERENCES

1. Carla B, Danila B, Paolo C, Patrizia PI, Riccardo S, Antonella M, et al. Clinical presentation and autoimmune characteristics of very young children at the onset of type 1 diabetes mellitus. J Pediatric Endocrinoly Metab. 2010;23:1151-7.
2. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. Geneva: WHO; 2006.
3. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002-2012. N Engl J Med. 2017;376:1419-29. doi: 10.1056/NEJMc1706291.
4. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. JAMA. 2014;311:1778-86. doi: 10.1001/jama.2014.3201.
5. Portuguese Society of Diabetology. Annual report of the National Diabetes Observatory. Lisbon: PSD;2016.
6. Fousteri G, Ippolito E, Ahmed R, Rahim Hamad A. Beta-cell Specific Autoantibodies: Are they Just an Indicator of Type 1 Diabetes? Curr Diabetes Rev. 2017;13:322-9. doi: 10.2174/1573399812666160427104157.
7. Direção Geral da Saúde. Crianças e jovens com Diabetes Mellitus Tipo 1 - Manual de Formação para apoio de Profissionais de Saúde e de Educação. Lisboa: DGS; 2019.
8. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G, Schober E, et al. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet. 2009;373:2027-33.
9. Gyurus EK, Patterson C, Soltesz G, László A, Zsuzsa A, Márta B, et al. Twenty-one years of prospective incidence of childhood type 1 diabetes in Hungary - the rising trend continues (or peaks and highlands?). Pediatr Diabetes. 2012;13:21-5. doi: 10.1111/j.1399-5448.2011.00826.x.
10. Sales Luis M, Alcafache M, Ferreira S, Fitas AL, Simões Pereira J, Caramalho Í, et al. Children with type 1 diabetes of early age at onset - Immune and metabolic phenotypes. J Pediatr Endocrinol Metab. 2019;32:935-41. doi: 10.1515/jpem-2019-0103.
11. Portuguese National Diabetes Progrqm – challenges and strategies. 2019.
12. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013;309:2473-9. doi: 10.1001/jama.2013.6285.
13. Skyler JS. Effects of oral insulin in relatives of patients with type 1 diabetes: The diabetes prevention trial-type 1. Diabetes Care. 2005;28:1068-76.
14. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014;383:69-82. doi: 10.1016/S0140-6736(13)60591-7.
15. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia. 2015;58:980-7. doi: 10.1007/s00125-015-3514-y.

16. Bonifacio E. Predicting type 1 diabetes using biomarkers. Diabetes Care. 2015;38:989-96.
17. Neves C, Neves JS, Oliveira SC, Oliveira A, Carvalho D. Diabetes Mellitus Tipo 1. Rev Port Diabetes. 2017;12:1-10.
18. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: A scientific statement of JDRF, the endocrine society, and the American diabetes association. Diabetes Care. 2015;38:1964-74. doi: 10.2337/dc15-1419.
19. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018;19:7-19. doi: 10.1111/pedi.12773.
20. Gan MJ, Albanese-O'Neill A, Haller MJ. Type 1 diabetes: Current concepts in epidemiology, pathophysiology, clinical care, and research. Curr Probl Pediatr Adolesc Health Care. 2012;42:269-91. doi: 10.1016/j.cppeds.2012.07.002.
21. Wolfsdorf JI, Glaser N, Agus M, Fritsch M, Hanas R, Rewers A, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Diabetic ketoacidosis and the hyperglycemic hyperosmolar state. Pediatr Diabetes. 2018;19:155-77.
22. Care D. Classification and diagnosis of diabetes: Standards of medical care in Diabetes-2018. Diabetes Care. 2018;41:S13-27.
23. Attard SM, Herring AH, Wang H, Howard AG, Thompson AL, Adair LS, et al. Implications of iron deficiency/anemia on the classification of diabetes using HbA1c. Nutr Diabetes. 2015;5:e166. doi: 10.1038/nutd.2015.16.
24. DiMeglio LA, Acerini CL, Codner E, Craig ME, Hofer SE, Pillay K, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Glycemic control targets and glucose monitoring for children, adolescents, and young adults with diabetes. Pediatr Diabetes. 2018;19:105-14. doi: 10.1111/pedi.12737.
25. Netto AP, Andriolo A, Filho FF, Tambascia M, Gomes MDB, Melo M, et al. Atualização sobre hemoglobina glicada (HbA 1c) para avaliação do controle glicémico e para o diagnóstico do diabetes: Aspectos clínicos e laboratoriais. J Bras Patol Med Lab. 2009;45:31-48.
26. Field A. Discovering statistics using SPSS. 3rd ed. London: Sage; 2009.
27. Akoglu H. User's guide to correlation coefficients. Turkish J Emerg Med. 2018;18:91-3.
28. Lenhard, W, Lenhard A. (2016). Calculation of Effect Sizes. [accessed Jun 2021] Available from: https://www.psychometrica.de/effect_size.html.
29. Bravis V, Kaur A, Walkey HC, Godsland IF, Misra S, Bingley PJ, et al. Relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort. BMJ Open. 2018;8:e020904. doi: 10.1136/bmjopen-2017-020904.
30. Ahmadov GA, Govender D, Atkinson MA, Sultanova RA, Eubova AA, Wasserfall CH, et al. Epidemiology of childhood-onset type 1 diabetes in Azerbaijan: Incidence, clinical features, biochemistry, and HLA-DRB1 status. Diabetes Res Clin Pract. 2018;144:252-9. doi: 10.1016/j.diabres.2018.09.009.
31. Perchard R, MacDonald D, Say J, Pitts J, Pye S, Allgrove J, et al. Islet autoantibody status in a multi-ethnic UK clinic cohort of children presenting with diabetes. Arch Dis Child. 2015;100:348-52. doi: 10.1136/archdischild-2014-306542.
32. Bilbao JR, Rica I, Vázquez JA, Busturia MA, Castaño L. Influence of sex and age at onset on Autoantibodies Against Insulin, GAD65 and IA2 in recent onset type 1 diabetic patients. Horm Res. 2000;54:181-5. doi: 10.1159/000053256.
33. Sabbah E, Savola K, Kulmala P, Veijola R, Vähäsalo P, Karjalainen J, et al. Diabetes-associated autoantibodies in relation to clinical characteristics and natural course in children with newly diagnosed type 1 diabetes. J Clin Endocrinol Metab. 1999;84:1534-9. doi: 10.1210/jcem.84.5.5669.
34. Borg H, Gottsäter A, Fernlund P, Sundkvist G. A 12-year prospective study of the relationship between islet antibodies and β-cell function at and after the diagnosis in patients with adult-onset diabetes. Diabetes. 2002;51:1754-62.
35. Lampasona V, Liberati D. Islet Autoantibodies. Curr Diab Rep. 2016;16:53. doi: 10.1007/s11892-016-0738-2.
36. Kerry A. McLaughlin, Carolyn C. Richardson, Aarthi Ravishankar, Christina Brigatti, Daniela Liberati, Vito Lampasona, Lorenzo Piemonti, Diana Morgan RGF and MRC. Identification of Tetraspanin-7 as a Target of Autoantibodies in Type 1 Diabetes. Diabetes Ther. 2016;2(March):1–35.
37. Andersson C, Kolmodin M, Ivarsson SA, Carlsson A, Forsander G, Lindblad B, et al. Islet cell antibodies (ICA) identify autoimmunity in children with new onset diabetes mellitus negative for other islet cell antibodies. Pediatric Diabetes. 2014;15(5):336-44.
38. Howson JM, Stevens H, Smyth DJ, Walker NM, Chandler KA, Bingley PJ, et al. Evidence that HLA class I and II associations with type 1 diabetes, autoantibodies to GAD and autoantibodies to IA-2, are distinct. Diabetes. 2011;60:2635-44. doi: 10.2337/db11-0131.
39. Turtinen M, Härkönen T, Parkkola A, Ilonen J, Knip M; Finnish Pediatric Diabetes Register. Sex as a determinant of type 1 diabetes at diagnosis. Pediatr Diabetes. 2018;19:1221-8. doi: 10.1111/pedi.12697.
40. Komulainen J, Kulmala P, Savola K, Lounamma R, Ilonen J, Reijonen H, et al. Clinical, Autoimmune, and Genetic Characteristics of very young children with Type 1 Diabetes. 1999;41:89-92.
41. Niechciał E, Rogowicz-Frontczak A, Piłaciński S, Fichna M, Skowrońska B, Fichna P, et al. Autoantibodies against zinc transporter 8 are related to age and metabolic state in patients with newly diagnosed autoimmune diabetes. Acta Diabetol. 2018;55:287-94. doi: 10.1007/s00592-017-1091-x.
42. Ardicli D, Kandemir N, Alikasifoglu A, Ozon A, Gonc N. Clinical characteristics of type 1 diabetes over a 40-year period in Turkey: Secular trend towards earlier age of onset. J Pediatr Endocrinol Metab. 2014;27:635-41. doi: 10.1515/jpem-2013-0320.
43. Cherubini V, Skrami E, Ferrito L, Zucchini S, Scaramuzza A, Bonfanti R, et al. High frequency of diabetic ketoacidosis at diagnosis of type 1 diabetes in Italian children: A nationwide longitudinal study, 2004-2013. Sci Rep. 2016;6:38844. doi: 10.1038/srep38844.
44. Chen YC, Tung YC, Liu SY, Lee CT, Tsai WY. Clinical characteristics of type 1 diabetes mellitus in Taiwanese children aged younger than 6 years: A single-center experience. Med Assoc. 2017;116:340-4. doi: 10.1016/j.jfma.2016.07.005.
45. Hanberger L, Åkesson K, Samuelsson U. Glycated haemoglobin variations in paediatric type 1 diabetes: The impact of season, gender and age. Acta Paediatr. 2014;103:398-403. doi: 10.1111/apa.12530.
46. Mazarello Paes V, Charalampopoulos D, Edge J, Taylor-Robinson D, Stephenson T, Amin R. Predictors of glycemic control in the first year of diagnosis of childhood onset type 1 diabetes: A systematic review of quantitative evidence. Pediatr Diabetes. 2018;19:118-26. doi: 10.1111/pedi.12530.
47. Viswanathan V, Sneeringer MR, Miller A, Eugster EA, DiMeglio LA. The utility of hemoglobin A1c at diagnosis for prediction of future glycemic control in children with type 1 diabetes. Diabetes Res Clin Pract. 2011;92:65-8. doi: 10.1016/j.diabres.2010.12.032.
48. Hathout EH, Hartwick N, Fagoaga OR, Colacino AR, Sharkey J, Racine M, et al. Clinical, autoimmune, and HLA characteristics of children diagnosed with type 1 diabetes before 5 years of age. Pediatrics. 2003;111:860-3.