Original article

Anti-tissue transglutaminase antibodies in patients with anti-glutamate dehydrogenase positive type 1 diabetes mellitus

Anticorpii anti-transglutaminază la pacienţii cu diabet zaharat tip 1 şi anticorpi anti-glutamat-dehidrogenază prezenţi

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Abstract

The purpose of our study is to determine the prevalence of celiac disease related antibodies (anti-tissue transglutaminase, anti-tTG IgA) in patients with type 1 diabetes mellitus (T1DM) and to compare these results with the general population. We also evaluated the prevalence of anti-tTG IgA in the absence and presence of anti-glutamate dehydrogenase antibodies (anti-GAD) in diabetic subjects. The autoantibodies were evaluated with respect to the clinical status and diabetes control of the patients. The study included 111 children and young adults, 62 boys and 49 girls, with a mean age of 13.42 ± 6.00 years and a duration of diabetes which ranged from 0 to15 years, and 161 control subjects, age and sex matched. Patients who were positive were offered a gastroduodenoscopic examination. Anti-GAD autoantibodies were positive in 68.5% (76/111) patients with T1DM. In T1DM patients with anti-GAD positivity, we found a prevalence of positive anti-tTG IgA 3.94% (3/76) and on T1DM patients without anti-GAD, the anti-tTG IgA positivity was 2.85% (1/35) (nonsignificant differences). Anti-tTG IgA antibodies positivity was higher in patients with T1DM (4/111; 3.6%), compared to controls (1/161; 0.6%). Serological markers are useful in identifying celiac disease patients with T1DM. Celiac disease (CD) related antibodies weren't more frequent in T1DM patients positive for anti-GAD and no age correlation was observed.

Keywords: celiac disease, diabetes mellitus, anti-tTGIgA, anti-GA

Rezumat

Studiul nostru a urmărit să determine prevalenţa anticorpilor asociaţi bolii celiace (anticorpii anti-tran-sglutaminază, anti-tTG IgA) la pacienţii cu diabet zaharat tip 1 (DZ tip 1) şi să compare aceste rezultate cu po-pulaţia generală. De asemenea, am evaluat frecvenţa anticorpilor anti-transglutaminază la pacienţii cu diabet

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zaharat în prezenţa şi absenţa anticorpilor anti-glutamat dehidrogenază. Prezenţa autoanticorpilor a fost evaluată în corelaţie cu controlul diabetului şi starea clinică a pacienţilor. Studiul a inclus 111 subiecţi diabetici, copii şi adulţi tineri, 62 băieţi şi 49 fete cu vârsta medie de 13.42 ± 6.00 ani şi durata a diabetului care a variat intre 0 si 15 ani şi 161 subiecţi control cu vârste şi sex similare. Pacienţilor pozitivi la examenul serologic li s-a oferit posibilitatea unui examen endoscopic. Pozitivitatea anti-GAD a fost detectată la 68.5% (76/111) pacienţi diabetici. La pacienţii cu DZ tip 1 anti-GAD pozitivi, rata de pozitivitate a anti-tTG-IgA a fost de 3.94% (3/76), iar la pacienţii anti-GAD negativi, rata de pozitivitate anti-tTG IgA a fost de 2.85% (1/35). Pozitivitatea anti-tTG IgA a fost mai mare la pacienţii cu DZ tip 1 (4/111; 3.6%), comparativ cu subiecţii din lotul de control (1/161; 0.6%). Markerii serologici sunt utili pentru identificarea pacienţilor cu boală celiacă şi cu DZ tip 1. Anticorpii asociaţi bolii celiace nu au fost mai frecvenţi la pacienţii cu DZ tip 1 şi anti-GAD pozitivi şi nu a fost observată nici o corelaţie cu vârsta.

***Cuvinte cheie:** boala celiacă, diabet zaharat, anti-tTG IgA, anti-GAD*

Introduction

The frequent association of type 1 diabetes mellitus (T1DM) with other autoimmune conditions (autoimmune thyroid disease, celiac disease, Addison's disease) can affect clinical management of the disease, especially at paediatric age (1). This clustering of diseases is probably due to a shared genetic background, where HLA and non-HLA genes are especially suspected and extensively studied as high genetic risk factors. It was found that the human leukocyte antigen (HLA) genotypes DR3-DQ2, DR4-DQ8 represented the highest-risk for T1DM. DR3-DQ2 genotype show a solid association with CD. Homozygosity for DR3-DQ2 bears an exposure to risk for the presence of anti- tTG autoantibodies in a population with T1DM (2). Celiac disease, a T-cell mediated gluten intolerance in genetically predisposed individuals, is represented and recognized by villus atrophy and malabsorption in the small intestine. The disease occurs after ingestion of foods containing gluten and related proteins by people genetically apt to CD (3). The disease is associated with organ-specific autoantibodies as anti-tissue transglutaminase IgA and IgG, anti-gliadin IgA and IgG and anti-endomysial IgA and IgG. Testing for these autoantibodies may be a helpful possibility to detect subclinical autoimmune disease and to prevent or delay the onset of clinical disease (4).

It has been confirmed that in patients with T1DM, both children and adults, the prevalence of CD varies from 2.3 to 11.1% compared to 0.5% in the general population (5-8). The average age at diagnosis of classical CD is commonly around 2-3 years, while the average age at diagnosis of T1DM is 7-8 years. The age at onset of T1DM is younger in patients with the double disease than in those with only T1DM (9). Children younger than 4 years at the onset of diabetes have a higher risk of CD than those older than 9 years. However, the risk of CD in children is independently and negatively associated with age at onset of diabetes (10). In patients with T1DM, diabetes is usually diagnosed first, CD preceding diabetes onset only in 10–25% (10) – generally, CD diagnosis on T1DM patients occurs, through the screening performed at diabetes onset, in 70–80% of patients with an average age of over 8 years .

Frequently, patients have no symptoms (10), but sometimes lack of stature development, sideropenic anemia or elevated transaminases can be symptoms of non-classical forms of the disease in patients with both T1DM and CD. In these patients, the risk of future complications (intestinal lymphomas, autoimmune diseases, infertility, and osteoporosis) depends on the time of exposure to gluten (5, 7). That is why serologic screening tests are vital in order to establish an early diagnosis of silent celiac disease. In prezent diagnosis is commonly performed by means of anti-tTG IgA (confirmed by EMA) or anti-tTG IgG if IgA-deficiency is present. Times indicated for screening test are at diabetes onset, yearly in the first 4 years of follow up and every

2 years in the successive 6 years of follow up (11, 12). In the presence of CD-related antibodies positivity it is recommended to perform bowel biopsy to confirm diagnosis of CD. Recent guide-lines of ESPGHAN Society (13) proposed that in evident CD-cases it is possible to avoid biopsy.

T1DM is an autoimmune type of diabetes. The beta cells of the pancreas are destroyed by the chronic progressive T-cell-mediated autoimmune process. We can find many markers of this process, like autoantibodies to islet cell (ICA), to glutamate decarboxylase (anti-GAD), and to insulin (IAA). One of these autoantibodies is present at any rate, even if these patients are subject to other autoimmune disorders (1, 14).

The purpose of our study was to determine the prevalence of anti-tTG IgA, as marker of CD, in patients with T1DM and to compare these results with the general population. We also evaluated the frequency of anti-tTG IgA in relation with the presence/absence of anti-GAD antibodies in diabetic subjects. The presence of antibodies was evaluated with special regard to the control of diabetes and to the clinical status of the patient.

Materials and methods

Selection criteria for T1DM patients and healthy controls

The study was carried out between December 2006 and December 2011 at the Diabetes Clinic of Clinical Emergency Hospital for Children from Galati. It included 111 patients with T1DM and 161 control subjects, originating from the same geographic area. All of them were included in the study after a member of family has signed informed consents and completed a validated questionnaire concerning clinical data (age, diarrhea, hypogonadism, anemia, weight loss, and dermatitis herpetiformis). The Ethical committee of the „Dunarea de Jos" University from Galati agreed to this study. The diagnosis of T1DM has been made on clinical history of diabetic ketoacidosis and requirement for insulin, respect-

ing the criteria of the American Diabetes Association (14). Diabetes patients had no intestinal disease, and were not treated with any medication that could affect our tests. The control subjects were chosen from among those who were sent to hospital for a general health examination. None of them had diabetes mellitus or intestinal disease. IgA selective deficiency was considered to be an exclusion criterion. The duration of diabetes was registered for years starting from the date of the diagnosis. All the patients were measured in height and body weight, and their body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Biochemical and antibodies assay

Venous blood samples were taken from all subjects to determine biochemical and antibodies assay, anti-tTG IgA, total IgA and glucose for each participant in our study. Additionally, only for T1DM subjects, anti-GAD and glycosylated hemoglobin (HbA1c) were measured. Glucose and glycosylated hemoglobin were determined after 12 hours of fasting. Anti-tTG IgA was measured using ELISA technique (Anti-tTransglutaminase IgA Antibodies, BioSystems S.A., Spain) and the normal range was 0-12 U/mL. The anti-tTG IgA was positive if the levels were above the normal ranges and negative if the levels were below normal ranges. Anti-GAD (normal range: 0-1.05 U/mL) was assayed by sequential ELISA method (Isletest-GAD, Biomerica INC, U.S.A) and they were defined as positive if they were higher than 1.05 U/mL, and negative if they were between the normal ranges.

To exclude IgA deficiency in patients and control subjects, total IgA level was detected by radial immunodiffusion technique (DIF-FU-PLATE, Biocientifica S.A, Argentina). IgA values were defined as abnormal if they were below or above the normal range.

HbA1c was measured using a fast ion exchange resin supplied by BioSystems S.A., Spain (normal range for healthy people: 4.4 - 6.4%).

For the intestinal histopathologycal analysis, we performed four biopsy specimens,

**Table 1. Descriptive statistics for clinical and paraclinical parameters
in T1DM pacients and control group**

Diagnostic	Parameters	N	Mean	Min	Max	Std. Dev.
T1DM	Age (years)	111	13.42	1.00	25.00	6.00
	Age at onset of diabetes (years)	111	10.13	1.00	24.00	5.10
	Duration of diabetes (years)	111	3.30	0.00	15.00	3.61
	BMI(kg/m^2)	111	18.68	9.50	31.40	3.82
	Hb A1c (%)	111	9.52	4.80	18.90	2.69
	Anti-GAD (U/mL)	111	1.21	0.40	3.50	0.58
	Anti-tTgIgA (U/mL)	111	17.51	2.00	800.00	99.87
	Glucose (mg/dL)	111	282.45	50.0	758.0	149.88
	IgA (mg/dl)	111	144.21	38.00	628.00	90.44
Controls	Age (years)	161	12.32	1.00	25.00	6.29
	Anti-tTgIgA (U/mL)	161	3.15	2.00	23.00	2.46
	Glucose (mg/dL)	161	81.96	55.0	99.00	9.50
	IgA (mg/dl)	161	136.96	38.00	450.00	74.38

which were taken during gastroduodenoscopy from the second part of duodenum. Standard histological stain (hematoxylin eosin) protocol was applied. Slides were graded by conventional histology as normal, with partial villous atrophy, and with subtotal villous atrophy.

Statistical analysis

For statistical analysis, collected data were assessed with SPSS for Windows 11.5. Using descriptive statistics, qualitative data were presented as number and percentage and quantitative data as means and standard deviations. The ANOVA and Student's t-test were used to analyze continuous variables. The degree of associations between variables was determined using simple correlations. P-value was considered statistically significant if it was less than 0.05.

Results

Clinical and paraclinical characteristics of the investigated groups are shown in *Table 1*.

In T1DM group, there were 49 females and 62 males, with a *sex ratio* males/female 1.25. The control group consisted of 61 females and 100 males, with a *sex ratio* males/female 1.63. The mean age of T1DM patients was higher than control subjects, but the difference was not statistically significant (p = 0.152). In terms of gender and age we did not find any statistical difference between patient and control groups (p>0.05). None of the patients and control subjects had IgA deficiency. Anti-tTG IgA positivity was detected in 4 out of 111 (3.6%) T1DM patients and only one out of the 161 control subjects (0.6%). All five subjects with anti-tTG IgA positivity were males. In terms of anti-tTG IgA positivity, between T1DM patients and control subjects there was no statistically significant difference (p = 0.180). Mean value of anti-tTG IgA in T1DM patients was higher than control subjects (17.51 vs 3.15 U/mL). We determined a statistically important difference between patients with or without anti-tTG IgA positivity only for the age at onset of diabetes. The mean age at onset of T1DM patients with anti-tTG IgA positivity was higher than T1DM without anti-tTG IgA positivity (15.25 vs 9.94). Also, there was no statistically significant difference between patients with or without anti-tTG IgA positivity in terms of age, duration of diabetes, BMI and HbA1c (p>0.05).
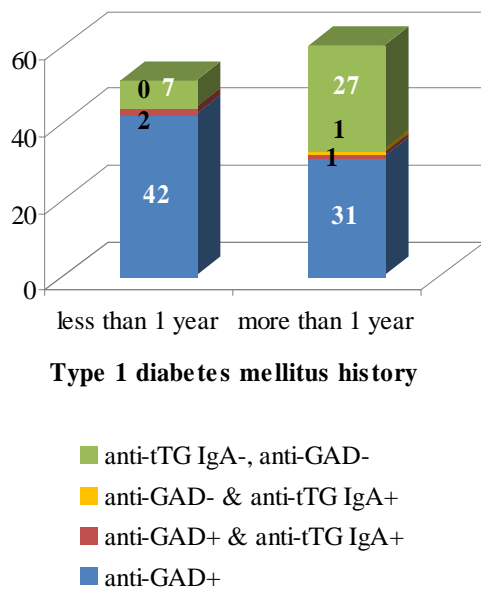
Type 1 diabetes mellitus history

- anti-tTG IgA-, anti-GAD-
- anti-GAD- & anti-tTG IgA+
- anti-GAD+ & anti-tTG IgA+
- anti-GAD+

Figure 1. Distribution of the investigated antibodies in T1DM patients

- Anti-GAD positive T1DM patients
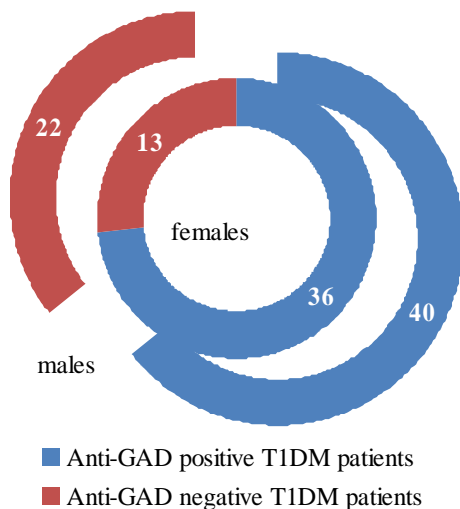- Anti-GAD negative T1DM patients

Figure 2. Sex distribution of anti-GAD positive and anti-GAD negative T1DM patients

Patients diagnosed with diabetes were split into two groups regarding of duration of the disease: the first group was the one having duration of disease less than one year (n = 51) and the second one having more than one year

(n = 60). Anti-tTG IgA positivity was present in four pacients: two belonging to the first group and two belonging to the second one - frequencies statistically not significant (p>0.05).

Anti-tTG IgA titers were not significantly correlated with diabetes duration (p = 0.749), with age at onset of diabetes (p = 0.418), with BMI (p = 0.589) and HbA1c (p = 0.755). In T1DM patients, anti-GAD positivity was detected in 76 subjects (68.5%), 40 males (24.7%) and 36 females (32.7%), mean value: 1.21.

Anti-GAD positivity was present in 44/51 patients whose duration of diabetes was less than one year and in 32/60 patients whose duration of diabetes was more than one year; the dissimilarities between these two groups were statistically not significant (p = 0.169). Also, there was a statistically significant difference between patients with or without anti-GAD positivity, in terms of age, gender, age at onset of diabetes, duration of diabetes and BMI (p < 0.05) (*Table 2*).

Anti-GAD titers were significantly correlated with age (p = 0.037), diabetes duration (p < 0.05) and with HbA1c (p = 0.015). Three from 76 patients with anti-GAD positivity (*Figure 1*) had anti-tTG IgA positivity, the fourth belonging to the anti-GAD negative T1DM group. In terms of anti-tTG IgA positivity, there was no statistically significant difference between the anti-GAD positive and anti-GAD negative patients (p = 0.317). At the same time, there wasn't a statistically important difference on T1DM patients with anti-GAD positivity, between the anti-tTG IgA positive and anti-tTG IgA negative in terms of age, gender, duration of diabetes, HbA1c and BMI (p>0.05).

Discussions

T1DM is often related to other autoimmune diseases, such as celiac disease. It is already known that the prevalence of CD is higher in diabetic patients than in non-diabetic population. The CD seroprevalence in diabetic population has different results in different countries: 2.9% in Austria (16), 6.7% in Germany (10), 21.3% in Czech Republic

**Table 2. Comparison of clinical and paraclinical parameters
in anti-GAD positive and anti-GAD negative T1DM patients**

Parameters	Anti- GAD negative T1DM patients (n=35)				Anti-GAD positive T1DM patients (n=76)				p-value[*]
	Mean	Min	Max	StdDev	Mean	Min	Max	StdDev	
Age (years)	16.89	6.00	25.00	5.27	11.83	1.00	24.00	5.66	0.000
Duration of diabetes (years)	6.38	0.00	15.00	4.26	1.89	0.00	9.00	2.10	0.000
Age at onset of diabetes (years)	10.43	3.00	20.00	4.48	10.00	1.00	24.00	5.38	0.683
Male/Female	22/13				40/36				-
HbA1c (%)	9.90	6.10	18.90	2.67	9.36	4.80	15.30	2.70	0.326
BMI (kg/m^2)	20.44	13.60	31.40	4.19	17.88	9.50	25.50	3.37	0.001
Anti-tTgIgA (U/mL)	7.54	2.00	98.00	21.06	22.11	2.00	800.00	119.83	0.478
Glucose (mg/dL)	293.83	63.00	758.00	151.73	277.21	50.00	700.00	149.74	0.590

* p-value is significant if < 0.05.

(17) and 10.5% in Brazil (18). Genetic, geographical and environmental factors can explain these variations. Differences between methods used for antibody detection could also cause this variability.

In our study, non-diabetic individuals had lower levels of anti-tTG IgA and lower proportions of positive subjects than diabetic individuals. Even if none of the subjects was confirmed for CD by histopathology, the found seroprevalence of 3.6% (4/111) indicates the presence of CD in T1DM children from Galati.

In our study, the average age of T1DM patients at diagnosis of celiac disease was 17.25 years. In a Brazilian study (18), the mean age of 11 years was reported. The involvement of factors such as: deficiency of awareness about CD in diabetes mellitus, deficiency of diagnostic facilities, late referral and population characteristics could determine this dissimilarity. Among our investigated subjects, CD prevails in males, which is in accordance to the mentioned Brazilian research (56.8% males versus 43.2% females) (18).

In literature, the anti-GAD frequency in T1DM has been reported more frequently in females than males (1, 19, 20) and varied from 25% to 70%. Anti-GAD frequency in our study was 68.5% (a little higher in females but without statistical difference versus males). Also, it is known that in more than 70% of children with recent onset T1DM anti-GAD is positive and it has a decreasing trend depending on decreasing number of residual beta cells with the duration of the disease (1, 21). In our study, in patients newly diagnosed with T1DM a slight increased anti-GAD positivity was observed compared to those with a longer duration of diabetes (over one year). Between the two groups, the dissimilarities weren't statistically significant. Similar to our results, Chang and his colleagues did not find any relations between duration of diabetes and gender and anti-GAD positivity (1, 22).

Regarding the association between anti-GAD positivity or negativity and anti-tissue transglutaminase autoimmunity in T1DM, we found no statistically significant correlation. On T1DM patients, typical CD is rare (12). Subclinical forms of disease, less symptomatic and without overt signs of malabsorption, are usually met and request specific therapy because the risk of long-term complications, such as infertility, anemia, osteoporosis, and malignancy.

Conclusions

The prevalence of CD among children and young adults with T1DM is significantly higher and associated with modest/atypical symptoms if we compare to non-diabetic subjects. In type 1 diabetic population, these find-

ings indicate that CD should be by any means taken into consideration. Diabetes specialists and gastroenterologists should strongly suspect CD. Habitual serological screening for CD in T1DM is mandatory because it may be the only way to detect asymptomatic patients.

The CD was seemingly lower in negative anti-GAD patients when compared to positive anti-GAD patients. Therefore, according to this result, a higher risk for CD could be predicted if anti-GAD is positive. Thus, patients with positive anti-GAD should be carefully and more frequently monitored. It has been proved that high morbidity and increased mortality is present in untreated celiac disease. All these facts make serologic screening necessary to detect CD on patients with T1DM. Undoubtedly, modern serology became a reliable noninvasive method for better targeting CD suspected patients although small intestinal biopsy has been the value test for diagnosis. In order to screen high risk groups and the general population, both IgG and IgA tTG antibodies have been extensively used, as recommended by their low cost, easy applicability and reasonably high sensitivity and specificity methods.

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