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Organ Specific Autoantibodies in Preclinical and Early Clinical Type 1 Diabetes in Turkey

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Abstract

Type 1 diabetes mellitus (T1D) patients (G1; n=73) and first degree relatives with islet cell antibody (ICA) values of ≥10 JDF u twice or ≥20 JDF u ones and loss of FPIR (G2; n=18) were screened for two other autoantibodies, anti-glutamic acid decarboxylase (GADA) and insulin autoantibodies (IAA), and for other organ-specific autoantibodies, anti-gastric parietal cell (anti-PCA) and anti-thyroid peroxidase (anti-TPO) as well. The two control groups consisted of healthy subjects (G3; n:55 and G4; n:13). In G1, positivity of ICA, GADA, IAA, anti-TPO and anti-PCA were 63%, 75.1%, 27.4%, 17.8% and 8.2%, respectively. In G2, positivity for GADA, IAA, anti-TPO and anti-PCA were 55.6%, 11.1%, 16.7% and 11.1%, respectively. None of the anti-TPO or anti-PCA positive cases had clinical or laboratory thyroid disease or pernicious anemia.

Other organ specific antibodies, in case they accompany GADA and/or IAA in high risk individuals, result in higher risk for T1D. Moreover, this condition may indicate future potential for developing thyrogastric autoimmune diseases.

In conclusion; autoantibodies are markers for autoimmune destruction in T1D, and for identification of subjects at risk for disease. Even at the time of diagnosis of T1D, screening for thyrogastric autoimmunity might be recommended for early detection of the relevant diseases.

Introduction

Type 1 (insulin dependent) diabetes (T1D) is an autoimmune disease characterized by selective beta cell destruction leading to progressive insulin deficiency and clinical hyperglycaemia, which in turn may cause a number of severe metabolic abnormalities and long-term complications (1). The disease is strongly associated with the presence of islet cell-specific autoantibodies, and these antibodies usually precede by years the development of overt diabetes (2). It has been demonstrated that islet cell antibodies (ICA), antibodies to glutamic acid decarboxylase (GADA) and insulin autoantibodies (IAA) are predictive markers of T1D.

ICAs, described by Bottazzo et al (3) were detected in 80% of newly diagnosed T1D patients (4) and until recently ICAs have formed the basis of risk assessment in T1D, many studies demonstrated the advantages of multiple combinations of autoantibody markers. Glutamic acid decarboxylase (GAD), a cytosolic enzyme, plays a role in the transformation of glutamate into gamma-aminobutyric acid and GADA was detected against 64.000Mr human islet cell antigen as an early marker

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of beta cell autoimmunity (5). Recent studies demonstrated that GAD65-Ab is identified in more adult patients than children with T1D (6). Antibodies reacting with human insulin (IAA) were detected in subjects with newly diagnosed T1D and who had not yet received insulin therapy (7). The highest levels of IAA have been detected in children developing T1D before 5 years of age. Combining two or more predictive antibody tests in series could achieve the best predictive value in T1D. The early detection of populations at risk of developing T1D should be regarded as an important tool in planning trials for prevention of diabetes.

T1D is frequently associated with organ-specific autoimmune diseases, including autoimmune thyroid disease (AITD) and pernicious anemia (8, 9). Abundant epidemiological data supports a genetic basis of T1D and AITD. Each disease is thought to be influenced by multiple susceptibility genes, as well as, environmental factors (10). The high prevalence of anti-thyroid peroxidase antibodies (anti-TPO) in patients with T1D prompts the necessity for further thyroid function evaluation (11). Patients with thyroid autoimmunity may also have higher antibody titers to beta cells (12).

Previous studies have shown a slightly higher prevalence of gastric parietal cell antibodies (anti-PCA) in T1D patients (13). Anti-TPO was also found to be more frequent in anti-PCA positive diabetic patients than in those without anti-PCA, suggesting an association between gastric and thyroid autoimmunity (14).

In this study, the prevalence of organ specific autoantibodies in new onset T1D patients and high-risk first degree relatives of patients with T1D were demonstrated.

Materials and methods

Study population

The study was conducted in a diabetes center in Turkey. Autoantibodies, including ICA, GADA, IAA, anti-TPO and anti-PCA were investigated in four different groups.

Early clinical type 1 diabetic group, Group 1 (G1) consisted of 73 consecutive patients with T1D 25 years of age or younger and having a disease duration of 3 months or less with no other (signs of clinical disease) known autoimmune disease. For detection of the high risk subjects, total 514 first degree relatives of T1D patients diagnosed under 25 years of age were screened for ICA using indirect immunofluorescence (IF) method. First-degree relatives (n:514) selected were 35 years or younger. The selected group consisted of 191 parents (mother/father; 121/70), 304 siblings (female/male; 153/151), and 19 offsprings (female/male; 9/10) without any known autoimmunity. We identified 22 individuals having ICA values of either ≥10 JDF u twice or ≥20 JDF u once. This group consisted of seven parents (mother/father; 2/5), 14 siblings (female/male; 6/8) and one offspring (male). Eighteen of them had a loss of first-phase insulin release (FPIR) less than third percentile of

Table 1. Characteristics of the study groups

Group	Number of subjects (n)	Sex (Female/Male)	Mean age
Early clinical T1D (Group 1)	73	38/35	15.1 ± 8.0
Preclinical T1D (Group 2)	18	5/13	26.5 ± 11.9
Control 1 (Group 3)	55	27/28	13.7 ± 11.4
Control 2 (Group 4)	13	6/7	28.2 ± 6.3

healthy normal individuals were considered as preclinical T1D and were defined as "Group 2" (G2). Because of the difference in mean ages of both groups (G1; mean age 15.1±8.1 and G2; mean age 26.5±11.9) two control groups G3 and G4 were constructed as two distinct groups of healthy individuals (control group 1: G3; n:55, mean age 13.7±11.6 and control group 2: G4; n:13, mean age 28.2±6.3). Characteristics of four groups are summarized in Table 1.

The study protocol was approved by the Ethical Board of the Istanbul Faculty of Medicine. A written informed consent was obtained from the participants or their parents.

ICA assay

ICA was detected by indirect IF method on cryostat sections of human pancreas from a cadaveric organ donor with blood group "0" as described by Bottazzo et al (3). Serum and control samples were incubated for 30 minutes. After a washing for 20 minutes in phosphate buffered saline (PBS), the sections were incubated with anti-human IgGAM-FITC (The Binding Side, Birmingham, UK) for 20 minutes. The sections were scored blindly by IF microscopy (Nikon Optiphot, Japan). Endpoint titers of test samples were converted to JDF u by comparison with a standard curve of log2 JDF u vs. log2 of endpoint titer of the standard sera. The threshold of detection was 4 JDF u (15).

IAA assay

IAA was assayed by IAA-100 (Biosource, Europe SA) radioimmunoassay trade kit. The presence of circulating anti-insulin antibodies was estimated on a semi-quantitative basis, by the determination of the binding of ^{125}I-Tyr-A14 to the serum fraction precipitated by the polyethylene glycol (PEG).

GADA assay

Isletest^{TM}-GAD (Biomerica, USA) trade kit was used for GADA detection. Briefly, GAD-specific IgG antibodies in sera react with the GAD antigen immobilized to microplate. After washing, enzyme-labeled goat anti-human IgG was added to wells and bound to GAD-antibody complex. After incubation and washing, substrate of the enzyme was added and colour changes were measured by ELISA reader (EL_x800 Bio-tek Instruments Inc. USA). The specificity of the assay was 87.1% and the sensitivity was 85%.

Anti-TPO assay

TPOAb RIA C.T (Biocode SA, Belgien), solid phase detection was used for determination of anti-TPO antibodies. The principle of the procedure was the challenge of the bound anti-TPO antibodies with the anti-TPO antibodies in serum samples. Detection limit of the assay was 7 IU/ml. Healthy subjects normally have anti-TPO antibody levels <20 IU/ml.

Anti-PCA assay

Slides with human gastric sections (Inova Diagnostics) were used for the determination of anti-PCA. Serum and control samples were incubated for 30 minutes in dark. After a washing for 5 minutes in PBS, the sections were incubated with IgG-FITC (Inova Diagnostics) for 30 minutes. The sections were evaluated by IF microscopy (Nikon Optiphot, Japan) according to the control samples.

Evaluation of FPIR

FPIR was assessed by an intravenous glucose tolerance test (IVGTT). A bolus glucose load (0.5 g/kg body weight) was given intravenously as a 20% dextrose solution. FPIR was calculated as 1+3 minutes insulin levels. FPIR <42 μu/ml (less than third percentile of normal healthy subjects) was considered loss of early phase insulin secretion (16).

Statistical analysis

Data were expressed as mean±SD. Statistical analysis was performed by Student's t and ANOVA; and Pearson or Spearman tests were used for correlation analysis whenever suitable. A p value of ≤0.05 was considered statistically significant.

Results

Autoantibodies in early clinical T1D

In G1, ICA <10 JDF u was present in 37%, 10–19 JDF u in 26%, and ≥20 JDF u in 37% of cases (Figure 1A). GADA was present in 75.4% with no difference in

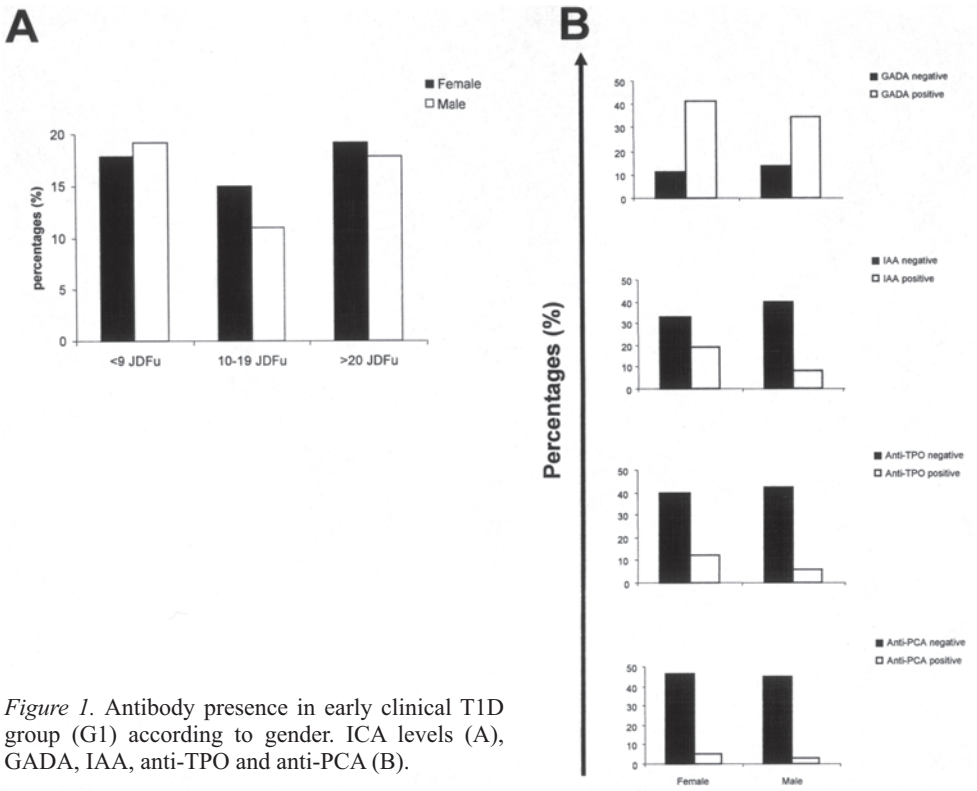

Figure 1. Antibody presence in early clinical T1D group (G1) according to gender. ICA levels (A), GADA, IAA, anti-TPO and anti-PCA (B).

gender (Figure 1B). On the other hand, IAA positivity observed in 20 diabetic patients in G1 (27.4%) was higher in females (Figure 1B). GADA positivity was more common in the >20 years of age subgroup and this was considered statistically significant (p= 0.001). 28.8% had one diabetes associated autoantibody, 47.9% had two and 13.7% had three (Figure 2). Correlation analysis revealed that titers of IAA tended to be higher in females compared to males (r= 0.26, p= 0.026). Furthermore, it was determined that GADA presence was positively correlated with age (r= 0.502, p<0.001).

Gender did not affect anti-TPO antibody positivity seen in 17.8% of the subjects (Figure 1B). Anti-PCA was present in 8.2% of the cases (Figure 1B). The titers of anti-TPO antibodies tended to increase with longer diabetes duration (data not shown). The presence of anti-PCA was positively correlated with anti-TPO positivity (r= 0.247, p= 0.039).

Compared to G1; ICA was not observed in G3, and GADA positivity was higher in G1 compared to G3 (p<0.001 and p<0.001, respectively). Similarly, IAA was not observed at all in G3 (p<0.001). The positivity in anti-TPO antibodies was observed at a rate of 17.8% in G1, as opposed to 1.8% in G3 (p<0.004). On the other hand anti-PCA in G1 was 8.2% as opposed to 3.6% in G3, but this difference did not reach to a statistical significance.

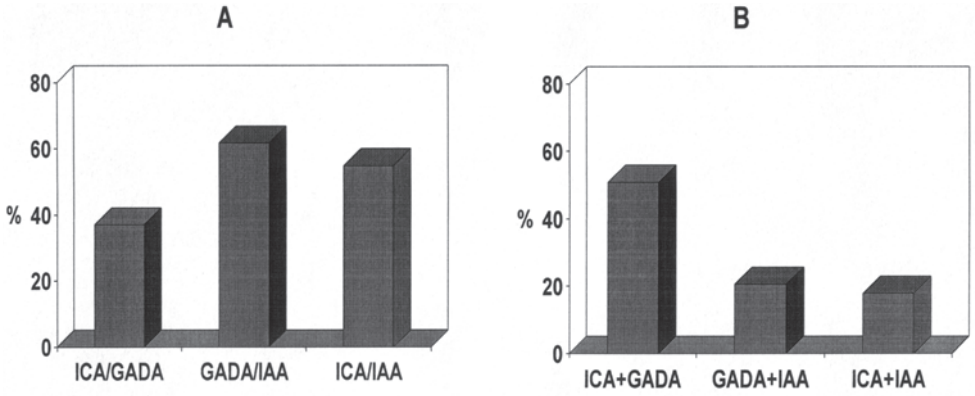

Figure 2. Prevalence of multiple autoantibodies in early clinical T1D Cases. (A). The percentages of ICA or GADA, GADA or IAA, ICA or IAA positive subjects in early clinical T1D. (B) The percentages of ICA and GADA, GADA and IAA, ICA and IAA positive subjects in early clinical T1D.

Assessment of high-risk individuals

For detection of relatives with high risk for diabetes, 514 relatives consisting of 191 parents (mother/father; 121/70), 304 siblings (female/male; 153/151) and 19 children (female/male; 9/10) were screened for ICA. The first screening results are summarized in Table 2.

Relatives with ICA values 10–19 JDF u were screened again for ICA in a second sample obtained within two weeks of the first sample and only 14 subjects (2.7%) consisting of 1 mother, 3 fathers, 9 siblings and 1 child had ≥10 JDF u by the second ICA screening. These 14 relatives taken together with 8 relatives having ICA values ≥20 JDF u underwent to an IVGTT. FPIR was lost (1+3 minutes insulin <42 μu/ml) in 18 of these subjects. We considered them as preclinical T1D patients, and they conducted G2.

Table 2. The distribution of the first ICA screening among first degree relatives of T1D patients

ICA titer	Mother	Father	Sibling	Offspring	Total
<10 JDF u	112 (24.6%)	59 (13.0%)	268 (59.0%)	17 (3.7%)	456 (100.0%)
10–19 JDF u	8 (16.0%)	9 (18.0%)	31 (62.0%)	2 (4.0%)	50 (100.0%)
≥20 JDF u	1 (12.5%)	2 (25.0%)	5 (62.5%)	0 (0.0%)	8 (100.0%)
Total	121 (23.6%)	70 (13.6%)	304 (59.1%)	19 (3.7%)	514 (100.0%)

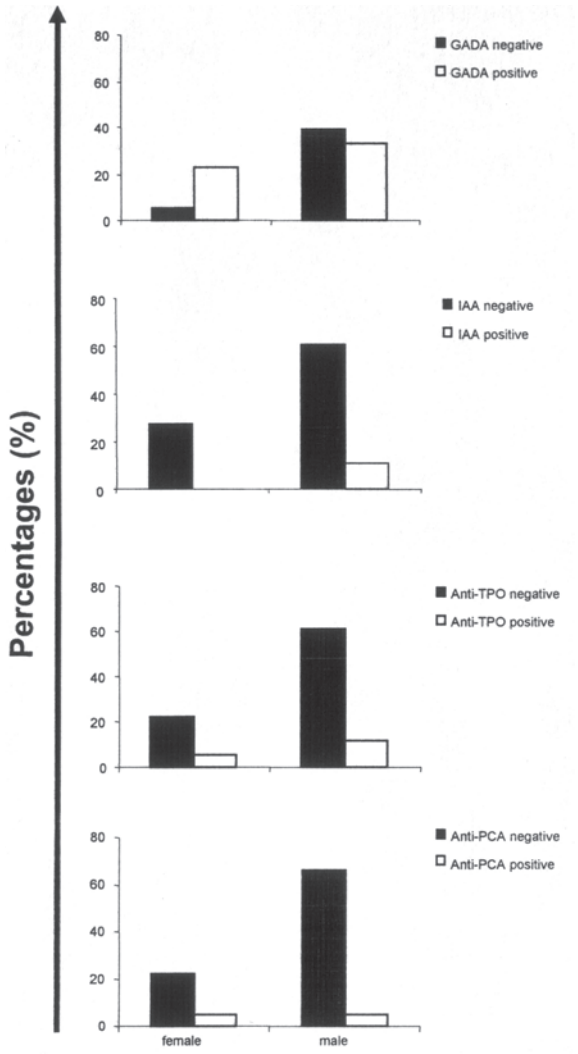

Figure 3. Antibody presence in preclinical T1D group (G2) according to gender.

Autoantibodies in preclinical T1D patients (G2)

In preclinical T1D group (G2) consisting of 18 subjects the presence of ICA and GADA showed no difference with respect to gender (Fig 3). IAA was present in 11.1% of the cases, but was not considered statistically significant because of the low number of the subjects in the group. 38.9% had only one diabetes associated antibody, where as 55.6% had two and 5.6% had three autoantibodies (Figure 4).

Anti-TPO antibody positivity was seen in 3 subjects, namely one female (5.6%) and two males (11.1%). Anti-PCA positivity was observed in 2 subjects, one female (5.6%) and one male (5.6%). Anti-TPO antibodies in this group were found to be positively correlated with anti-PCA in positivity and titers as well ($p<0.001$, $r=0.791$ and $p=0.001$, $r=0.707$, respectively) (Figure 3).

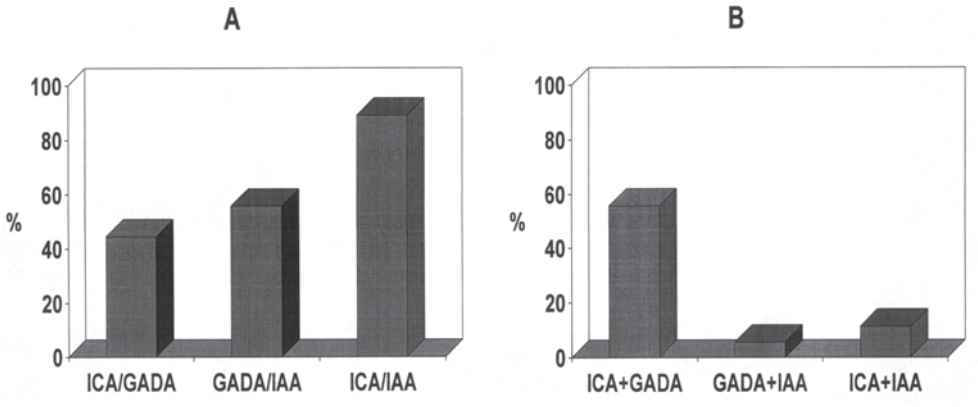

Figure 4. Prevalence of multiple autoantibodies in preclinical T1D cases. (A). The percentages of ICA or GADA, GADA or IAA, ICA or IAA positive subjects in preclinical T1D. (B) The percentages of ICA and GADA, GADA and IAA, ICA and IAA positive subjects in preclinical T1D.

None of the assayed antibodies were positive among the 13 members of G4 except for anti-PCA positivity in one female (7.7%).

High ICA positivity (≥20 JDF u) observed at a rate of 61.1% in G2 was not observed at all in G4 ($p<0.001$). GADA positivity was higher (55.6 %) in G2, but was not detected at all in G4 (0%) ($p<0.002$). On the other hand, the increase of IAA positivity observed in G2 (11.1%) compared to G4 (0%) was not deemed statistically significant.

The difference in positivity of anti-TPO and anti-PCA in G2 and G4 (16.7% vs 0% and 11.1% vs 7.7%, respectively) was also not considered statistically significant. None of the anti-TPO or anti-PCA positive cases in all study groups had any clinical or laboratory thyroid disease or pernicious anemia.

Discussion

In this study the prevalence of several islet-specific and other organ-specific autoantibodies in Turkish subjects with preclinical T1D were evaluated.

T1D is a chronic autoimmune disease with a long prodromal period. Screening subjects with high risk for the disease, especially relatives of T1D patients, in this time period gives opportunity to plan trials for the prevention of the disease. Strategies to identify subjects at risk for T1D are largely based on the detection of autoantibodies directed to beta cell antigens. Prediction of the disease is still largely based on ICAs. In this study ICA was screened for detecting subjects at risk for T1D and ICA, GADA, IAA, anti-TPO and anti-PCA antibodies were compared in preclinic T1D, newly diagnosed T1D patients and control subjects.

Several studies showed high frequencies of diabetes-specific autoantibodies in first degree relatives of T1D patients (17, 18). In Denis study it was also shown that the frequency of ICA and GADA was higher among siblings as compared to parents

of patients with T1D. Our study demonstrated slightly higher titers of ICA in fathers (5.7±11.9 JDF u) compared to mothers (2.4±4.5 JDF u) and siblings (3.4±7.5 JDF u), but in contrast to the literature, ICA positivity did not differ among relatives.

Studies performed in first degree relatives of T1D patients determined ICA levels ≥ 20 JDF u in 0.9 to 3.0% of the screened subjects (19, 20, 21). In this study, high titers of ICA (≥20 JDF u) in 1.6% of the first degree relatives were also found, but titers were not different among the subjects according to the relativity.

T1D is characterized by autoimmune destruction of beta cells starting before clinical disease and during this destruction several autoantibodies (ICA, GADA and IAA) directed to islet antigens detected in serum samples of the subjects. One study assessed the prevalence of GADA and ICA in 171 T1D patients and showed the rates of GADA and ICA to be 64.9% and 46.2%, respectively (14). We also detected prevalences of GADA and IAA to be 75.4% and 27.4% in T1D subjects similar to the literature (22, 23, 24).

It has been shown that GADA and IAA in recent onset T1D patients were more frequent among female (25), similarly our findings support that IAA titers were higher in females compared to males ($p=0.026$, $r=0.26$). The same study demonstrated higher GADA titers in older patients as shown in our study ($p<0.001$, $r=0.502$).

Many studies investigated organ-specific antibodies like anti-TPO and anti-PCA in T1D patients and found higher prevalences of these antibodies compared to controls (26). In a study performed in Thailand anti-TPO antibodies were detected 30% in 50 T1D cases and the frequencies were higher in females (27). A study in Giessen demonstrated higher thyroid autoimmunity in girls with T1D and the positive correlation with age (28). Many other studies also found higher prevalences of thyroid autoimmunity in T1D patients (29, 30). In our study higher thyroid autoimmunity in T1D patients were also found compared to control subjects ($p<0.004$).

In a study investigating patients with T1D having disease duration of 16.4±10.4 for gastric autoimmunity demonstrated high frequency of anti-PCA in older cases, but gender and age at onset of diabetes did not influence the appearance of anti-PCA (14). The same study also suggested an association between gastric and thyroid autoimmunity. In an other study performed by the same group, it was shown that thyrogastric antibodies were highly prevalent in T1D, especially in patients with persisting ICA (31). Although the higher frequency of anti-PCA in diabetic group (8.2%) compared to controls (3.6%), this study did not demonstrate any statistical significancy possibly according to the low number of subjects. The lower incidence of anti-PCA in this study compared to previous studies was considered for the younger age of the cases. However, a positive correlation between anti-PCA and anti-TPO antibodies was found.

A lot of studies focused on the first degree relatives of T1D patients. It was found that life-table analysis showed a 43% risk of T1D for those with ICA ≥10 JDF u, rising to 53% for those with ICA ≥20 JDF u and the risk was increased with the number of autoantibodies (2). Subjects having ICA levels >80 JDF u were shown to develop diabetes within a follow-up of 7 years (32).

GADA combined with ICA was found to increase the sensitivity and specificity of risk assessment in diabetes (33). In a study GADA was found in 43% of ICA positive first degree relatives and the levels of GADA was influenced by age, gender and ICA status (34). Many studies demonstrated the correlation of ICA and GADA in T1D (35, 36). Although the higher frequency of GADA in relatives with ICA ≥20 JDF u once or ≥10 JDF u twice, we did not find any correlation between ICA and GADA, but GADA correlated positively with age which is also demonstrated by previous studies (data not shown).

The prevalence of IAA was also found to be higher in ICA positive first degree relatives of T1D subjects like in patients with T1D (37, 38). Higher prevalence of IAA in siblings (mean age 16.6 years) compared to parents (mean age 44.1 years) was detected (39).

The presence of IAA was shown in 11.1% of relatives considered as having risk for T1D, the higher mean age of the study group (mean age 26.5±11.9) may contribute to a relatively lower prevalence of this antibody as IAA has more predictive value for subjects under five years of age. We could not measure more specific autoantibodies, like IA-2 or IA-2 ϐ due to technical reasons.

There are many studies demonstrating extrapancreatic (gastric, thyroid etc) autoantibodies in first degree relatives of patients with T1D with positive ICA levels but no diabetes (40, 41). Anti-TPO and anti-PCA prevalence in subjects at risk for T1D (16.7% and 11.1%, respectively) were also higher compared to controls (0% and 7.7%, respectively) in this study, however the difference did not reach to any significancy.

In conclusion; the detection of autoantibodies targeting antigenic determinants of the islet cells are important markers for the autoimmune destruction of the beta cells in T1D, and for identification of the subjects at risk for the disease. Moreover, the study pointed out that even at the time of diagnosis of T1D, screening for thyrogastric autoimmunity might be recommended for early detection of the relevant diseases.

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