Relative Frequency of Islet Autoimmunity in Children and Adolescents with Autoimmune Thyroid Disease

Putarek NR et al. Islet Autoimmunity in Autoimmune Thyroiditis

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What is Known
• Two most common autoimmune endocrine diseases, diabetes mellitus type 1 (T1D) and autoimmune thyroid diseases (AITD) - autoimmune thyroiditis and Graves’ disease, are often found in the same patient and/or within the same families.
• Thyroid autoimmunity was widely studied in T1D patients, but few studies examined islet autoantibodies and the risk of development of T1D among AITD.

What is New
• This is the first comprehensive study that included children/adolescents with both AITDs (autoimmune thyroiditis and Graves’ disease) and in which islet cell autoimmunity was estimated by measuring three islet autoantibodies.
• Observed relative frequency of T1D development in AITD patients was much higher than in the general Croatian population (3.7% vs. 0.2%).
• This study was the first to evaluate islet autoimmunity and glucose metabolism in family members of AITD patients with islet autoantibodies. As five of 20 family members were found to have impaired glucose tolerance/islet cell autoimmunity, we propose that the family members of AITD patients have an increased risk of developing T1D. However, our observation should be evaluated and confirmed in a more significant number of patients.

Abstract
The present study aims to investigate islet autoimmunity and susceptibility to type 1 diabetes (T1D) in children/adolescents with autoimmune thyroid disease (AITD), and family members of AITD patients with islet autoimmunity. Islet-cell cytoplasmic, glutamic-acid decarboxylase, and tyrosine-phosphatase autoantibodies were measured in 161 AITD patients (127 with autoimmune thyroiditis (AT); 34 with Graves’ disease (GD)), 20 family members of AITD patients with islet autoimmunity, and 155 age-matched controls. Islet autoimmunity was found in 10.6% of AITD patients, significantly more frequent than in controls (1.9%; p=0.002). A higher prevalence of islet autoantibodies was found in females with AT (p=0.013) but not in males (p=0.16) and AT (p=0.001) but not in GD patients (p=0.19), compared to corresponding controls. Two or three islet autoantibodies were found concurrently in six AITD patients with islet autoimmunity. They all developed T1D and had significantly higher islet autoantibody titers (p<0.001) than AITD patients with single islet autoantibodies but normal glucose metabolism. T1D was found in 0.7% of AITD patients compared to 0.2% of the age-matched, general Croatian population. Islet autoantibodies were found in 5/20 family members of AITD patients with islet autoimmunity, among which two developed T1D. None of the controls was positive to more than one islet autoantibody or developed T1D.

Conclusion: Children/adolescents with AITD (particularly females and patients with AT) represent a risk group for islet autoimmunity and T1D, as well as family members of AITD patients with positive islet autoantibodies, but the last observation must be examined in a more significant number of patients.

Keywords: Autoimmune thyroid disease, islet autoimmunity, screening, diabetes mellitus type 1, children

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Introduction

Autoimmune endocrine diseases are organ-specific diseases in which the immune response target organs are endocrine glands (1). The most common of these diseases is diabetes mellitus type 1 (T1D) and two autoimmune thyroid diseases (AITD): autoimmune thyroiditis (AT) and Graves’ disease (GD). Of all autoimmune endocrinopathies that co-occur, ATID and T1D are far more often found in the same person or family (1,2). This phenotype is classified as a variant of the autoimmune polyendocrine syndrome type 3 (APS3v) (3,4).

Thyroid autoimmunity was widely studied in T1D, and thyroid autoantibodies (AAb) were found in about 8-44% of patients with T1D (5). One study carried out a clinical form of APS (5). On the other hand, few studies examined islet autoimmunity and risk for the development of T1D among AITD patients, and only four were conducted in children and adolescents (6–9). One study was performed in children with AT and GD (6) and three others in children with AT (7–9).

Among studies conducted up to date, these were exclusively carried out in patients with AT (10,11) or GD (12,13), while others included patients with both conditions (14–19). Islet autoimmunity was assessed by measuring different diabetes-associated AAb as serological markers of β-cell autoimmunity, which included glutamic acid decarboxylase (GAD), islet cell cytoplasmic autoantibodies (ICA), insulin (IAA), perforin, and zinc-transporter 8 (ZnT8) AAB. T1D, type 1 diabetes; T2D, type 2 diabetes; TPO, thyroid peroxidase; Tg, thyroglobulin; IAA, islet autoantibodies; ICA, islet cell antibodies; IA2, islet cell autoantibodies.

Parameters in the study

In line with growing interest in the early detection of persons and groups at risk for T1D development, this study aimed to assess the relative frequency of hormonal markers of autoimmunity to islet cells (ICA, GAD, IA2) in children and adolescents with AITD as a group and separately among AT and GD patients. Additionally, we wanted to determine the relative frequency of T1D in the same group of patients and family members (parents, siblings) of patients with AITD and islet autoimmunity. In AITD patients and family members who tested positive for islet AAb, glucose metabolism was assessed to evaluate for T1D.

Patients and methods

Patients

The prospective observational study included 161 patients with AITD divided into two groups [127 with AT (29 males and 98 females, aged 4.17-19.0 years) and 34 with GD (7 males and 27 females, old 6.5-21.9 years)]. All patients were treated at the Department of Pediatric Endocrinology and Diabetes, University Hospital Center Zagreb. Patients are recruited as consecutive patients from the outpatient clinic from June 2012 to December 2014 and followed up until June 2018. Patients affected by any syndromic or another known genetic disease (e.g., Turner, Down, or Klinefelter syndrome, as well as polyglandular syndromes) were excluded from the study.

The control group consisted of 155 patients (52 males and 103 females, aged 4.0-21.5 years) admitted to the Department of Pediatrics, University Hospital Centre Zagreb, for evaluation of other non-chronic diseases whose clinical history was negative for thyroid autoimmunity and other autoimmune disorders, and who had no family history of T1D and AITD. Additionally, 20 family members (18 parents and two siblings) of patients with AITD and positive islet AAb were included in the study.

AT diagnosis was based on elevated titters of IAA against thyroid peroxidase (TPO) and thyroglobulin (Tg) and thyroid ultrasound examination consistent with this diagnosis. Since measurement of TSH receptor antibodies was unavailable, the diagnosis of Graves disease was based on clinical and biochemical findings of hyperthyroidism, thyroid ultrasound, and Doppler examination. Only patients with persistent biochemical results of hyperthyroidism and requiring antithyroid medication during follow-up of 3.5-6 years were labeled as GD patients for hyperthyroidism due to AT.

The University Hospital Center Zagreb Ethics committee and the University of Zagreb School of medicine ethics committee approved the study protocol following the Declaration of Helsinki, and informed consent was obtained from all participants and/or their parents.

Parameters in the study

In both patient and control groups, the titers of Tg, TPO AAb, and islet AAb (GAD, IA2, and ICA) were assessed at the time of evaluation. Islet AAb were also measured in 20 family members (10 mothers, eight fathers, and two sisters) of 10 AITD patients with islet autoimmunity. In 16 patients with AITD and islet autoimmunity, four of their family members with islet autoimmunity, and three control subjects with islet autoimmunity glucose metabolism was evaluated with oral glucose tolerance test (OGTT) and HbA1c (using the immupass plus method in Siemens Alc Vantage Analyzer, Siemens Healthcare GmbH, Erlangen, Germany) according to ISPAD criteria (22).

Determinations of GAD and IA2 AAb were performed by commercial ELISA kits (EUROIMMUN, Germany). In 2010 Clinical Institute of Laboratory Diagnosis, University Hospital Merkur, Zagreb, participated in Diabetes Assay Standardization Program - DASP. Sensitivities and specificities were 88% and 94%, respectively, for GAD, and 72% and 99%, respectively, for IA2 AAb. The cut-off for positive results was set at 5 Units/mL for GAD and 10 Units/mL for IA2 antibodies (23).

Detection of ICA autoantibodies was performed by indirect immunofluorescence. Scores of fluorescence intensities were then calculated into Juvenile Diabetes Foundation units (JDF). Results >5 JDF units were considered positive. ICA assays were validated by repeated participation in the immunology of diabetes workshops and proficiency testing programs of the University of Florida (Gainesville, FL) with >95% sensitivity, specificity, consistency, and validity (24). The quality of our performance was validated by continuous yearly participation in Instand EQA schemes. The cut-off values of positivity for TPO and Tg AAb were 20.0 Units/mL and 60.0 Units/mL, respectively. The manufacturer provided reference ranges and cut-off values for the enzyme-linked immunosorbent assay (ELISA Brahms GmbH, Hemmingdorf, Germany) methods, with results higher than the cut-off values set by the manufacturer were considered positive.

Statistical analysis

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Data are presented in tables using descriptive statistics (frequencies, means, and standard deviations). The patient groups were compared using the appropriate tests, depending on the data type and distribution (Chi-square test, Fisher exact test, and the Mann-Whitney U test for unpaired data). Statistical Package for the Social Sciences version 21.0 (IBM SPSS Statistics, USA) was used for calculations, and p<0.05 was considered significant.

Results

Islet AAb and glucose metabolism in AITD patients

Islet autoimmunity was significantly more frequent in patients with AITD (10.6%) than in the control group (1.9%; p=0.002). The relative frequency of AAb in AT patients (18.5%; p=0.001) but not in GD patients (5.9%, p=0.19), as compared to controls (Table 1). AITD patients with islet autoimmunity were slightly younger at the time of evaluation (median 11.6 years) than those without islet autoimmunity (median 12.8 years). Still, the difference was not statistically significant (Mann-Whitney test: U=1071, z=-1.26; p=0.21).

Relative frequencies of all islet AAb were significantly higher in AITD patients than in controls (ICA p=0.04; GAD p=0.002; IA-2 p=0.02; Table 2). The clinical and laboratory characteristics of patients with AITD and islet autoimmunity are summarized in Table 3. Three out of 17 AITD patients with islet autoimmunity (patients #1-3) were positive for three islet AAb, and an additional three patients (patients #4-6) were positive for two islet AAb. In contrast, none of the control subjects was positive for more than one islet AAb (Tables 2 and 3).

There was no statistically significant difference in the frequency of islet autoimmunity between sexes (5.6% males and 12% females with AITD, p=0.27). A statistically significant difference in total islet autoimmunity was found in females with AITD compared to females in the control group (12% vs. 2.9%, p=0.011) but not among males with AITD (5.9% vs. 0%, p=0.16) as compared to males in the control group.

When analyzed separately, statistically significant differences were found in frequencies of GAD (7.2%) and IA-2 AAb (5.6%) in females with AITD compared to females in the control group (p=0.005 and 1%; p=0.06, respectively). Statistically significant differences were not found in frequencies of ICA AAb in females with AITD (6.4%) compared to females in the control group (1.9%; p=0.10), as well as in frequencies of all three AAb in males with AITD (ICA 2.8%; GAD 2.8%; IA-2 0%) compared to males in the control group (0%).

At the time of evaluation, T1D was diagnosed in 1/16 AITD patients with islet autoimmunity (patient #1, HbA1c 8.8%, 83.6 mmol/mol (Table 3), and one patient had impaired glucose tolerance and normal HbA1c (patient #3, Table 3). The remaining 14 patients had normal blood glucose levels in OGTT and normal values of HbA1c. During the 6-year follow-up, 5/16 AITD patients with islet AAb (4 females and one male, patients #2-6; Table 3) developed T1D. Patient #1 was lost to follow-up, and her glucose metabolism was not investigated. Six patients who developed T1D all tested positive for two or three AAb. All these patients were younger than 15 at the T1D diagnosis. The relative frequency of T1D in our AITD patient cohort was 3.7%, while in patients younger than 15 years of age, the relative frequency was 4.8%.

According to the Croatian registry of diabetes in children and adolescents, the prevalence of T1D in the general age-matched Croatian population is 0.2% (unpublished data).

AITD patients with islet autoimmunity who developed T1D had significantly higher titers of islet AAb in comparison to AITD patients with islet autoimmunity and normal glucose metabolism (Mann-Whitney test: N=16, Table 3: ICA – U = 6.5; z=-2.50; p=0.01; GAD– U = 7.0; z=-2.44 p=0.02; IA2– U = 7.5; z=2.51; p=0.01). None of the patients in the control group with positive islet AAb developed T1D during the follow-up.

Islet autoantibodies and glucose metabolism in family members of patients with AITD and islet autoimmunity

Positive islet AAb was found in 5/20 family members of patients with AITD and islet autoimmunity (3/9 fathers and 2/10 mothers). The mother of patient #1 was positive for three islet AAb and was diagnosed with T1D at the age of 19. The other four patients (2/9, 4b, 11a, and 11b) with positive islet AAb had normal levels of glucose in OGTT and normal values of HbA1c at the time of evaluation. After the first evaluation, HbA1c was measured every 6-8 months. The father of patient #2, with positive GAD and ICA AAb, developed T1D after six years of follow-up at the age of 46 (Supplement 1).

Antithyroid AAb in AITD patients

AITD patients with islet autoimmunity had higher TPO and Tg AAb titers compared to AITD patients without islet autoimmunity, but the difference was not statistically significant (Mann-Whitney test: Tg-Ab– U=1126, z=-0.28; p=0.78; TPO – U=1768, z=-2.05; p=0.04).

Discussion

Screening for the risk of T1D is gaining more attention worldwide. The long-term vision for T1D screening programs is to identify individuals at risk of T1D and to offer them interventions to delay or prevent the condition (25). But other essential factors, such as matching the long-term vision for T1D screening programs and currently achievable clinical benefits, drive the current recommendations for screening. It is shown that screening programs significantly reduce diabetes ketoacidosis (DKA) rates, usually to less than 5%, and reduce hospitalization when coupled with long-term monitoring (26-29). DKA prevention at diagnosis has potential lifelong benefits, including avoidance of acute morbidity, neurocognitive impairment, and mortality (30,31). Other non-less worthy benefits are to prepare children and families for a smoother transition to insulin therapy and advance preventative treatments through clinical trial recruitment (25).

In the study, we analyzed three islet AAb (ICA, GAD, and IA2) in children and adolescents with AITD (AT and GD) to assess this group of patients as a possible target for a T1D screening program. All of our patients were positive for thyroid AAb before inclusion in the study, allowing us to select and follow the patients that developed thyroid autoimmunity before the onset of T1D. We found that 10.6% of AITD patients were positive for one or more islet AAb, significantly more than in controls (1.9%; p=0.002). This difference was entirely due to antibodies found in AT patients compared to controls (11.8% vs. 1.9%; p=0.003), as we found no significant difference in islet autoimmunity between the GD patients and controls.

However, due to the small number of patients in the GD group, the relationship between GD and islet autoimmunity cannot be excluded.
When analyzing islet AAb separately, all three AAb were significantly more frequent in AITD patients compared to the control group (ICA p=0.002, IA-2 p= 0.001), and GAD AAb was found only in the AITD patients but not in controls. Few studies reported the frequency of ICA autoimmunity in patients with ATID; only four included children and adolescents (6–9). Bright et al. found ICA AAb in 2.3% of children with ATID compared to 0% of controls (6). In studies conducted in adult AITD patients, ICA positivity ranged from 0 - 4.9% (12,13,15,17). Only one study evaluated the frequency of IA2 AAb in children with AT (but not with GD) and found them to be more common than in control subjects (3.39% vs. 1.16%, p=0.012) (7), as was confirmed in our study. In one study on adult patients, IA2 AAb was found more frequently in patients with GD (18).

GAD positivity was assessed in three studies conducted on children with AT (7,9). In two (8,9), GAD AAb was found significantly more often in children with AT than in controls (9.8-10.6% vs. 0-3.3%, p<0.003 and p=0.036, respectively), as was was confirmed in our study. However, in the survey by Pilia et al., the difference was not significant (7). Several studies analyzed GAD autoimmunity in adult patients with AT (10,11,14–16,19) and GD (12,13,15,16). Relative frequency of GAD AAb ranged from 2.8-6.6% (10,11,14–16,19) in patients with AT. Although the correlation between GAD AAb and AT was found in some studies (15), it was not always statistically significant (16). In adult GD patients, GAD AAb was found in 6-13% of patients (12,13,15,16), significantly more common than controls in some studies (15,16). However, the evaluation only sometimes included control subjects (10–14).

We did not find significant differences in islet autoimmunity between males and females with AITD. However, females with AITD were positive to islet AAb (GAD and IA-2 AAb, but not to ICA AAb) significantly more often than males in the control group. On the other hand, in males with AITD, we did not find any difference in islet autoimmunity compared to controls. As thyroid autoimmunity and ATID are more common in females, the female gender per se is proposed as a risk factor for the positive association between islet autoimmunity and thyroid autoimmunity (32).

In 16 patients with ATID who were positive for islet AAb, the susceptibility for T1D development was assessed. Upon initial evaluation, one patient was diagnosed with T1D, and five developed T1D during the follow-up period of six years (12,13,15). However, we cannot exclude that more patients would develop diabetes if the follow-up were longer. In Bright et al. study, one of two children with AT and positive ICA AAb developed diabetes after one year, and two children with AT and negative ICA AAb after four and six years, respectively. Pilia et al. (7) reported that over two years of follow-up, 19 children with AT and islet autoimmunity developed T1D (one positive to GAD AAb and the other to GAD and IA-2 AAb). Lethagen et al. (10) found reduced ability for insulin secretion in GAD-positive AT patients and concluded that GAD AAb might be a marker of subclinical insulin. During the follow-up of 4 years, 2/15 of their GAD AAb-positive patients (compared to 1/426 GAD AAb negative patients) were diagnosed with diabetes (p=0.008) (10). Lethagen et al. (13) followed nine GAD AAb-positive patients (two also ICA-positive) for 27-70 months. One patient, who was positive for both islet AAb, developed diabetes; Maugender et al. (12) found a high frequency of GAD AAb (6.1% of AT patients) but a low progression toward diabetes (only one patient). Aseyo et al. (11) studied insulin sensitivity and secretion patterns in GAD AAb positive and GAD AAb negative AT patients. They concluded that it is not likely that the presence of GAD AAb per se is associated with a disturbance in glucose metabolism. A significant relationship between the higher titer of GAD AAb and abnormalities of glucose metabolism was found in the studies by Marhawa et al. (18) and Mogurengthi et al. (16). Kawasaki et al. (15) did not report similar findings. The observed relative frequency of T1D development in our patients with ATID was compared to that in the Croatian general population. In our cohort, T1D was diagnosed in 3.7% of ATID patients, much more frequent than in the general population in the same age groups (0.2%) (31, Croatian registry of diabetes in children and adolescents, unpublished data).

In our study, AITD patients who developed T1D had significantly higher titers of GAD AAb (p=0.02) than ATID patients with islet autoimmunity and normal glucose metabolism. Moreover, we noticed significantly higher titers of ICA and IA-2 AAb (both p=0.01) in this patient group.

We further measured TPO and Ig AAb titer and found higher titers in AITD patients with islet autoimmunity compared to ATID patients without islet autoimmunity. However, the difference was not statistically significant, as was observed in some other studies (15,17). On the other hand, Marwanch et al. (12) found that GAD AAb levels increased with an increasing titer of TPO AAb.

Islet autoimmunity and susceptibility to T1D were evaluated in 20 first-degree family members of patients with AITD and positive AAb. Prospective long-term studies on a large number of subjects are required to examine the factors responsible for islet destruction, insulin deficiency, and evolution toward diabetes in patients with ATID and the correlation to AT or GD separately.

To the best of our knowledge, our study was the first to evaluate islet autoimmunity and glucose metabolism in family members of AITD with islet AAb, indicating their increased risk for developing T1D. Still, this observation must be verified in more extensive studies.

### Study Limitations

The measurement of anti-TSH receptor antibodies were not available in our institution at that time and therefore not performed at diagnosis of GD. However, in a group of GD patients with clear clinical, biochemical, and ultrason signs of disease, we did not find significantly different only those who did not develop hypothyroidism during follow-up.

Moreover, we did not test family members of AITD patients without positive islet AAb for the development of T1D. It would be necessary to confirm the results found in family members of patients with ATID on a more significant number of subjects,
both those with and without islet autoimmunity, to determine the risk for glucose metabolic impairment in relatives of patients withAITD.

**Authorship Contribution**

Concept: Natasa Rojnic Putarek, Miroslav Dumic, Design: Natasa Rojnic Putarek, Miroslav Dumic; Data Collection or Processing: Natasa Rojnic Putarek, Nevena Krnic, Maja Baretic; Analysis or Interpretation: Jadranka Knezevic-Cuca, Venna Kusec; Supervision: Miroslav Dumic; Final Version of the Manuscript: read and approved by all the authors.

**References**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Patients with islet autoimmunity (%)</th>
<th>Chi-square/p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AITD (n=161)</td>
<td>17 (10.6%)</td>
<td>9.91 /0.002</td>
</tr>
<tr>
<td>AT (n=127)</td>
<td>15 (11.8%)</td>
<td>11.39 / 0.0007</td>
</tr>
<tr>
<td>GD (n=34)</td>
<td>2 (5.9%)</td>
<td>1.68 / 0.19</td>
</tr>
<tr>
<td>Control group (n=155)</td>
<td>3 (1.9%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: The frequency and percentage of islet autoimmunity in patients with AITD subdivided into groups: AT and GD compared to controls. The results of chi-square and p-value (<0.05) between the patients positive to islet autoimmunity in all groups compared to controls are presented.
Table 2. The frequency and percentages of islet autoantibodies in AITD patients and controls are presented. Statistical significance for Chi-square or Fisher between the two groups is presented.

<table>
<thead>
<tr>
<th>Islet AAb n(%)</th>
<th>AITD (n=161)</th>
<th>Controls (n=155)</th>
<th>P (Chi-square or Fisher’s exact*)</th>
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<tr>
<td>ICA</td>
<td>9 (5.6%)</td>
<td>2 (1.3%)</td>
<td>0.04</td>
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<tr>
<td>GAD</td>
<td>10 (6.2%)</td>
<td>0 (0%)</td>
<td>0.002*</td>
</tr>
<tr>
<td>IA-2</td>
<td>8 (5.0%)</td>
<td>1 (0.6%)</td>
<td>0.02</td>
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One islet AAb (n)

<table>
<thead>
<tr>
<th>Islet AAb (n)</th>
<th>AITD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>GAD</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>IA-2</td>
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<td>1</td>
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Two islet AAb (n)

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<th>Islet AAb (n)</th>
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<th>Controls</th>
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<tbody>
<tr>
<td>ICA + GAD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ICA + IA-2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>GAD + IA-2</td>
<td>1</td>
<td>0</td>
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Three islet AAb (n)

<table>
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<th>Islet AAb (n)</th>
<th>AITD</th>
<th>Controls</th>
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<tbody>
<tr>
<td>ICA + GAD + IA-2</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

AAb – autoantibodies; ICA - islets cell cytoplasmic autoantibody; GAD - glutamic acid decarboxylase autoantibody; IA-2 - tyrosine-phosphatase autoantibody; AITD – autoimmune thyroid disease

* Statistical significance for Fisher's exact test
Table 3. Clinical characteristics, thyroid and islet autoantibodies titers, and HbA1c in 17 AITD patients and three control subjects with islet autoimmunity.

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Dg</th>
<th>Age et AITD (yr)</th>
<th>Sex</th>
<th>TPO (U/mL)</th>
<th>Tg (U/mL)</th>
<th>Age at evaluation</th>
<th>ICA (JDF)</th>
<th>GAD (U/mL)</th>
<th>IA2 (U/mL)</th>
<th>Age at T1D dg (yr)</th>
<th>HbA1c % (mmol/mol)**</th>
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<tr>
<td>1</td>
<td>AT</td>
<td>11.8</td>
<td>F</td>
<td>&gt;2000</td>
<td>44.2</td>
<td>12.9</td>
<td>330</td>
<td>676</td>
<td>258.6</td>
<td>12.9</td>
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<td>AT</td>
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<td>F</td>
<td>&gt;2000</td>
<td>0.2</td>
<td>8.8</td>
<td>290</td>
<td>859</td>
<td>136.1</td>
<td>10.3</td>
<td>8.3</td>
</tr>
<tr>
<td>3</td>
<td>GD</td>
<td>6.5</td>
<td>F</td>
<td>&gt;2000</td>
<td>73.1</td>
<td>9.5</td>
<td>370</td>
<td>2059</td>
<td>1882</td>
<td>8.0*</td>
<td>7.3</td>
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<tr>
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<td>285</td>
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<td>1245</td>
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<td>AT</td>
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Dg – diagnosis; yr – year; AT - autoimmune thyroiditis; GD – Graves disease; AITD – autoimmune thyroid disease, M – male; F – female; TPO - thyroid peroxidase; Tg - thyroglobulin; ICA – islet cell cytoplasmic autoantibody, GAD - glutamic acid decarboxylase autoantibody; IA2 - tyrosine-phosphatase autoantibody; NT – not tested

**Bold - patients who developed T1D during the investigation period; * impaired glucose tolerance at the time of evaluation; **HbA1c % (mmol/mol) at the time of T1D diagnosis or the last HbA1c measured in patients with normal glucose metabolism.