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

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What is already known on this topic?

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

What this study adds?

- This is the first comprehensive study that included children/adolescents with both AITDs (AT and GD) and in which islet cell autoimmunity was estimated by measuring three islet AAbs.
- The observed relative frequency of T1D development in patients with AITD 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 patients with AITD and islet AAbs. As five of 20 family members were found to have impaired glucose tolerance/islet cell autoimmunity, we propose that family members of AITD patients have an increased risk of developing T1D. However, these findings should be evaluated and validated in studies with larger sample sizes.

Abstract

Objective: The aim of the present study was to investigate islet autoimmunity and susceptibility to type 1 diabetes (T1D) in children/adolescents with autoimmune thyroid disease (AITD, and in family members of AITD patients with islet autoimmunity.

Methods: Islet-cell cytoplasmic, glutamic-acid decarboxylase, and tyrosine-phosphatase autoantibodies (AAbs) 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.

Results: 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 AAbs was found in females with AITD ($p = 0.011$) but not in males ($p = 0.16$) and in AT ($p = 0.013$) but not in GD patients ($p = 0.19$), compared to corresponding controls. Two or three islet AAbs were found concurrently in six AITD patients with islet autoimmunity. They all developed T1D and had significantly higher islet AAbs titers ($p = 0.01$) than AITD patients with single islet AAbs but normal glucose metabolism. T1D was found in 3.7% of AITD patients compared to 0.2% of the age-matched, general Croatian population. Islet AAbs were found in 5/20 family members of AITD patients with islet autoimmunity, among whom two developed T1D. None of the controls was positive for more than one islet AAb or developed T1D.

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Conclusion: Children/adolescents with AITD, particularly females and patients with AT, appear to represent a risk group for islet autoimmunity and T1D, as do family members of AITD patients with positive islet AAbs. However, these findings should be validated in larger studies.
Keywords: Autoimmune thyroid disease, islet autoimmunity, screening, diabetes mellitus type 1, children

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 type 1 diabetes mellitus (T1D) and two autoimmune thyroid diseases (AITD), autoimmune thyroiditis (AT) and Graves' disease (GD). Of all autoimmune endocrinopathies that co-occur, AITD 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 polyglandular syndrome type 3 (3,4).

Thyroid autoimmunity has been widely studied in T1D, and thyroid autoantibodies (AAb) were found in 8-44% of patients with T1D (5), while 50% of these patients developed a clinical form of AITD (5). In contrast, few studies have examined islet autoimmunity and risk of developing T1D among patients with AITD, and only four of these studies were conducted in children and adolescents (6,7,8,9). One study was performed in children with AT and GD (6) and three others in children with AT (7,8,9). Among studies conducted in adults, some were exclusively carried out in patients with AT (10,11) or GD (12,13), while others included patients with both conditions (14,15,16,17,18,19). 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 AAb (ICA), tyrosine-phosphatase (IA2), insulin (IAA), proinsulin and zinc-transporter 8 AAb. These antibodies are age-dependent, with IAA and IA2 more commonly seen in children under ten, while GAD is associated with older age and the female gender (20,21).

In line with growing interest in the early detection of people and groups at risk for T1D development, this study aimed to assess the relative frequency of humoral 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. In addition, 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 patients with AITD and family members who tested positive for islet AAb, glucose metabolism was assessed to evaluate for T1D.

Methods

Patients

This 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, aged 6.5-21.9 years)]. All patients were treated at the Department of Pediatric Endocrinology and Diabetes, University Hospital Center, Zagreb. Patients were 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, such as 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 recruited to the study.

AT diagnosis was based on elevated titers of AAb against thyroid peroxidase (TPO) and/or thyroglobulin (Tg) and thyroid ultrasound examination consistent with this diagnosis. Since measurement of thyroid-stimulating hormone (TSH) receptor antibodies was unavailable, the diagnosis of GD 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, ranging 3.5-6 years, were labeled as GD to exclude hyperthyroidism due to AT.

The University Hospital Center Zagreb Ethics Committee and the University of Zagreb Faculty of Medicine Ethics Committee approved the study protocol (no: 641-01/22-02/01, date: 07.12.2022), the study was performed in line with 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 in three control subjects with islet autoimmunity, glucose metabolism was evaluated with oral glucose tolerance test (OGTT) and glycated hemoglobin (HbA1c) using the immunoassay method on the Siemens A1c Vantage Analyzer (Siemens Healthcare GmbH, Erlangen, Germany) according to ISPAD criteria (22).

Determinations of GAD and IA2 AAb were performed by commercial enzyme-linked immunosorbent assay (ELISA) kits (Euroimmun, Germany). In 2010 the Clinical Institute of Laboratory Diagnosis, University Hospital Merkur, Zagreb, participated in Diabetes Antibody Standardization Program. 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 AAbs was performed by indirect immunofluorescence. Scores of fluorescence intensities were then calculated into Juvenile Diabetes Foundation (JDF) units. 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, USA) with >95% sensitivity, specificity, consistency, and validity (24). The quality of our performance is 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 ELISA (ELISA Brahms GmbH, Henningsdorf, Germany) methods, with results higher than the cut-off values set by the manufacturer considered positive.

Statistical Analysis

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's exact test, and the Mann-Whitney U test for unpaired data). Statistical Package for the Social Sciences, version 21.0 (IBM Inc., Armonk, NY, USA) was used for calculations, and a $p < 0.05$ 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 frequency was significantly higher only in AT patients (11.8%; $p = 0.001$) but no different in GD patients (5.9%, $p = 0.19$), when 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), but this was not 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 $p = 0.002$; IA2 $p = 0.02$, 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).

Table 1. The frequency and percentage of islet autoimmunity in patients with AITD (subdivided into groups: AT and GD) and control subjects. 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

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

AITD: autoimmune thyroid disease, AT: autoimmune thyroiditis, GD: Graves' disease

Table 2. The frequency and percentages of islet autoantibodies in AITD patients and controls are presented. Statistical significance for chi-square or Fisher's exact test between the two groups is presented

Islet AAb n (%)	AITD (n = 161)	Controls (n = 155)	p (chi-square or Fisher's exact*)
ICA	9 (5.6%)	2 (1.3%)	0.04
GAD	10 (6.2%)	0 (0%)	0.002*
IA-2	8 (5.0%)	1 (0.6%)	0.02
One islet AAb (n)			
ICA	3	2	
GAD	5	0	
IA-2	3	1	
Two islet AAb (n)			
ICA + GAD	1	0	
ICA + IA-2	1	0	
GAD + IA-2	1	0	
Three islet AAb (n)			
ICA + GAD + IA-2	3	0	

*Statistical significance for Fisher's exact test.
AAb: autoantibodies, ICA: islets cell cytoplasmic autoantibody, GAD: glutamic acid decarboxylase autoantibody, IA2: tyrosine-phosphatase autoantibody, AITD: autoimmune thyroid disease

There was no significant difference in the frequency of islet autoimmunity between sexes (5.6% males and 12% females with AITD; p = 0.27). A significant difference in proportion with 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, significant differences were found in the frequencies of GAD (7.2%) and IA2 AAb (5.6%) in females with AITD compared to females in the control group (0%; p = 0.005 and 1%; p = 0.06, respectively). No differences was found in the frequency of ICA AAb in females with AITD (6.4%) compared to females in the control group (1.9%; p = 0.10), nor was there a difference in the frequencies of any of the three diabetes-associated AAbs in males with AITD (ICA 2.8%; GAD 2.8%; IA2 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 9.8%; 83.6 mmol/mol) (Table 3), and one patient had impaired glucose tolerance and normal HbAlc (patient #3, Table 3). The remaining 14 patients had normal blood glucose levels on OGTT and normal values of HbA1c. During the 6-year follow-up, another 5/16 AITD patients with islet AAb (4 females and one male, patients #2-6; Table 3) developed T1D. Patient #17 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 years 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 AAbs 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 islets AAb and was diagnosed with T1D at the age of 19 years. The other four parents (#2b, 4b, 11a, and 11b) with positive islet AAb had normal levels of glucose on 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 years (Supplement Table 1).

Table 3. Clinical characteristics, thyroid and islet autoantibodies titers, and HbA1c in 17 AITD patients and three control subjects with islet autoimmunity

Patient#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	1	2	3
Dg	**AT**	**AT**	**GD**	**AT**	**AT**	**AT**	AT	AT	AT	AT	AT	AT	AT	AT	GD	AT	AT	Control group		
Age at AITD dg (yr)	**11.8**	**10.1**	**6.5**	**8.0**	**11.4**	**8.1**	13.5	11.3	19.0	11.5	12.3	7.8	13.2	12.2	15.4	13.7	17.3			
Sex	**F**	**F**	**F**	**F**	**F**	**M**	F	F	F	F	F	F	F	F	F	M	F	F	F	F
TPO (U/mL)	**> 2000**	**> 2000**	**> 2000**	**40**	**> 2000**	**> 2000**	269	> 2000	159	> 2000	1191	115	> 2000	96	279	46	1112	0.18	0	0
Tg (U/mL)	**44.2**	**0**	**71.1**	**140**	**25**	**584**	755	147	0	0	28.2	0	177.2	155	27	0	643	1.3	0	0
TSH (µU/L)	**17.3**	**18.4**	**< 0.01**	**11.3**	**34.3**	**> 100**	16.	10.1	4.6	18.8	9.2	3.2	27.4	10.1	< 0.01	1.4	4.0	2.2	1.6	3.6
fT4 (pmol/L)	**10.2**	**11.9**	**44.3**	**9.8**	**9.2**	**3.1**	13.0	14.4	18.7	8.6	12.0	16.6	9.2	11.6	48.6	15.6	10.9	16.3	17.2	14.4
Age at evaluation (yr)	**12.9**	**10.8**	**8.0***	**9.5**	**11.4**	**11.2**	16.8	14.3	20.4	14.2	16.0	11.3	14.9	14.2	16.0	16.6	20.1	12.7	10.5	11.0
ICA (JDF)	**330**	**290**	**370**	**285**	**65**	**50**	45	< 5	< 5	50	< 5	65	95	< 5	60	25	30	< 5	50	35
GAD (U/mL) (IU/mL)	**676**	**859**	**2509**	**0**	**68.6**	**149**	0	22.7	17.7	23.7	0	0	0	27.4	0	0	1193	0	0	0
IA2 (U/mL)	**258.6**	**136.1**	**1882**	**1245**	**226**	**0**	16.7	0	0	0	22.3	0	0	0	0	21.0	0	13.6	0	0
Age at T1D dg (yr)	**12.9**	**12.6**	**10.3**	**12.9**	**14.8**	**13.3**														
HbA1c % (mmol/mol)**	**9.8 (84)**	**8.3 (67)**	**6.8 (51)**	**8.5 (69)**	**7.3 (56)**	**7.8 (62)**	5.5 (37)	5.2 (33)	5.0 (31)	5.2 (33)	5.3 (34)	4.7 (28)	4.8 (29)	5.2 (33)	5.3 (34)	5.0 (31)	NT	4.8 (29)	5.2 (33)	NT

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.
Dg: diagnosis, yr: year, AT: autoimmune thyroiditis, GD: Graves disease, AITD: autoimmune thyroid disease, M: male, F: female, TPO: autoantibodies against thyroid peroxidase, Tg: autoantibodies against thyroglobulin, TSH: thyroid-stimulating hormone, fT4: free thyroxin, ICA: islet cell cytoplasmic autoantibody, GAD: glutamic acid decarboxylase autoantibody, IA2: tyrosine-phosphatase autoantibody, NT: not tested, HbA1c: glycated hemoglobin

Antithyroid AAb in AITD Patients

Patients with AITD with positive islet autoimmunity had higher TPO and Tg AAb titers compared to AITD patients without islet autoimmunity, but the difference was not significant (Mann-Whitney test: Tg-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). However, other essential and currently achievable clinical benefits drive the current recommendations for screening. It has been shown that screening programs significantly reduce diabetes ketoacidosis (DKA) rates, usually to less than 5%, and reduce hospitalization when coupled with long-term monitoring (26,27,28,29). DKA prevention at diagnosis has potential lifelong benefits, including avoidance of acute morbidity, neurocognitive impairment, and mortality (30,31). Other no less worthy benefits would be to prepare children and families for a smoother transition to insulin therapy and advance preventative treatments through clinical trial recruitment (25).

In the present study, we analyzed three islets 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 significantly more AITD patients were positive for one or more islet AAb compared to controls. This difference was entirely due to antibodies found in AT patients, as there was no significant difference in islet autoimmunity between the GD patients and controls. However, due to the small number of patients in the GD group, an association 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 and GAD AAb was found only in the AITD patients but not in controls.

Few studies have reported the frequency of ICA autoimmunity in patients with AITD and only four included children and adolescents (6,7,8,9). Bright et al. (6) found ICA AAb in 2.3% of children with AITD compared to 0% of controls. 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 excluded 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 in adult patients, IA2 AAb was found more frequently in patients with AT and GD (18).

GAD positivity was assessed in three studies conducted in children with AT (7,8,9). 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 again confirmed in our study. However, Pilia et al. (7), found no significant difference in rates of GAD AAb positivity. Several studies analyzed GAD autoimmunity in adult patients with AT (10,11,14,15,16,19) and GD (12,13,15,16). Relative frequencies of GAD AAb ranged from 2.8-6.6% (10,11,14,15,16,19) in patients with AT. Although a correlation between GAD AAb and AT was found in some studies (15), it was not always significant (16). In adult GD patients, GAD AAb was found in 6.1-13% of patients (12,13,15,16), significantly more common than controls in some studies (15,16). However, the evaluation only sometimes included control subjects (10,11,12,13,14).

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

In 16 patients with AITD 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 (Table 3). However, we cannot exclude that more patients would develop diabetes if the follow-up were longer. In the study of Bright et al. (6), 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, 2/19 children with AT and islet autoimmunity developed T1D (one positive to GAD AAb and the other to GAD and IA2 AAb). Lethagen et al. (10) found reduced insulin secretion capacity in GAD-positive AT patients and concluded that GAD AAb might be a marker of subclinical insulitis. During the follow-up of 4 years, 2/15 (13%) of their GAD AAb positive patients compared to 11/426 (2.6%) GAD AAb negative patients were diagnosed with diabetes ($p = 0.08$) (10). Hallengren 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. Maugendre et al. (12) found a high frequency of GAD AAb (16/150 GD patients) but a low progression toward diabetes (only one patient). Aksoy 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 M Marhawa et al. (8) and Moriguchi et al. (16). Kawasaki et al. (15) did not report similar findings. The observed relative frequency of T1D development in our patients with AITD was compared to that in the Croatian general population. In our cohort, T1D was found in 3.7% of AITD patients, much more frequent than in the general population in the same age groups (0.2%) (33, Croatian registry of diabetes in children and adolescents, unpublished data).

In the present study, AITD patients who developed T1D had significantly higher titers of GAD AAb than AITD patients with islet autoimmunity and normal glucose metabolism. Moreover, we noticed significantly higher titers of ICA and IA-2 AAb in this patient group.

We further measured TPO and Tg AAb titer and found higher titers in AITD patients with islet autoimmunity compared to AITD patients without islet autoimmunity. However, the difference was not statistically significant, as was observed in some other studies (15,17). On the other hand, M Marwaha et al. (8) found that GAD AAb levels increased with an increasing titer of TPO AAb.

Islet autoimmunity and susceptibility to T1D were analyzed in 20 first-degree family members of patients with AITD and positive islet AAb (Supplement Table 1). Positive islet AAb was found in 25%, although one of the mothers was already diagnosed with T1D, but interestingly one father developed T1D during follow-up. This suggests that family members of patients affected with AITD and islet autoimmunity might have a higher risk for T1D.

Study Limitations

Study limitations should be noted. The measurement of anti-TSH receptor antibodies were not available in our institution at that time and therefore not performed as part of the diagnosis of GD. However, in a group of GD patients with clear clinical, biochemical, and ultrasound signs of disease, we included 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 AITD in a much larger number of subjects, including both those with and without islet autoimmunity, to determine the risk for glucose metabolic impairment in relatives of patients with AITD.

Conclusion

In conclusion, AITD patients may be a potentially significant group for targetted T1D screening. Our results suggest that females with AT are at especially high risk for T1D development, as are patients with a higher titer of two or more islet AAb, and family members of AITD with positive AAb. Prospective long-term studies on a larger number of subjects are required to examine the factors responsible for islet destruction, insulin deficiency, and progression toward diabetes in patients with AITD and the correlations with 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. However, this observation must be verified in much larger studies.

Ethics

Ethics Committee Approval: The University Hospital Center Zagreb Ethics Committee and the University of Zagreb Faculty of Medicine Ethics Committee approved the study protocol (no: 641-01/22-02/01, date: 07.12.2022).

Informed Consent: Informed consent was obtained from all participants and/or their parents.

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Authorship Contributions

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Click for Supplement Table 1. Distribution of islet AAb in 20 family members of 10 AITD patients with islet autoimmunity

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