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# Screening for Associated Autoimmunity in Type 1 Diabetes Mellitus With Respect To Diabetes Control

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## Summary

As an autoimmune disease, type 1 diabetes mellitus (DM) can be associated with other autoimmune disorders. The aim of this study was to detect subclinically associated autoimmune thyroid disease, coeliac disease, and Addison's disease. The presence of autoantibodies was evaluated with special regard to the control of diabetes and to the clinical status of the patient. Fifty-one type 1 diabetic patients (22 men, 29 women, mean age 37±11 years, mean duration of diabetes 16±13 years) were included into this study. Specific antibodies to islet antigens – glutamic acid decarboxylase (GAD65), protein thyrosine phosphatase IA-2α, and to thyroid autoantigens – thyroid microsomal peroxidase (TPO) and thyroglobulin (TG) and also thyroid stimulating hormone (TSH) were measured by RIA. Autoantigens of the small intestine – tissue transglutaminase autoantibodies (ATTG), IgA and IgG antibodies to gliadin (AGA-IgA, AGA-IgG) were evaluated by ELISA. Endomysial autoantibodies (EMA) and adrenal cortex antibodies (ACA) were detected by indirect immunofluorescence microscopy. Eleven new cases of thyreopathy (22 % of patients) were detected by the assessment of thyroid autoantibodies and TSH. Two new cases of thyreotoxicosis were diagnosed during the study. Coeliac disease was diagnosed in at least two cases. Addison's disease was not diagnosed, although the ACA were positive in two patients. No influence of single or combined autoantibody positivity on the control of diabetes was found if normal organ function was preserved. In both patients with thyreotoxicosis the control of diabetes was worsened and improved after treatment. The screening of autoantibodies in type 1 diabetic patients could reveal subclinical cases of AITD or coeliac disease. Subclinical forms of these disorders have no influence on diabetes control. However, impaired organ function may be associated with the worsened control of diabetes as we demonstrated on two newly diagnosed cases of thyreotoxicosis. We suggest the need for the follow-up of patients with positive autoantibodies because further deterioration of the respective organs can be expected.

**Key words**

Type 1 diabetes mellitus • Autoimmune thyroid disease • Coeliac disease • Addison's disease • Autoimmunity



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## Introduction

Type 1 diabetes mellitus (DM) is an autoimmune disease (Lernmark 1999, Redondo and Eisenbarth 2002). It can be associated with other autoimmune endocrine disorders as well as autoimmune impairment of non-endocrine tissue. The associated autoimmune disease may influence the control of diabetes by impairing function of the respective organ.

Autoimmune thyroid disease (AITD) is the most frequent autoimmune disease associated with type 1 diabetes mellitus. The screening and diagnosis of AITD are based on the assessment of autoantibodies to thyroid peroxidase (anti-TPO) and thyroglobulin (anti-TG). The prevalence of these autoantibodies is dependent on gender, age of patient, and age at the onset of diabetes. It also varies in different geographic regions and is known to be higher in regions with higher iodine intake (De Block *et al.* 2001, Landin-Olsson *et al.* 1989, 1992, Premawardhana *et al.* 2000, Vondra *et al.* 1996, Perros *et al.* 1995). The assessment of thyroid stimulating hormone (TSH) allows the evaluation of the thyroid gland function. Dyslipidemia and arrhytmia are the main features frequently accompanying impaired thyroid gland function in non-diabetic subjects. Moreover, thyreotoxicosis can worsen metabolic control of diabetes and increase its lability often with a need for increased insulin dosage, and hypothyreosis can lead to increased frequency of hypoglycemia in diabetic patients.

Coeliac disease (CD) can also be associated with type 1 DM, and in most cases it is present in its subclinical form. At the onset of type 1 diabetes, the prevalence of coeliac disease has been reported between 2.5-3 % (Saukkonen *et al.* 1996, Fraser-Reynolds *et al.* 1998). In its advanced stages CD causes dyspepsia, weight loss, and malabsorption resulting in severe complications, e.g. peripheral neuropathy, ataxia, intellectual deterioration and brain atrophy, dental enamel defects, infertility, neoplastic complications, and others (Aine *et al.* 1990, Collin *et al.* 1996, Luostarinen *et al.* 1999, 2001, Mustalahti *et al.* 1999). Anti-tissue transglutaminase (ATTG) and endomysial autoantibodies (EMA) are the most sensitive and specific markers of coeliac disease. The assessment of anti-gliadin autoantibodies (AGA) can also be used, but their sensitivity and specificity depends on the type and purification of antigen. A gluten-free diet leads to normalization of histology and a decrease in clinical symptoms.

Autoimmune adrenalitis and Addison's disease have been described in association with type 1 diabetes mainly as a part of the autoimmune polyendocrine syndrome type II (APS-II) (Falorni *et al.* 2002). It is far less frequent than AITD or coeliac disease, but in clinically manifest cases, it leads to low plasma cortisol concentration accompanied by the symptoms of hypocorticalism and may potentially be life-threatening.

The aim of our study was to evaluate the autoantibody profile in type 1 diabetic patients and to detect subclinical forms of the associated autoimmune diseases. The influence of autoimmune abnormalities on the control of diabetes was studied with special regard to the effect of the autoimmune processes not deteriorating the function of the target organs.

## Methods

Fifty-one type 1 diabetic patients were included in our study. Their characteristics are shown in Table 1. The control group for the screening of coeliac disease consisted of sixty healthy subjects (24 men, 36 women) matched for age, sex, and BMI.

**Table 1.** The characteristics of type 1 diabetic patients included in the study.

| | **Age (years)** | **Diabetes duration (years)** | **BMI ($kg.m^{-2}$)** | **$HbA_{1c}$ (%)** | **C-peptide (nmol/l)** |
|---|---|---|---|---|---|
| *Men (n = 22)* | 35 ± 10 | 17 ± 14 | 23.4 ± 3.2 | 8.1 ± 2.3 | 0.28 ± 0.21 |
| *Women (n = 29)* | 39 ± 12 | 15 ± 13 | 23.5 ± 2.6 | 7.6 ± 1.6 | 0.40 ± 0.50 |
| *All patients (n = 51)* | 37 ± 11 | 16 ± 13 | 23.5 ± 2.8 | 7.8 ± 1.9 | 0.36 ± 0.40 |

Data are means ± S.D. Differences between males and females were not statistically significant.

Glycated hemoglobin $A_{1C}$ was measured using the Imx GHg Assay System (ABBOTT) with a normal range of 4.6-6.6 %. C-peptide and TSH were measured using routine RIA assays (Immunotech, Czech Republic). Normal range for C-peptide and TSH is 0.4-0.9 nmol.l$^{-1}$ and 0.5-3.5 IU.l$^{-1}$, respectively.

GAD65 and IA-2α autoantibody assays were performed with RIA kits (Cis-Bio International, France) and a concentration of more than 1 IU.ml$^{-1}$ was considered to be positive. TPO and TG autoantibodies were measured with RIA kits (Immunotech, Czech Republic) with normal values being less than 50 IU.ml$^{-1}$ and 100 IU.ml$^{-1}$, respectively.

Screening for gliadin (AGA), endomysial (EMA), and tissue transglutaminase autoantibodies (ATTG) was performed at the Department of Clinical Biochemistry, Laboratory of Gastroenterology. Gliadin autoantibodies were measured by ELISA by a method developed in this laboratory. Purified α-gliadin separated by ion-exchange chromatography on SP-Sephadex was used as an antigen (Kocna *et al.* 1983). The results are expressed as an index using normal values less than 30, both for AGA-IgA and AGA-IgG. EMA were detected by indirect immunofluorescence microscopy on the primate smooth muscle slices (Immco, USA). Both sensitivity and specificity reach almost 100 % in active coeliac disease. ATTG antibodies were measured by ELISA kit (Genesis, UK). Normal values for IgA ATTG autoantibodies are less than 10 IU.ml$^{-1}$.

Adrenal cortex autoantibodies (ACA) were measured by indirect immunofluorescence microscopy on primate adrenals sections (The Binding Site, UK).

The results were statistically evaluated using the t-test for normally distributed unpaired data and Mann-Whitney rank sum test for not-normally distributed data. Pearson's correlation was applied to compare the relationship between autoantibodies and control of diabetes. The data are expressed as means ± standard deviation.

**Table 2.** Serum levels of GAD65 and IA-2α autoantibodies and their prevalence in Type 1 diabetic patients.

| Ab | Anti-GAD65 (IU.ml$^{-1}$) n (%) | Anti-IA-2α (IU.ml$^{-1}$) n (%) | One Ab only n (%) | Both Ab n (%) |
|---|---|---|---|---|
| *Men* | 13.1 ± 31.9 | 0.55 ± 0.96 | 10 (45 %) | 3 (14 %) |
| *(n = 22)* | 12 (55 %) | 4 (18 %) | | |
| *Women* | 34.0 ± 48.4 | 3.6 ± 6.9 | 13 (45 %) | 8 (28 %) |
| *(n = 29)* | 20 (69 %) | 9 (31 %) | | |
| *All patients* | 25.0 ± 43.0 | 2.2 ± 5.4 | 23 (45 %) | 11 (22 %) |
| *(n = 51)* | 32 (63 %) | 13 (25 %) | | |

Data are means ± S.D. The number of cases positive in one or both antibodies is included.

## Results

No statistically significant differences in the mean concentrations of anti-GAD and anti-IA-2α were observed between men and women with type 1 diabetes, although there was a slight trend towards higher values in females (Table 2).

Similarly, no significant differences in the mean values of anti-TPO and TSH were found between men and women in the diabetic group (Table 3). However, the mean anti-TG concentration was significantly higher in diabetic women compared to diabetic men ($p<0.05$). The elevated TSH levels were also present in all three men positive for anti-TPO and anti-TG. In women, increased anti-TPO and anti-TG concentrations were detected in two patients, where one patient had elevated TSH while the other had a TSH concentration below the normal values. The results of thyroid tests are shown in Table 3.

The mean concentration of AGA-IgG was comparable in patients and controls, while AGA-IgA was significantly higher in the patients than in the controls ($p<0.01$, Table 4). The frequency of ATTG positivity and its mean concentration was significantly higher in the patients compared to the control group ($p<0.03$).

No significant differences were observed between diabetic men and women in the concentrations

of AGA-IgA, AGA-IgG, and ATTG (Table 4). EMA positivity was found with positivity of all other detected markers of coeliac disease only in one diabetic male, and positivity of all CD markers except EMA was observed in four other male patients. In diabetic women, the positivity of EMA together with ATTG and AGA-IgG was present in one patient and all CD markers except EMA were positive in another female.

**Table 3.** Serum levels of TSH as well as TPO and TG antibodies and their prevalence in type 1 diabetic patients.

| | Anti-TPO (IU.ml$^{-1}$) n (%) | Anti-TG (IU.ml$^{-1}$) n (%) | TSH (IU.l$^{-1}$) n (%) | 1 Ab only n (%) | Both Ab n (%) |
|---|---|---|---|---|---|
| *Men* | 235.2 ± 573.7 | 105.4 ± 217.0 | 3.4 ± 3.6 | 0 (0 %) | 3 (14 %) |
| *(n = 22)* | 3 (14 %) | 3 (14 %) | 5 (23 %) | | |
| *Women* | 325.3 ± 1214.2 | 276.5 ± 585.9* | 3.7 ± 7.4 | 7 (24 %) | 2 (7 %) |
| *(n = 29)* | 6 (21 %) | 5 (17 %) | 4 (14 %) | | |
| *All pts* | 257.7 ± 574.3 | 222.7 ± 896.7 | 3.6 ± 6.0 | 7 (14 %) | 5 (10 %) |
| *(n = 51)* | 9 (18 %) | 8 (16 %) | 9 (18 %) | | |

Data are means ± S.D. The number of cases positive in one or both antibodies and the number of patients with TSH elevations (TSH >3.5 IU.l$^{-1}$) is included (* - difference between men and women, $p<0.05$).

**Table 4.** Comparison of the mean concentration of autoantibodies and their prevalence of positivity in type 1 diabetic patients and controls. The number of positive cases is included (difference between patients and controls, $^{x}$ $p < 0.01$, $^{y}$ $p < 0.03$).

| | AGA-IgA (index) n (%) | AGA-IgG (index) n (%) | ATTG (IU.ml$^{-1}$) n (%) | EMA n (%) | ≤ 2 Ab n (%) | > 2 Ab n (%) |
|---|---|---|---|---|---|---|
| *DM men* | 24.3 ± 19.0 | 38.3 ± 22.3 | 19.4 ± 56.3 | 1 (5 %) | 11 (50 %) | 5 (23 %) |
| *(n = 22)* | 8 (36 %) | 13 (59 %) | 8 (36 %) | | | |
| *DM women* | 18.4 ± 12.1 | 41.9 ± 17.9 | 8.6 ± 10.7 | 1 (3 %) | 20 (69 %) | 2 (7 %) |
| *(n = 29)* | 4 (14 %) | 19 (66 %) | 9 (31 %) | | | |
| *All DM patients* | 19.9 ± 14.5* | 39.5 ± 19.7 | 12.6 ± 35.2$^{\#}$ | 2 (4 %) | 31 (61 %) | 7 (14 %) |
| *(n = 51)* | 12 (24 %) | 32 (63 %) | 17 (33 %) | | | |
| *Controls* | 14.3 ± 9.3 | 36.3 ± 24.4 | 3.7 ± 6.3 | 0 (0 %) | | |
| *(n = 60)* | 4 (7 %) | 29 (48 %) | 4 (7 %) | | | |

Data are means ± S.D. The number of positive cases is included (difference between patients and controls, * $p<0.01$, $^{\#}$ $p<0.03$).

In diabetic men and women, the prevalence of positivity in the respective groups of antibodies is comparable (Figs 1 and 2).

Positivity of ACA was found in two women with type 1 DM (4 %). Both were anti-GAD positive, whereas higher anti-IA-2α and ATTG were found only in one. Impaired function of the adrenal glands in these patients was not observed. Their autoantibody profile is shown in Table 5.

No relationship was found between the control of diabetes evaluated by $HbA_{1C}$ and autoantibody positivity (Fig. 3). A significant inverse relationship was observed between the concentration of anti-GAD65 or anti-IA-2α and duration of diabetes ($r = –0.47$, $p<0.002$ and $r = –0.56$, $p<0.01$, respectively). An inverse relationship was found between serum C-peptide concentration and duration of diabetes ($r = –0.49$, $p<0.02$), and between $HbA_{1C}$ and the age of patients ($r = –0.41$, $p<0.02$). C-peptide concentration was inversely related to $HbA_{1C}$ values ($r = –0.46$, $p<0.02$). Mean values of C-peptide concentration in groups of patients with one and two positive autoantibodies (both islet and thyroid) were not

significantly different. A positive correlation was observed between AGA-IgA and AGA-IgG concentrations ($r = 0.30$, $p<0.02$).

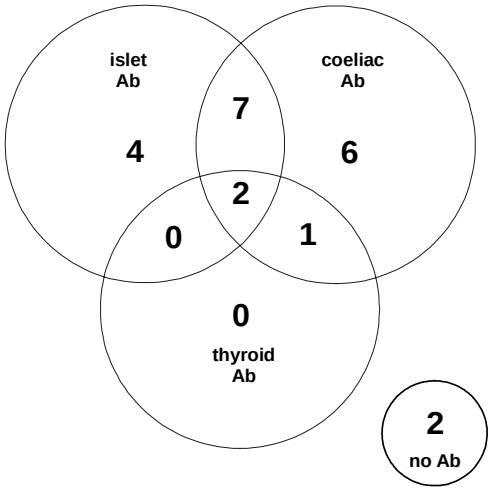


**Fig. 1.** The number of cases with positivity of specific autoantibodies in men with type 1 diabetes mellitus.

During our study, two patients developed thyreotoxicosis. The first patient was a female previously only slightly positive in anti-TG (102.4 IU.ml$^{-1}$), negative in anti-TPO antibodies (28.6 IU.ml$^{-1}$) with normal thyroid hormone concentrations (TSH 1.84 IU.l$^{-1}$) and with satisfactory control of diabetes ($HbA_{1C}$ 6.7 %). Tachycardia and weight loss developed together with an increase in $HbA_{1C}$ to 8.4 %. She was re-evaluated, and concentrations of both anti-TPO and anti-TG antibodies (20.4. and 76.4 IU.ml$^{-1}$, respectively) were normal but TSH level (0.001 IU.l$^{-1}$) was suppressed. AITD was supported by ultrasonographic examination. The second female patient was previously negative in anti-TPO (15.8 IU.ml$^{-1}$) and anti-TG (27.1 IU.ml$^{-1}$) with normal TSH (3.03 IU.l$^{-1}$). Diabetes control was unsatisfactory ($HbA_{1C}$ 8.7 %), but sudden progressive worsening of diabetes control ($HbA_{1C}$ up to 11.3 %) was accompanied by positive anti-TPO (88.3 IU.ml$^{-1}$) and anti-TG (133.5 IU.ml$^{-1}$) antibodies. Newly diagnosed thyreotoxicosis (TSH not detectable) was confirmed. The control of diabetes improved during carbimazol treatment in both patients.

**Table 5.** Autoantibody profile in two female patients with ACA positivity.

| Diabetes duration (years) | Age (years) | Anti-AD65 (IU.ml$^{-1}$) | Anti-IA-2α (IU.ml$^{-1}$) | AGA-IgA (index) | AGA-IgG (index) | ATTG (IU.ml$^{-1}$) | EMA n (%) | Anti-TPO (IU.ml$^{-1}$) | anti-TG (IU.ml$^{-1}$) | TSH (IU.l$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| *2* | 43 | 149.0 | 12.2 | 24.9 | 28.1 | 10.3 | – | 28.5 | 15.3 | 0.15 |
| *1* | 35 | 46.2 | 0.2 | 10.9 | 12.9 | 7.2 | – | 38.4 | 17.5 | 1.25 |

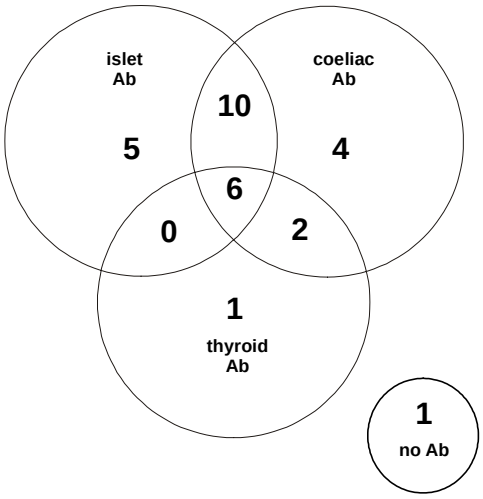


**Fig. 2.** The number of cases with positivity of specific autoantibodies in women with type 1 diabetes mellitus.

## Discussion

The prevalence of islet autoantibodies to GAD65 and IA-2α was found in more than 60 % of our type 1 diabetic patients and there was a decrease in their concentrations with increasing duration of diabetes. This is comparable with other reports (Feeney *et al.* 1997, Gorus *et al.* 1997, Vandewalle *et al.* 1995, Yamada *et al.* 1997, Cinek *et al.* 2000). A decrease of C-peptide concentration confirms the progression of insulitis and destruction of B-cells. The worsening of diabetes control in C-peptide negative patients may reflect the inability of the organism to cope with an abnormal metabolic situation, although a sufficient amount of exogenous insulin is being supplied.

In our study, the positivity of thyroid

autoantibodies was detected in almost 30 % of examined patients. This percentage is much higher than in the normal Czech population, where it reaches less than 10 % (Zamrazil *et al.* 1989, Trunecká *et al.* 1990). Based on autoantibody positivity and TSH concentration, autoimmune thyroid disease (AITD) was newly diagnosed in 11 patients, predominantly in women. This represents more than 20 % of the studied patients and is in agreement with previous findings (Vondra *et al.* 1996). The positivity of autoantibodies is more frequent in follow-up studies, because their concentration fluctuates and sometimes positivity is present only temporarily (Vondra *et al.* 1996). AITD was supported by ultrasonographic findings in four of the eleven patients. In these patients, high autoantibody concentration was present along with significantly elevated TSH.

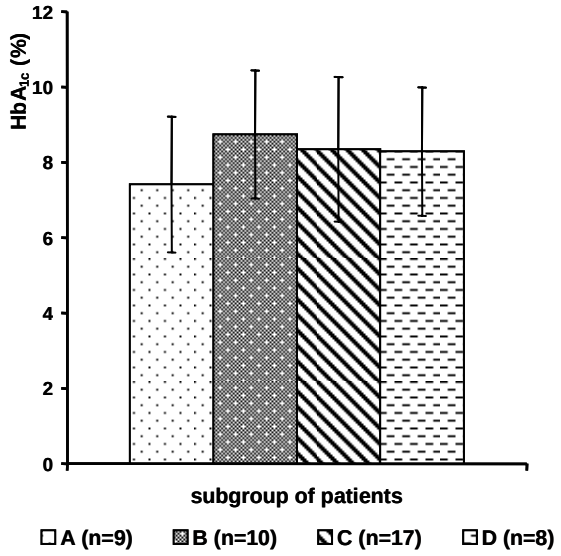


**Fig. 3.** Comparison of the control of diabetes in subgroups evaluated by autoantibody positivity:
**A** – patients with positive islet autoantibodies (n=9)
**B** – patients with positive coeliac autoantibodies (n=10)
**C** – patients with simultaneous positivity of both islet and coeliac autoantibodies (n=17).
**D** – patients with simultaneous positivity of islet, coeliac, and thyroid autoantibodies (n=8). Subgroups sorted by Figs 1 and 2. Differences are not statistically significant.

In the screening for coeliac disease, all four autoantibodies were positive in only one patient, three autoantibodies were positive in six patients, whereas 75 % of patients had at least one positive marker. This is in agreement with recently published results (Kocna *et al.* 2002). The positivity of EMA, which is considered the most specific marker for coeliac disease, was found in two patients who refused to undergo enterobiopsy. However, the diagnosis of coeliac disease was almost certain as they had simultaneous positivity of three and two other autoantibodies, respectively. In addition, chronic dyspepsia and low BMI were present in their clinical picture and improvement was observed after an appropriate dietary regimen. The subclinical form of coeliac disease may possibly be present in the remaining five patients exhibiting positivity in three autoantibodies. In our study, AGA-IgG had very low specificity and their concentrations did not significantly differ in patients and controls. AGA-IgA was a more specific marker but care should be taken in evaluating these autoantibodies in patients with IgA deficiency, because false negativity may occur.

Positivity of ACA with normal adrenal function was found in only two cases. Although they did not express any symptoms of adrenal insufficiency during the study and the levels of free urine cortisol were normal in these subjects, the role of these autoantibodies should be evaluated in further follow-up.

No relationship between positivity and/or concentration of the islet, thyroid, and coeliac autoantibodies was observed in our study except that between AGA-IgA and AGA-IgG. We found that single or combined autoantibody positivity had no influence on the control of diabetes if normal organ function was preserved. However, impaired organ function may be associated with the worsened control of diabetes. This was demonstrated by two newly diagnosed thyreotoxicoses.

In summary, the screening of autoantibodies in type 1 diabetic patients could reveal subclinical cases of AITD or coeliac disease, but its predictive value for progression to clinical manifestation is limited. The follow-up of patients with positive autoantibodies is necessary because further deterioration of the respective organs may occur. In the case of sublinical form of the disease, autoantibody positivity might have no influence on the control of diabetes. However, the changes in organ function may worsen diabetes control as was demonstrated in two patients with AITD. Further research will be necessary to test these relationships in a prospective follow-up study.

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