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Autoimmune thyroiditis in non-obese subjects with initial diagnosis of Type 2 diabetes mellitus

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ABSTRACT. Autoimmune thyroiditis is often associated with Type 1 diabetes mellitus (T1DM). In non-obese adult-onset diabetes diagnosed initially as Type 2 diabetes mellitus (T2DM), there is a proportion of cases with so far undiagnosed T1DM. The objective of this study was to estimate the frequency of autoimmune thyroiditis (AT) among non-obese (BMI <30.0 kg/m^2) patients with T2DM and to compare the frequency of AT in subgroups of patients according to the presence of glutamic acid decarboxylase antibodies (GADA), insulin requirement, and post-breakfast C-peptide levels. The study included 118 adult patients (55 men and 63 women) with the initial diagnosis of T2DM and age at the onset of diabetes >35 yr. Median of age was 66 yr (range 39-82), and median duration of diabetes was 9 (range 1-27) yr. AT was diagnosed using thyroid peroxidase antibodies, TG-antibodies, US and TSH levels. Nineteen per cent of the subjects were found to have AT, and the frequency of AT did not significantly differ between the groups of GADA+ and GADA- subjects. There was no difference in the frequency of AT between the group treated with hypoglycemic agents and/or diet and the group requiring insulin. The frequency of AT was higher in the group with post-breakfast C-peptide levels ≤0.8 nmol/l compared to the group with post-breakfast C-peptide levels >0.8 nmol/l (37% *vs* 16%), however the group with post-breakfast C-peptide levels ≤0.8 nmol/l had longer duration of diabetes.

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INTRODUCTION

Two major types of diabetes mellitus (DM) are identified: Type 1 diabetes mellitus (T1DM), with beta-cell destruction leading to absolute insulin deficiency and Type 2 diabetes mellitus (T2DM), with insulin resistance and usually relative (rather than absolute) insulin deficiency (1). Regarding adult-onset diabetes, a particular problem area still remains in the classification of DM. Here, the major problem is to distinguish the T2DM from a slowly progressing form of the T1DM that was first characterized in the early 1980's (2) and called latent autoimmune diabetes (AT) in adults (LADA) later (3). The typical patient with LADA is 25-yr old or older, non-obese and presents with what clinically appears to be T2DM (4). Subsequent clinical course, characterized by a slowly progressing development of insulin deficiency and the presence of serological markers of autoimmunity (mainly the glutamic acid decarboxylase antibodies – GADA), suggests that it is a form of T1DM (5, 6).

AT is often associated with typical T1DM. This has been reported in children (7-10) as well as in younger adults (11-13). AT has been documented using different diagnostic criteria that are a combination of the presence of thyroid peroxidase antibodies (TPOAb) or TG-antibodies (TGAb), assessment of thyroid function, US imaging of the thyroid and fine needle biopsy in some studies.

Though the association of AT with T1DM is clear, little is known about the risk of autoimmune thyroiditis in LADA. Here, the main problem still re-

Key-words: Autoimmune thyroiditis, T2DM, glutamic acid decarboxylase antibodies, thyroid peroxidase antibodies, TG-antibodies, C-peptide.

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mains of how to provide a clear definition of LADA, the diagnosis of AT being far from easy as well. A small number of studies investigated the prevalence of thyroid antibodies in adult patients who were initially diagnosed as having T2DM. Gambelunghe *et al*. (14) found a higher prevalence of TPOAb in GADA+ subjects with initial diagnosis of T2DM compared to GADA- subjects. In another study, Groop *et al.* (15) examined a group of patients with T2DM who were controlled with diet or oral hypoglycemic agents and a group of age-matched T2DM patients who required insulin to control their hyperglycemia. The frequency of TPOAb was significantly higher in those diabetic patients who required insulin than in those who could be controlled without insulin (15).
The objective aim of this study was to estimate the frequency of AT in non-obese T2DM patients and to compare the frequency of AT in subgroups of patients divided according to the presence of GADA, insulin requirement and post-breakfast C-peptide levels.

MATERIALS AND METHODS

Study population

The study population consisted of 118 adult patients (55 men and 63 women) who were randomly selected from a population of patients treated at an outpatient diabetes care unit in a district of Prague (Prague 9). The selection criteria were as follows: 1. initial diagnosis of T2DM and treatment with hypoglycemic agents and/or diet for at least 6 months after onset; 2. age at onset of diabetes >35 yr and 3. BMI <30 kg/m^2. The duration of diabetes ranged from 1-27 yr, and none of the patients suffered from a diabetic renal disease in the stage of chronic renal failure. The study protocol was approved by the local Ethical Committee, and written informed consent was obtained from all study participants.

US procedures

Thyroid US was performed by 2 experienced investigators using the Toshiba SSA 270A and Hitachi equipment using a 7,5 MHz probe. AT was defined as the finding of diffuse or focal reduced echogenicity on US (16).

Thyroid function

Thyroid function was determined using TSH and free FT_4 levels measured with EIA (Roche Diagnostics, Analyser ES 300, normal range for TSH: 0.27 - 4.2 mIU/l, normal range for FT_4: 12.0 – 22.0 pmol/l). The level of TSH >4.2 was consistent with hypothyroidism.

Autoimmune thyroiditis

AT was defined as the presence of thyroid antibodies (TPOAb or TGAb) together with typical finding on US of hypoechogenic thyroid gland, or as hypothyroidism with US finding of hypoechogenic thyroid gland.

C-peptide

C-peptide level was determined using IRMA (Immunotech, Czech Republic) after overnight fast and 60 min after administration of standardized breakfast consisting of 1650 kJ, and 46 g carbohydrate, 19 g fat and 13 g protein. The optimal cut-off value for post-breakfast C-peptide was found using receiver operating characteristic (ROC) at maximum likelihood ratio at the value 0.80 nmol/l.

Antibody assays

TPOAb and TGAb were measured using ELISA (Milenia Biotec, Germany; the cut-off limit for positivity was based on the upper normal limit of the kit, which was 125 IU/ml for TPOAb and 250 IU/ml for TGAb). GADA were measured using 2 commercial ELISA kits: ELIAS (Germany), with positive values >1500 mU/ml and after ending of production with Diaplets Roche, with positive values >50 ng/ml. The sensitivity of ELIAS kit has been reported to be equivalent to 26% and the specificity of 100% (17). The sensitivity of Diaplets Roche kit has been reported of 69% and the specificity of 98% (18). The results of Diaplets Roche were compared with those obtained with ELIAS in 40 samples using the Spearman's correlation with respect to non-Gaussian data distribution. The comparison found significant correlation ($r=0.581$, $p<0.001$).

Statistical analysis

The results are presented as medians and 25-75 percentiles or SE, or as percentages. Statistical analysis was performed using the Statgraphics software, chi-square test, Fischer's exact test, respectively; Mann-Whitney test were carried out. *P* values less than 0.05 were considered as significant.

RESULTS

AT was found in 23 subjects (19%), and the frequency of AT appeared to be higher in women (16 cases, 25%) compared to men (7 cases, 13%), $p<0.10$. TPOAb were found in 14 subjects (12%), the frequency of TPOAb was significantly higher in women: 12 women (19%) *vs* 2 men (4%), $p<0.05$. The median of positive TPOAb titres was 235 (range 155-5050) IU/ml. TGAb were found in 8 subjects (7%), the frequency of TGAb was similar in men and in women: 6 women (9%) and 2 men (4%). The median of positive TGAb titres was 1745 (range 365 - 3780) IU/ml. New diagnosis of hypothyroidism was established in 11 cases (9%), in 2 cases overt hypothyroidism and in 9 cases subclinical hypothyroidism.
We found GADA in 12.7% non-obese patients with initial diagnosis of T2DM. GADA+ subjects were found to have longer duration of diabetes than those who were GADA-, with no difference in BMI and age. The frequency of AT did not significantly differ in GADA+ and GADA- subjects, the frequency of TPOAb, however, tended to be higher in GADA+ individuals (Table 1).
Table 2 shows a comparison of the group of pa-

Table 1 - *Autoimmune thyroiditis, thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), C-peptide levels and basic clinical characteristics in GADA- and GADA+ subjects. Data are presented as absolute numbers, ratios, percentages or as medians (25-75 percentiles).*

	Total	GADA-	GADA+	Significance
Male:female	55:63	49:54	6:9	NS
BMI (kg/m^2)	25.5 (23.7/27.3)	25.5 (23.8/27.3)	25.2 (21.2/27.4)	NS
Age (yr)	66 (58-70)	66 (58-70)	67 (58-70)	NS
Diabetes duration (yr)	8.5 (4-15)	8.0 (4-15)	14.0 (5-19)	$p<0.03$
Fasting C-peptide (nmol/l)	0.74 (0.55-1.06)	0.77 (0.60-1.09)	0.51(0.08-0.69)	$p<0.02$
Post-breakfast C-peptide (nmol/l)	1.44 (1.02-2.10)	1.47 (1.06-2.10)	0.96 (0.06-1.47)	$p<0.02$
TPOAb Male:female	14 (11.9%) 2:12*	10 (9.7%) 2:8	4 (26.7%) 0:4	$p<0.06$
TGAb Male:female	8 (6.7%) 2:6	6 (5.8 %) 2:4	2 (13.3%) 0:2	NS
Autoimmune thyroiditis Male:female	23 (19.5%) 7:16**	18 (17.4%) 7:11	5 (33.3%) 0:5	NS

*$p<0.05$ for frequency of men compared to women; **$p<0.10$ for frequency of men compared to women.

tients treated with hypoglycemic agents and/or diet with the group of patients who required insulin to reduce their hyperglycemia. The criterion used to start insulin therapy was unsatisfactory compensation of diabetes with oral hypoglycemic agents regarding to glycosylated hemoglobin (HbA_{1c}) and post-prandial glycemia levels. However, in our study there was not significant difference of HbA_{1c} in insulin requiring (median 9.8, SE=2.1%) and non-insulin requiring subjects (median 8.6%, SE=2.5). The subjects who required insulin had longer duration of diabetes and higher frequencies of GADA. They did not, however, differ from the non-insulin requiring subjects in other characteristics (BMI, age). The subjects who required insulin tended to have a higher frequency of AT.

Table 3 shows the comparison of the subjects when divided according to the post-breakfast C-peptide levels. Both subgroups did not differ in age and BMI, however the diabetes of the subjects with post-breakfast C-peptide ≤0.8nmol/l lasted longer. The frequencies of AT and TPOAb presence were higher in the group with post-breakfast C-peptide ≤0.8nmol/l.

Table 2 - *Autoimmune thyroiditis, thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), glutamic acid decarboxylase antibodies (GADA), C-peptide levels and basic clinical characteristics in subjects who required treatment with insulin (insulin requiring) and those who did not (non-insulin requiring). Data are presented as absolute numbers, ratios, percentages or as medians (25-75 percentiles).*

	Non-insulin requiring	Insulin requiring	Significance
Male:female	40:48	15:15	NS
BMI (kg/m^2)	25.3 (23.8-27.3)	25.7 (23.5-27.1)	NS
Age (yr)	66 (58-70)	66 (55-70)	NS
Diabetes duration (yr)	7 (2-11)	16 (10-19)	$p<0.001$
Fasting C-peptide (nmol/l)	0.82 (0.64-1.15)	0.39 (0.21-0.63)	$p<0.001$
Post-breakfast C-peptide (nmol/l)	1.78 (1.24-2.23)	0.76 (0.41-1.14)	$p<0.001$
GADA	8 (9.1%)	7 (23.3%)	$p<0.05$
TPOAb Male:female	8 (9.1%) 1:7**	6 (20.0%) 1:5°	NS
TGAb Male:female	6 (6.7%) 1:5	6.8% 1:1	NS
Autoimmune thyroiditis Male:female	14 (15.9%) 3:11*	9 (30.0%) 4:5	p<0.10

*$p<0.05$ for frequency of men compared to women; **$p<0.10$ for frequency of men compared to women.

Table 3 - *Autoimmune thyroiditis, thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), glutamic acid decarboxylase antibodies (GADA) and basic clinical characteristics in the group with post-breakfast C-peptide >0.8 nmol/l compared to the group with post-breakfast C-peptide ≤0.8nmol/l. Data are presented as absolute numbers, ratios, percentages or as medians (25-75 percentiles).*

	Post-breakfast C-peptide >0.8 nmol/l	Post-breakfast C-peptide >0.8 nmol/l	Significance
Male:female	45:52	10:9	NS
BMI (kg/m2)	25.6 (23.8-27.3)	25.0 (23.5-27.1)	NS
Age (yr)	66 (61-70)	63 (54-70)	NS
Diabetes duration (yr)	8 (4-13)	17 (8-19)	$p<0.004$
GADA	10 (10.3%)	5 (26.3%)	$p<0.08$
TPOAb Male:female	8 (8.2%) 1:7*	6 (31.6%) 1:5*	$p<0.02$
TGAb Male:female	6 (6.2%) 1:5	2 (10.5%) 1:1	NS
Autoimmune thyroiditis Male:female	16 (15.5%) 5:11	7 (36.8%) 2:5	$p<0.05$

*$p<0.05$ for frequency of men compared to women.

DISCUSSION

In the present study, the frequency of AT in non-obese patients with initial diagnosis of T2DM was almost 20%. These results are consistent with the frequency of AT in adult patients with T1DM that has been reported from 20-40% (11-13, 19). The incidence of autoimmune thyroiditis in the non-diabetic adult population varied from 5-10%, with significantly higher incidence in women (20). On the other hand, 2 studies of autopsy material reported notably higher incidence of lymphocytic AT of about 30% (21, 22). There is also a study in the population of blood donors that reported a very high prevalence of TPOAb in female at age 55-64 about 30% (23). We have recent data obtained from a randomly selected population from one region of the Czech Republic. The incidence of TPOAb was 8.9% in the whole adult population, whereas there were 21.1% TPOAb+ women in the age group of 50-65 yr (24). The incidence of AT in our population of non-obese patients with initial diagnosis of T2DM seems to be similar to that found in the general population.

The comparison of our results with published data on patients with an initial diagnosis of T2DM faces difficulties due to the fact that different studies used different selection criteria and suited different populations. Furthermore, most of the studies estimated only the frequency of thyroid peroxidase antibodies and based their diagnosis of AT on insufficient parameters. The use of US is associated with higher sensitivity for the diagnosis of AT disease (25).

In the study of Gambelunghe *et al.* (14), a significantly higher prevalence of TPOA in GADA+ subjects was found. The population characteristics were similar to the ones in our study, the only difference being the subjects' age (median 58; range 30-72 yr) that was higher in our study. In the study of Gambelunghe *et al.*, (14) 67 subjects were GADA+ and 174 subjects were GADA-, with prevalence of TPOA 23.9% and 5.2%, respectively ($p<0.001$). Compared with these results, our study found the TPOAb to be more frequent in GADA- subjects (9.7%). Another study by Tuomi *et al.* (26), investigated 33 GADA+ and 69 GADA- subjects with characteristics and median of age similar to our study. In this study, the frequency of TPOAb in GADA- individuals was 19% and no statistically significant difference in TPOA frequencies was found between GADA+ and GADA- groups (26). It is not clear whether those GADA- subjects have T2DM as the positivity of GADA in the group of cases of non-obese T2DM may not be the sole autoimmunity marker (6). Approximately 25% non-obese T2DM subjects may have other markers of autoimmune diabetes than GADA (3). The relatively high frequency of TPOAb in our GADA- subjects could be attributed to the fact that our population was older – the prevalence of AT has been reported to increase in elderly subjects (27), mainly in women (22).

Groop *et al.* (15) studied 312 subjects with T2DM, whose diabetes was diagnosed after the age of 35 yr and found significantly higher frequency of TPOAb in subjects who required treatment with insulin compared to those who did not require such treatment (33.7 *vs* 19.8%, respectively). The difference in the frequency of TPOAb between in-

sulin requiring and non-insulin requiring subjects found in our study was not statistically significant (9% *vs* 20%). This lack of consistence could be explained by the small size of our sample. The other reason might be lower BMI of the subjects who do not require insulin in our study compared to the data from Groop *et al*. Subjects with lower BMI might have had relatively higher percentage of unknown T1DM.

In the subgroup with lower levels of post-breakfast C-peptide, higher frequencies of TPOAb and higher frequencies of AT were observed. On the other hand, the subjects in this subgroup also had a longer duration of diabetes. In a study of children with T1DM, Radetti *et al*. (10) found that the prevalence of AT increased with the duration of diabetes. In another study of 151 adult patients with autoimmune polyglandular syndrome (28), the median of diabetes duration in which AT disease first occurred was 11 yr. This is, however, the problem of our study, as well as of many other studies, with insufficient duration of diabetes. Later development of AT cannot be ruled out in some of the patients classified as not having this disorder in the study.

In conclusion, we found that the frequency of AT in non-obese subjects with initial diagnosis of T2DM was 19%, and that AT occurred in both, GADA+ and GADA- subjects. No significant difference in the frequency of AT between insulin-treated and non-insulin-treated subjects was found. When the patients were divided according to their post-breakfast C-peptide levels, we found that the group with post-breakfast C-peptide ≤0.8 nmol/l had higher frequency of AT and higher frequency of thyroid peroxidase antibody. On the other hand, the subjects in this subgroup (C-peptide ≤0.8 nmol/l) had also longer duration of diabetes. This work demonstrated the presence of a heterogenity among non-obese cases with initial diagnosis of T2DM with onset after the age of 35 yr. We found relatively high frequency of AT in our study, but this result is related to relatively high median of age of our study population and to the risk of development of AT in non-obese patients with initial diagnosis of T2DM is probably similar to general population.

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