

Autoantibodies to thyroid peroxidase in patients with type 1 diabetes in Taiwan

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Abstract

Objective: Type 1 diabetes mellitus is frequently associated with autoimmune thyroid disease (ATD). Genetic susceptibility to autoantibody formation in association with ATD and type 1 diabetes mellitus has been described with varying frequencies, but there is still debate about the situation in the Chinese population. We have, therefore, investigated the prevalence of anti-thyroid peroxidase (anti-TPO) in type 1 diabetic patients, and compared the effect of anti-glutamate decarboxylase (anti-GAD) on the thyroid autoimmunity in patients with type 1 diabetes mellitus in Taiwan.

Subjects and methods: Two hundred and forty-three subjects with type 1 diabetes mellitus and seventy unrelated normal controls were recruited for the detection of anti-TPO. Two hundred and seventeen sera from two hundred and forty-three type 1 diabetic patients were tested for anti-GAD. RIA and immunoprecipitation were used for anti-TPO and anti-GAD detection respectively.

Results: The intra-assay and interassay coefficients of variation of anti-TPO detected by the RIA method ranged from 5.5% to 11.1%. Among 243 type 1 diabetic patients, 53 (21.8%) were positive for anti-TPO. Compared with those without thyroid autoimmunity, there was a female preponderance for the type 1 diabetic patients with thyroid autoimmunity (female:male, 99:91 vs 37:16 respectively). Among the type 1 diabetic patients with thyroid autoimmunity, anti-TPO tended to occur in those of older age or with long-standing disease. The frequency of anti-GAD was 45.6% (99 of 217), without gender preponderance (males:females, 18.0% vs 27.6%). Compared with those with negative anti-GAD, no significant difference of anti-TPO positivity for the type 1 diabetic patients with positive anti-GAD was found.

Conclusion: Our data indicated that the RIA method for anti-TPO detection is sensitive and precise for routine clinical use. The presence of anti-TPO in 21.8% of our type 1 diabetic patients confirmed the strong association of ATD and type 1 diabetes mellitus without ethnic differences. The absence of correlation between anti-TPO and anti-GAD in our type 1 diabetic patients suggested genetic heterogeneity in the role of autoimmunity of type 1 diabetes mellitus and ATD among races.

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Introduction

The association of type 1 diabetes mellitus with autoimmune thyroid disease (ATD) has been well documented in many populations (1–7). The occurrence of thyroid autoantibodies against microsomes (AMA) and thyroglobulin (ATA), frequently seen in Hashimoto's thyroiditis and Graves' disease, has been reported in type 1 diabetes mellitus with varying frequency (2, 6–8). However, AMA tends to correlate better with thyroid dysfunction than does ATA (9). Although most type 1 diabetic patients are clinically euthyroid, screening for ATD by measuring AMA levels has been recommended as there is a risk of developing future thyroid dysfunction (2, 7). Recently, detection of antibodies against thyroid peroxidase (TPO), a major

antigen for microsomal autoantibody, appears to obviate the need for AMA and ATA measurements because of the improvement in specificity and sensitivity of the method (10).

The prevalence of antibodies to islet-cell (ICA) and glutamate decarboxylase (anti-GAD) is significantly increased in patients with ATD compared with that of unaffected controls (11, 12). Genetic susceptibility of autoantibody formation associated with ATD and type 1 diabetes mellitus has been described in the literature (12–15); however, there is still debate about the situation in the Chinese population (7, 16–18). In the present study, we aimed to determine the frequency and titers of anti-TPO in Taiwanese patients with type 1 diabetes mellitus, and to compare the frequency of anti-TPO in the presence or absence of anti-GAD.

Subjects, materials and methods

Subjects

Sera were collected from 243 subjects with type 1 diabetes mellitus and 70 unrelated normal controls after informed consent was obtained. All the individuals recruited in this study were Han Chinese living in northern Taiwan. The type 1 diabetic patients were recruited from the Diabetic Clinic of the National Taiwan University Hospital, Taipei in the period 1990–1991 ($n=78$, subset 1), and in the period 1994–1995 ($n=165$, subset 2). The mean age of type 1 diabetic subjects was 17.8 (range 6–46) years, and the diagnosis of type 1 diabetes mellitus was based on the published criteria (19) with a typical clinical history of diabetic ketoacidosis and a requirement for insulin (20). The control subjects were recruited from those who visited the hospital for general health examination. All the subjects were clinically euthyroid.

Thyroid peroxidase antibody

Autoantibodies to thyroid peroxidase (anti-TPO) were measured by radioimmunoassay (TPO Antibodies Radioimmunoassay Kit, Sorin Biomedica, Saluggio, Italy). The assay is based on the competition between anti-TPO in patient's sera and the solid-phase Fab to TPO (mouse monoclonal) for the fixed and limited number of labeled TPO binding sites. After incubation, the amount of anti-TPO present in the patient's sera is inversely related to that of labeled TPO bound to the solid phase. The results of anti-TPO were obtained according to a calibration curve. The upper normal limit, determined as the mean+2s.d. of the data obtained from the sera of the controls in the study, was 11.4 arbitrary units (AU)/ml for anti-TPO, in agreement with the reference range suggested by the manufacturer (<10 AU/ml). The intra- and interassay coefficients of variation of the anti-TPO were calculated for 5 assays. The anti-TPO titers were also compared with AMA titers determined by passive hemagglutination (PH) assays (Fujirebio, Tokyo, Japan) in the sera of patients of subset 1 as previously reported (8).

Antibodies to glutamate decarboxylase (anti-GAD)

The presence of anti-GAD antibodies was tested in 217 samples from the type 1 diabetic patients with an

immunoprecipitation method (21). Briefly, *in vitro* transcription of a plasmid DNA containing human GAD₆₅ sequences (provided by Dr Dyrberg at Novo Nordisk A/S Industry, Denmark) and translation with [³⁵S]meth-ionine labeling was performed according to the manufacturer's instructions (TNT™ T7 Coupled Reticulocyte Lysate System, Promega, Madison, WI, USA). Subsequently, 10 µl serum were incubated with the labeled GAD₆₅ overnight at 4 °C. After adsorption to protein A-sepharose beads, the antibody-bound fraction of labeled GAD₆₅ was eluted and separated by polyacrylamide gel electrophoresis, and then autoradiographed. The band was deemed to be positive if the intensity of the band area exceeded the mean+2s.d. of the values from healthy controls.

Statistical analysis

Comparison between means was performed by Student's *t*-test and comparison between frequencies was carried out by chi-squared test. A *P* value of 0.05 or less was interpreted as significant for the analysis.

Results

Intra-assay and interassay variation of anti-TPO

The intra-assay coefficients of variation of the anti-TPO assay were 5.5% at a mean value of 2.7 AU/ml ($n=5$); 6.7% at 77.3 AU/ml ($n=5$) and 6.7% at 768.9 AU/ml ($n=5$). The interassay coefficients of variation were 6.9% at a mean value of 3.9 AU/ml ($n=5$); 6.0% at 54.9 AU/ml ($n=5$) and 11.1% at 370.1 AU/ml ($n=5$).

Comparison of anti-TPO and AMA

The sera of 78 subjects of subset 1 were available for analysis (8). There was a highly significant positive correlation ($r=0.889$, $P<0.001$) between the titers of anti-TPO determined by RIA and AMA determined by PH.

Demographic characteristics among type 1 diabetic patients and thyroid autoimmunity

Anti-thyroid peroxidase antibodies were detected in 53 (21.8%) out of 243 patients and 2 (2.9%) out of 70

Table 1 Frequencies and titers of anti-TPO in subjects with type 1 diabetes and normal controls. Data are shown as number (percentage).

Subjects	<i>n</i>	Anti-TPO titers (AU/ml)				
		<11.4	11.4–100	100–200	200–500	>500
Type 1 diabetes	243	190 (78.2)	20 (8.2)	13 (5.3)	12 (5.0)	8 (3.3)
Normal	70	68 (97.1)	2 (2.9)	0	0	0

Table 2 Demographic characteristics of type 1 diabetic patients positive or negative for anti-TPO. Data are presented as means \pm s.d.

	Positive	Negative	P value
Number of patients	53	190	
Male:female	16:37	91:99	<0.05*
Age at diagnosis (years)	13.4 \pm 7.8	10.4 \pm 7.3	NS
Duration (years)	7.8 \pm 6.2	6.6 \pm 4.4	NS
BMI	19.5 \pm 3.1	19.6 \pm 3.0	NS

*By χ^2 -test.

NS, not significant.

normal controls. None of these subjects had any symptoms of thyroid disease. The distribution of anti-TPO titers and positive frequencies of type 1 diabetic patients were variable as shown in Table 1. The presence of significant anti-TPO titers (>11.5 AU/ml) was thought to indicate thyroid autoimmunity. As shown in Table 2, 16 out of 107 males (15.0%) and 37 out of 136 females (27.2%) had thyroid autoimmunity. A female predominance was noted in the cases with thyroid autoimmunity ($P < 0.05$). When patients were subdivided into those who had had the disease for a short duration (duration less than 1 year) and those of long-standing (more than 1 year) disease, this association of female predominance was also true for the group with long-standing type 1 diabetes mellitus (male: female 13.8%:29.3%; $P < 0.01$), but not for the shorter-duration group (Table 3). There was a significantly higher frequency of anti-TPO positivity in older than in younger groups (43.8% in the age group more than 25 years, 27.2% in the age group of 10 to 25 years, 15.6% in the age group less than 10 years; $\chi^2 = 9.5$, $P < 0.01$). Except for the difference mentioned above, there was no remarkable difference in the body mass index (BMI).

Anti-TPO and anti-GAD in type 1 diabetes mellitus

The frequency of anti-GAD was 45.6% (99 out of 217), and no difference between males and females (18.0% vs 27.6%) was found. The occurrence of positivity of anti-TPO in the presence of anti-GAD was 23.2% (23 out of

99 subjects positive for anti-GAD). This difference was not statistically significant compared with 22.0% (26 of 118) among cases negative for anti-GAD. There was also no significant difference in patients with positive anti-GAD among those with shorter or long-standing duration of disease as shown in Table 3.

Discussion

Type 1 diabetes mellitus is strongly associated with other organ-specific diseases such as ATD, pernicious anemia, and idiopathic adrenal insufficiency (1, 4, 22, 23), while ATD has been reported to be the most common coexisting autoimmune disease with type 1 diabetes mellitus (24). There is also an increased prevalence of thyroid antibodies in type 1 diabetic patients with ATD (2, 3, 7, 13). The reasons for the increased frequency remain obscure. It was thought to result from a generally increased propensity to react against certain antigens, or from a genetically impaired ability to acquire tolerance to some autoantigens, or perhaps from certain common antigens present in the tissues prone to autoimmune disease (25). Among the thyroid autoantibodies, anti-TPO autoantibody, in addition to AMA and ATA, has recently become available for the determination of thyroid autoimmunity (10, 26). Anti-TPO assayed by monoclonal antibody-assisted RIA appears to be a more sensitive and specific marker for ATD than the conventional PH method (26). In the present study we have found an excellent correlation between AMA measured by PH and anti-TPO activity measured by RIA in sera ($r = 0.889$; $P < 0.001$), in accordance with a previous report (27). The intra-assay coefficients of variation of the anti-TPO assay ranged from 5.5% to 6.7%, while the interassay coefficients of variation ranged from 6.0% to 11.1% at different anti-TPO titers ($n = 5$), also indicating that the RIA measurement utilized in our study is a precise method of quantifying anti-TPO.

The prevalence of AMA in type 1 diabetic patients varied from 11.9% to 22% (2, 7, 28). As for the Chinese population, Tsai and Lee (7) and Chuang *et al.* (8) reported that 22% and 27.7% respectively of type 1 diabetic patients were positive for AMA, while Wong reported an absence of thyroid autoimmunity in Chinese children with type 1 diabetes mellitus (17).

Table 3 Prevalence of anti-TPO and anti-GAD in type 1 diabetic patients according to sex and duration of disease. Data are shown as number (percentage).

Antibody (+)	Duration <1 year				Duration >1 year			
	Total (n = 14)	Male (n = 7)	Female (n = 7)	P value	Total (n = 203)	Male (n = 87)	Female (n = 116)	P value
Anti-TPO	3	2 (28.6)	1 (14.3)	NS	46	12 (13.8)	34 (29.3)	<0.01
Anti-GAD	8	4 (57.1)	4 (57.1)	NS	91	35 (40.2)	56 (48.3)	NS

NS, Not significant.

Moreover, 10% to 24% of type 1 diabetic patients who were clinically euthyroid have been reported to be positive for anti-TPO (14, 15, 29). From the sera of 243 type 1 diabetic patients in the present study, 53 (21.8%) were positive for anti-TPO. The data confirmed the reports of Chuang and Tsai and their coworkers, and were in accordance with previous reports, indicating no ethnic difference in the association of ATD and Chinese type 1 diabetes mellitus.

High titers of anti-TPO were highly suggestive of ATD, and correlated well with thyroid dysfunction (30). The positive anti-TPO titers in our type 1 diabetic patients showed wide distribution. Among the patients with positive anti-TPO, the anti-TPO titers were mostly below 500 AU/ml (84.9%; 45 out of 53), whereas only 15.1% of patients had titers greater than 500 AU/ml (Table 1). Although there was no apparent thyroid disease in the tested subjects at the time of investigation, further thyroid function evaluation should be regularly followed up because of the high risk of development of thyroid dysfunction in the future.

It is well known that organ-specific endocrine autoimmunity develops more frequently in women, including type 1 diabetes mellitus with thyroid autoimmunity (8). The production of anti-TPO is inheritable in an autosomal fashion in women but not in men (31). In our study, female preponderance with a male/female ratio of 16/37 ($P < 0.05$) was also found in the type 1 diabetic patients with thyroid autoimmunity. The association was also valid for the subgroup of long-standing type 1 diabetic patients, but not for the shorter-duration group. We also found a tendency for anti-TPO to occur with increasing age, in agreement with the report by Verge *et al.* (14). Taken together, a subgroup of type 1 diabetic patients associated with thyroid autoimmunity might present in the female patients of older age or with long-standing disease. The gene or other genetic predisposing factors responsible for this interesting phenomenon need to be investigated further. As for human leukocyte antigen (HLA) genes, we have previously identified that HLA DR3 and DR4 are associated with the susceptibility to type 1 diabetes mellitus regardless of thyroid autoimmunity in a Taiwanese population (8). With similar analysis in the present study, there was no difference in the HLA DR typing between the type 1 diabetic patients with or without positivity of anti-TPO (data not shown).

Varying frequencies of anti-GAD have been reported ranging from 25% to 70% (14, 32–34), compared with a frequency of 45.6% (99 out of 217) in our study. In contrast to previous reports describing a higher frequency of anti-GAD in females compared with males, we failed to show a difference between the two sexes. There was also no difference in the occurrence of positive anti-GAD among patients with different duration of disease and different age at diagnosis. In view of the association of anti-GAD and type 1 diabetes mellitus with thyroid autoimmunity, Kawasaki *et al.* demonstrated

that high levels of anti-GAD were present in type 1 diabetic patients with ATD (12). Martino *et al.* (32) revealed a significantly higher frequency of anti-TPO among anti-GAD positive than among anti-GAD negative adults with newly-diagnosed type 1 diabetes mellitus, whereas Verge *et al.* showed a similar association but without statistical significance (14). In the present study, we could not find any correlation between the presence of anti-TPO and anti-GAD. This may reflect the ethnic difference in the role of anti-TPO and anti-GAD immunity for type 1 diabetes mellitus with thyroid autoimmunity.

In conclusion, the utilization of a monoclonal antibody-assisted RIA for detection of anti-TPO has proved to be a sensitive and precise method for clinical routine use. The presence of anti-TPO in 21.8% of our patients with type 1 diabetes prompts the necessity for further thyroid function evaluation. The absence of a correlation between anti-TPO and anti-GAD in the Taiwan type 1 diabetic patients indicates an ethnic difference or genetic heterogeneity in the roles of autoimmunity of type 1 diabetes mellitus and ATD played by these two autoantibodies.

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