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Glutamic acid decarboxylase (anti-GAD) & tissue transglutaminase (anti-TTG) antibodies in patients with thyroid autoimmunity

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***Background & objectives*: Several autoimmune disorders have been reported to be associated with autoimmune thyroiditis and may coexist with other organ-specific autoantibodies. The aim of the present study was to evaluate the presence of tissue transglutaminase (anti-TTG) and glutamic acid decarboxylase (anti-GAD) antibodies in patients suffering from autoimmune thyroiditis as diagnosed by anti-thyroid peroxidase (anti-TPO) antibodies, which may indicate high risk for developing celiac disease or type 1 diabetes mellitus.**

***Methods*: Five thousand children and 2800 adults were screening as part of a general health examination done on a voluntary basis in four different parts of Delhi. A total of 577 subjects positive for anti-TPO antibody constituted the cases. Equal number of age and sex matched anti-TPO antibody negative controls were randomly selected from the same cohort to form paired case control study. The cases and controls were further divided into two groups as follows: group-1 (children and adolescent <18 yr), group-2 (adults >18 yr). Serum samples of cases and controls were analysed for thyroid function test (FT3, FT4, and TSH), anti-TTG and anti-GAD antibodies.**

***Results*: A total of 1154 subjects (577 cases and 577 controls) were included in this study. Hypothyroidism was present in 40.2 per cent (232) cases compared to only 4.7 per cent (27) in controls (P<0.001). Anti-TTG and anti-GAD antibodies were present in 6.9 and 12.5 per cent subjects among cases compared to 3.5 per cent (P=0.015) and 4.3 per cent (P=0.001) in controls, respectively. Only anti-GAD antibody were significantly positive in cases among children and adolescents (P =0.0044) and adult (P=0.001) compared to controls. Levels of anti-TTG and anti-GAD antibodies increased with increasing titre of anti-TPO antibody.**

***Interpretation & conclusions*: Our findings showed high positivity of anti-GAD and anti-TTG antibodies among subjects with thyroid autoimmunity. It is, therefore, important to have high clinical index of suspicion for celiac disease or type 1 diabetes mellitus in patients with autoimmune thyroiditis.**

Key words Anti-GAD antibody - anti-TPO antibody - anti-TTG antibody - thyroid autoimmunity

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Tolerance to self antigen is acquired by elimination of autoreactive T-cells[1] and breakdown of this tolerance leads to activation of these cells and consequent autoimmunity. Various mechanisms postulated are genetic susceptibility, release of sequestrated self antigen by infection or molecular mimicry[2]. This loss of immune tolerance leading to exposure of various tissue specific peptides, results in the development of autoimmune disorders. Presence of auto-antibodies, have a strong predictive value in cases with organ-specific autoimmune disorders, which are known to have a long preclinical phase[3]. Among patients with type 1 diabetes mellitus (T1DM), evidence based recommendations exist for evaluation of thyroid and gut autoimmunity[4]. However, no such recommendations exist for patients with autoimmune thyroiditis, which is a common disorder[5,6]. In view of limited reports showing association between thyroid autoimmunity, T1DM and celiac disease, especially in children and adolescents[7-13], we undertook this study to evaluate presence of tissue transglutaminase (TTG) and glutamic acid decarboxylase (GAD) antibodies among patients with thyroid autoimmunity.

Material & Methods

The present study was carried out as part of school health examination and general health check-up on voluntary basis for members of Resident Welfare Associations from four different regions (South, North, West and East) of Delhi between January 2009 - December 2010. A total of 5000 children and 2800 adults were evaluated for detailed clinical and biochemical evaluation. Exclusion criteria included subjects with known systemic disorders or medications. Children (n=236) and adults (n=341) who were found to be positive for anti-thyroid peroxidase (anti-TPO) antibodies (>34 IU/l) were enrolled as case subjects. Equal number of age (± 1 yr) and sex matched anti-TPO antibody negative controls were selected from the same cohort for paired case-control study. The study subjects (cases) and controls were further divided into two groups as follows: group-1 (children and adolescents ≤ 18 yr), group-2 (adults >18 yr). Serum samples of cases and controls were analysed for thyroid function test (FT3, FT4, and TSH), anti-TTG and anti-GAD antibodies. The study protocol was approved by the Ethics Committee of the Army Research and Referral Hospital, Delhi Cantt and informed consent was obtained from participants or parents of children.

Thyroid function tests were performed by electrochemiluminiscence assay and normal ranges for FT3, FT4 and TSH were 2.8-7.1 pmol/l, 12.0-22.0 pmol/l, and 0.27-4.20 mIU/l, respectively. Subjects with TSH >4.5 mIU/l were defined either as subclinical hypothyroidism if FT4 was normal or overt hypothyroidism if FT4 was low. Anti-TPO antibodies were measured by using electrochemiluminiscence kits from Roche (Germany) with normal range from 0.0-34.0 IU/l. Anti-TTG and anti-GAD antibodies were measured by enzyme immunoassay (ELISA) kit supplied by Immunodiagnostic System (USA) as per the manufacturer's protocol. Anti- TTG and anti-GAD antibody levels of >20 and >1.05 U/ml were considered positive. Intra- and inter-assay coefficients of variation were 3.5 and 8.6 per cent for anti TTG antibodies, and 4.5 and 4.8 per cent for anti-GAD antibody, respectively.

Statistical analysis was carried out using EPI2003 (CDC, Atlanta, USA). Data were presented as mean ± SD or number (%) unless specified. All paired data were analysed by paired t-test. If Barlett's chi-square test for equality of population variances was <0.05, then Kruskal-Wallis test was applied. All non parametric paired data (case and control) were analysed by McNemar test (*http://www.graphpad.com/quickcalcs/mcnemar1.cfm*).

Results

Mean age, thyroid function tests and results of anti-TTG and anti-GAD antibody estimation among cases and controls in different age groups are depicted in Table I. Hypothyroidism was present in 40.2 per cent (232) cases when compared to 4.7 per cent (27) in controls (P<0.001). Overt hypothyroidism was present in 13.1 per cent (n=76) of cases and none in controls. The remaining subjects among hypothyroid group had subclinical hypothyroidism. Overall, FT3 and FT4 levels were significantly lower and TSH levels were significantly higher in cases than in controls. Though FT4 levels were also lower compared to controls in group-1 but was not statistically significance (Table I).

Levels of anti-TTG (6.9%) and anti-GAD (12.5%) antibodies were significantly higher in cases when compared to controls (3.5%, P=0.015; 4.3%, P=0.0001, respectively). However, when the difference in prevalence of TTG antibodies was assessed separately in the two age categories, it failed to attain significance; whereas that for GAD antibodies persisted unchanged (Table I). There was no significant difference in anti-TTG and anti-GAD antibody levels between males and females (5.3 vs. 5.1%, P=0.92; and 7.1 vs. 9.7%,

Table I. Thyroid function, anti-TTG and anti-GAD antibodies in groups 1 and 2 patients

Group	Test	Cases*	Controls	*P* value
Child and adolescent group (<18 yr) (n=572)	FT3 (pmol/l)	4.30 ± 0.81	4.68 ± 0.79	<0.001
	FT4 (pmol/l)	15.75 ± 2.31	16.05 ± 2.54	0.18
	TSH (mIU/l)	5.44 ± 7.59	2.64 ± 1.19	<0.001
	anti-TTG positive (U/ml)	15 (6.4)	8 (3.3)	0.21
	anti-GAD positive (U/ml)	25 (10.6)	8 (3.3)	0.003
Adult group (> 18 yr) (n=682)	FT3* (pmol/l)	4.26 ± 1.12	4.51 ± 0.75	<0.001
	FT4* (pmol/l)	14.69 ± 5.06	16.12 ± 2.51	<0.001
	TSH* (mIU/l)	6.76 ± 10.41 (3.80)	2.19 ± 1.86 (2.11)	<0.001
	anti-TTG positive (U/ml)	25 (7.4)	12 (3.5)	0.051
	anti-GAD positive (U/ml)	47 (13.8)	17 (5.0)	0.001

*Positive for anti-thyroid peroxidase antibodies (>34 IU/l)
FT3, free triiodothyronine; FT4, free tetraiodothyronine; TSH, thyroid stimulating hormone; TTG, tissue transglutaminase; GAD, glutamic acid decarboxylase
Values in parentheses are percentages

Table II. Anti-TTG and anti-GAD antibodies according to anti-TPO titre

Anti-TPO titre (IU/l)	Number of subjects	Anti-GAD antibody No. (%)	Anti-TTG antibody No. (%)
Negative (<34)	528	23 (4.3)	16 (3.0)
Mild positive (34.1-102)	180	16 (8.9)	10 (5.6)
Strongly positive >102	446	58 (13.0)	33 (7.4)
P value*		<0.00001	0.023

*Chi-square for trends
TPO, thyroid peroxidase; TTG, tissue transglutaminase; GAD, glutamic acid decarboxylase

P=0.17, respectively). However, anti-TTG and anti-GAD antibody levels increased with increasing titre of anti-TPO antibody (Table II).

Discussion

High prevalence of autoimmune thyroiditis with increasing age, female gender and in subjects with hypothyroidism has been well established[4,5,14]. The overall presence of anti-TTG antibody was significantly higher among cases than in controls. However, when stratified into children and adults, there was no significant difference. There are limited reports on an increasing prevalence of celiac disease in patients with autoimmune thyroid disorders with rates ranging from 2.0-7.8 per cent[7,9-14].

High occurrence of anti-TTG antibody (6.4%) was found in children with autoimmune thyroiditis in this study. Limited reports on high occurrence of anti-TTG antibody in children with T1DM[15] and autoimmune thyroiditis[13,16] are available. There are two reports available from Turkey and Italy, where anti-TTG antibodies have been observed in 7.9 and 7.8 per cent children with autoimmune thyroid disease[13,16]. Conversely, in patients with celiac disease, anti-TPO antibody and autoimmune thyroiditis have been reported to range from 10.5-14.6 per cent[7,17].

In the present study, anti-TTG antibodies were seen in 3.3 per cent in children and adolescent control group which was similar to that reported earlier (2.5%)[15]. In contrast, Sari *et al*[13] did not find anti-TTG positivity in their control group in Turkish children. In a study from northern India anti-TTG antibodies were reported in 10.6 per cent suspected cases of celiac disease through a structured questionnaire in 3-17 yr old children[18].

Anti-TTG antibodies were found in 6.3 per cent adult subjects with thyroid autoimmunity in the present study, which is similar to that reported from Turkey[19].

Table III. Prevalence of markers of celiac disease in subjects with autoimmune thyroiditis

Author	Year	Place	Method	% Prevalence (n)
Collin *et al*[24]	1994	Finland	Anti-endomysium antibodies and test for malabsorption	0.4 (83)
Cuoco *et al*[12]	1999	Italy	Anti-gliadin and anti-endomysium antibodies	4.3 (92)
Valentino *et al*[25]	1999	Italy	Anti-endomysium antibodies	3.3 (150)
Sategna-Guidetti *et al*[21]	1999	Italy	Anti-endomysium antibodies	3.3 (152)
Volta *et al*[26]	2001	Italy	Anti-endomysium antibodies	3.2 (220)
Hadithi *et al*[27]	2007	Netherlands	Anti-TTG antibody and jejunal biopsy	15 (104)
Guliter *et al*[19]	2007	Turkey	Anti-TTG antibody and jejunal biopsy	7.8 (136)
Present study	2011	India	Anti-TTG antibody	6.3 (577)

However, a wide range of markers of celiac disease in subjects with thyroid autoimmunity has been reported (Table III). Conversely, high prevalence of anti-thyroid antibodies (13.9-29.7%) among patient with celiac disease has also been reported[20,21]. It has been suggested that celiac disease might have a role in the development of thyroid autoimmunity[22]. The present study showed a positive relation between anti-TTG antibody and anti-TPO antibodies. However, Jiskra *et al*[23] did not show any significant relation between anti-TPO and anti-TTG-antibodies.

Anti-GAD antibody positivity was significantly higher among cases (12.5%) than in controls (4.3%), which persisted even after stratification according to age groups. A low positivity of anti-GAD antibodies (5.1-7.9%) has been reported among non diabetic patients with autoimmune thyroid disease[28,29].

We perhaps for the first time have observed high positivity of anti-GAD antibodies (10.6%) in children with autoimmune thyroiditis.

In our study anti-GAD antibody positivity among adults was significantly higher among cases than in controls (13.8% vs. 5.0% P=0.002). A wide geographic variation in has been reported in anti-GAD positivity in patients with Hashimoto's thyroiditis[28,29]. High anti-GAD antibody positivity has been reported among patients with Graves' disease (10-13%)[30].

The limitation of the study included absence of histological evaluation of intestinal biopsy to confirm diagnosis of celiac disease and long term follow up of anti-GAD and anti-TTG antibody positive patients to assess the functional significance of these antibodies in the predisposition to celiac disease or type-1 diabetes mellitus.

In conclusion, our study showed high anti-GAD and anti-TTG positivity among subjects with thyroid autoimmunity. It is, therefore, important to have high clinical index of suspicion for these two disorders in patients with autoimmune thyroiditis.

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