

Seropositivity to anti-thyroid peroxidase and anti-thyroglobulin autoantibodies in hypo and hyper-thyroidism: Diagnostic and epidemiological significance

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Abstract

Background: Autoantibodies to thyroid antigens in general, and thyroid peroxidase in particular, are considered as the hallmark of autoimmune thyroid dysfunctions. While a pro-inflammatory response is considered as predominant in hypothyroidism, in Grave's disease it is a predominant antibody response.

Objectives: The objective of the present study was to evaluate seropositivity to TPO and TG antigens in these two conditions with apparently diversified patterns of immune responses.

Methods: An Enzyme linked immunosorbent assay was employed for detection of Anti-Thyroid Peroxidase (TPO) and Anti-Thyroglobulin (TG) auto-antibodies in sera of patients with Hypo and Hyperthyroidism.

Results: Total seropositivity to both TPO Ab/ TG Ab was found to be 69.09% in hypo and 66.66% in hyperthyroidism; it was 54.54% and 53.33% for hypo and hyperthyroidism respectively for TPO Ab alone. A substantially less seropositivity of 41.81% and 40% was observed for Hypo and Hyperthyroid respectively for TG Ab. Analysis of seropositivity in relation to age at onset revealed higher sero-positivity (53.33%) in early onset (≤ 35 years) group in hypo for TPO Ab. Functional significance of TPO Ab in the two thyroid conditions has been discussed particularly in the light of their diagnostic utility and epidemiological significance.

Conclusion: It is concluded that higher sera positivity was observed for TPO compare to TG. These variations may be due to differences in antigenic characteristics of these two thyroid antigens.

Keywords: Thyroid dysfunction, Anti-TPO, Anti-TG, Auto-antibodies- Epitopic differences

Introduction

Auto-immune thyroid disorders are considered as the most common organ specific autoimmune conditions having a prevalence rate of about 5% with female preponderance (4 females: 1 male).^{1,2} The frequently observed thyroid disorders are hypothyroidism resulting in increased serum TSH levels with reduced T4/T3 hormone production while the hyperthyroidism is associated with over-production of T4/T3 hormones and decreased TSH levels (< 0.30 $\mu\text{IU/mL}$). Laboratory diagnosis of these thyroid disorders is based on quantitative estimation of serum TSH as well as the levels of thyroid hormones.³ Auto-immune status of hypo- and hyperthyroidism is generally determined by demonstrating auto-antibodies to various thyroid antigens viz., thyroid peroxidase enzyme (previously referred to as microsomal antigen), thyroglobulin and the Alpha and Beta units of receptor to thyroid stimulating hormone.⁴ The importance of detecting these antibodies lies not only in determining auto-immune status for diagnostic purpose but also for epidemiological studies. These antibodies have also been shown to be prognostic indicators of severe form of disease, for the sero diagnosis of hyperthyroidism can be done by detection of autoantibodies to TSH receptor or alternatively detection of anti-TPO Abs has also been recommended in view of cumbersome bioassay for anti-TSHR antibodies.⁴ In the present

study screening for auto-antibodies to TPO and TG was performed on serum samples from patients with hypo and hyperthyroidism employing ELISA kits from M/S Omega Genesis Cambridge U.K). The objective of the study was not only to determine auto-immune status of patients belonging to these two clinical conditions but also to compare the seropositivity to these antigens in the patients.

Materials and Methods

Patients visiting the out-patient ward of the Department of Medicine were selected. Institutional Ethics committee approval was obtained for carrying-out this study. After provisional diagnosis the patients were advised to undergo thyroid profile test. Two ml of intravenous blood was drawn into vacutainers without anti-coagulant. The serum obtained from the clot was used for estimation of Thyrotropic-stimulating hormone (TSH), Total tri-iodothyronine (TT3) and Total tetra-iodothyronine (TT4). Normal values for these hormones were as follows: TSH 0.27-5.0 $\mu\text{IU/mL}$, Total T4 60-120 nmol/L and total T3 0.6-3.3 nmol/L. Patients with TSH more than upper limit of normal range and T4/T3 levels less than the lower limit of normal range were considered as suffering from hypothyroidism. Cases whose TSH levels were less than the lower limit of normal TSH range along with elevated TT4/TT3 levels were diagnosed as suffering from

hyperthyroidism. TT3 and TT4 estimations were performed by a third generation chemiluminiscent assay (Biomerieux, France, 3rdGeneration). Demographic details of the patients were recorded in a proforma and it was also noted whether the patient was already diagnosed as suffering from thyroid dysfunction and was on therapy. A total of 85 patients (55 hypothyroid and 30 hyperthyroid) were selected. The number of female cases was 77 and males 8. Sera from cases diagnosed as suffering from hypo- and hyperthyroidism were tested for anti-TPO IgG and anti-TG IgG auto-antibodies employing commercially available ELISA Kits for quantitative assay for anti-thyroid peroxidase IgG and anti-thyroglobulin IgG antibodies.

Enzyme –linked Immunosorbent assay for TPOAb

IgG and TGAb IgG: Detection of autoantibodies was carried out in 96 well microplates precoated with TPO/TG antigens. Patients' sera diluted 1:100 were dispensed into the wells in 100 microlitre quantity. Positive and negative controls supplied with the kit were also added (100µl) in duplicate along with standard dilutions for plotting the standard curve. The microplate was incubated for 30 minutes. The wells were decanted by inverting the plate on a blotting paper and were thoroughly washed with washing buffer (100mM Tris buffered saline) to remove unbound serum components. In the next step rabbit anti-human IgG conjugated to horse radish peroxidase was added (100µl) to the well which binds to surface bound antibodies in the second incubation for 15 minutes. In

order to remove unwanted conjugate washing was performed with washing solution. Subsequently a solution containing 3, 3', 5, 5' tetramethyl benzidine (TMB) enzyme substrate (100µl) was added to trace the specific antibody binding and incubated for 15 minutes. This was followed by addition of 100µl of stop solution (0.25M sulphuric acid) to each well which terminates the reaction and provides the appropriate pH for color development. The optical densities of the standards, controls and samples are measured in a microplate reader at 450 nm. A 620 nm filter can be used as a reference wavelength. The antibody titres in the patients sera were determined from the standard curve plotted using standard dilutions provided with the kit and expressed as units/ml. Values above 75 IU/ml were considered as consistent with the presence of autoimmune thyroid disease while sera having below 75 IU/ml were considered as sero-negative. Similarly a cut-off value of ≥ 100 U/mL was used for TG Ab seropositivity.

Results

A total of 85 patients (55 hypothyroid and 30 hyperthyroid) were included in the study; of these 5 were males and 50 females in hypothyroid group and 3 males out of 30 in hyperthyroid cases (Table 1). The mean ages with stand deviations were 35.27 ± 11.7 and 37.23 ± 13.66 years in hypo- and hyperthyroid categories respectively. Reference value ranges for serum TSH, total T3 and total T4 have also been provided (Table 1)

Table 1: Demographic details of the patients and Reference value range of serum TSH, total T3 and total T4

Groups	Hypothyroidism	Hyperthyroidism
Number	55	30
Mean Age	35.27 ± 11.7	37.23 ± 13.66
Gender(male/female)	5/50	3/27
TSH (μ IU/mL)	0.27-5.0	0.27-5.0
TT3(nmol/L)	0.6-3.3	0.6-3.3
TT4(nmol/L)	60-120	60-120

Table 2: Total Sero Positivity for TPO Ab and TG Ab IN Hypo and Hyperthyroid cases

Category		*TPOAB	*TGAB	TPO+TG	Total
Hypothyroid	55	15	8	15	38(69.09%)
Hyperthyroid	30	8	4	8	20(66.66%)

*TPO AB ALONE, *TG ALONE, TPO+TG

The total seropositivity together with TPO Ab as well as TG Ab in hypothyroid group was found to be 69.09% (38 cases out of 55). This includes cases positive for either TPO Ab or TGAb alone as well as those sero-positive for both of these auto-antibodies. Similar analysis in hyperthyroid group revealed a total seropositivity of 66.66% (20 out of 30 cases). The percent prevalence rate for TPO Ab alone in hypothyroid cases was 54.54% (30 cases out of 55) and 53.33% (16 out of 30 cases) (Table 3) in hyperthyroid condition. Compared to this a relatively low seropositivity was recorded for TG Ab; 41.81% for hypo- and 40% for hyperthyroid group (Table 3).

Table 3: Prevalence of auto-antibodies to TPO and ATG antigens in cases with Hypo and Hyperthyroidism

Category	Autoantibodies to TPO Ab		
	Total	Positive	Negative
Hypothyroid	55	30(54.54%)	25(46.46%)
Hyperthyroid	30	16(53.33%)	14(46.66%)
Category	Autoantibodies to TG Ab		
	Total	Positive	Negative
Hypothyroid	55	23(41.81%)	32 (58.18%)
Hyperthyroid	30	12(40%)	18(60%)

Seropositivity to TPOAb AND TG Ab in relation to age at onset of AITD: Analysis of seropositivity to TPO Ab and TG Ab by categorizing seropositive patients according to age at onset of disease revealed that 53.33% cases (30 out of 55) of hypothyroid group had an age at onset ≤ 35 years (Table 4); whereas it was 46.66% (14 out of 30) in cases with age at onset > 35 years. However in cases with hyperthyroidism, the late onset group had high percent seropositivity for TPOAb (62.50%) in contrast to 37.50% in early onset group (Table 5).

Table 4: Prevalence of Autoantobodies to TPO and TG in relation to age at onset of the disease in Hypothyroid cases

Category	TPO Ab		TG Ab	
	Positive	Negative	Positive	Negative
≤ 35 Years	16(53.33%)	14(56%)	10(43.47%)	21(65.62%)
≥ 35 Years	14(46.66%)	11(44%)	13(56.52%)	11(34.37%)
Total	30	25	23	32

Table 5: Prevalence of auto antibodies to TPO and TG in relation to age at onset of the disease in hyperthyroid cases

Category	TPO Ab		TG Ab	
	Positive	Negative	Positive	Negative
≤ 35 Years	6(37.5%)	9(64.28%)	7(58.33%)	9(50%)
≥ 35 Years	10(62.5%)	5(35.71%)	5(41.66%)	9(50%)
Total	16	14	12	18

The prevalence rate of TGAb was 43.47% in early onset hypothyroid group while it was considerably higher (56.52%) in late onset group (>35 yrs). The percent seropositivity in hyperthyroid group with age at onset ≤ 35 was 58.33% compared to 41.66% in late-onset group (Table 5).

Discussion

The number of female cases was much higher with a ratio of 10 females to 1 male. This is accordance with other studies where female preponderance has been reported.^{5,6,7} The high vulnerability of females (particularly of young age) to autoimmune thyroid dysfunction is attributed to hormonal imbalance at puberty, high level of estrogen is also likely to cause rise in TSH.⁸ Another reason that is offered is skewed X-chromosome inactivation for high susceptibility of females to autoimmune thyroid dysfunction.

The objective of the present study was to compare the relative seropositivity to TPO Ab and TG Ab in patients suffering from hypo and hyperthyroidism. There are limited numbers of such comparative analysis in literature and this is the first report on this aspect in patients from own country. The prevalence of TPO Ab as well as TG Ab together in the present study was 69.09% in hypo and 66.66% in hyperthyroid cases indicating high seropositivity when these auto-antibodies are evaluated together in the patients (Table

2). However, seropositivity to of TPO Ab alone was 54.54% in hypo and 53.33% in hyper cases compared to a low seropositivity of 41.81% for hypo and 40% for hyper for TG Ab indicating higher sensitivity and specificity of TPO Ab in not only diagnosing cases of these two thyroid conditions but also its potential application in epidemiological studies in order to know the trend of the disease in population.^{9,10}

The total seropositivity for (TPOAb/ TG Ab alone plus TPO Ab + TG Ab) reported in the present study is comparable to those reported on thyroid dysfunctions in other studies. There are reports indicating that the seropositivity to thyroid antigens to be generally above 60% in hypothyroid group.⁸ Nevertheless in the present study the percent seropositivity for TPO Ab in the hypothyroid group was 54.54% while it was 53.33% in patients with hyperthyroidism. In another study on newly diagnosed cases of hypothyroidism, seropositivity to TPO Ab was reported to be around 60% which is somewhat higher than that observed in the present study.¹⁰ In studies where RIA has been

employed for serological detection of TPO auto-antibodies seropositivity was found to be between 40-50% of cases.^{11,10} Various factors may influence seropositivity of disease in the patients studied as well as regional and ethnic variations. In a similar study reported about 59% of the patients seropositive in coastal region where sea food is having high iodine concentration the percentages are relatively high.⁷

The importance of TPO Ab in epidemiological studies can be realized from the findings that these autoantibodies are present in healthy individuals 7-9 years before these individuals develop hypothyroidism. Whereas in cases of Graves disease this period was 6 months to 2 years before the onset of the disease. This was reported by Hutfluss et al in a retrospective study carried out on healthy individuals emphasizing the need for autoantibodies screening in healthy individuals in women with first trimester pregnancy.¹²

Seropositivity in relation to age at onset: The seropositivity to TPO auto-antibodies appear to be somewhat higher in early age at onset cases of hypothyroidism though this difference was not statistically significant (Table 4). However, in cases with hyperthyroidism, the late onset group had a high sero-positivity for TPOAb (62.50%) in contrast to 37.5% in early-onset group. There appears to be a difference for age at onset related seropositivity. The prevalence rate of TGAb was 43.47% in early onset hypothyroid group while it was higher (56.52%), (Table 5) in late onset group (>35 years). It is likely that with advancing age the prevalence rate of TG Ab may increase. Sinclair⁴ inferred that TG auto-antibody may be more useful in monitoring and treatment of thyroid carcinoma. However, further studies are required with relatively large number of cases to draw more convincing conclusion about age-related prevalence rate of TG Ab as well as TPO Ab in these disorders.

Hypo and Hyperthyroidism are the frequently observed thyroid dysfunctions, cell-mediated immunity is believed to play a major role in the damage to follicular cells in hypothyroidism and this damage is complimented to a certain extent by thyroid peroxidase auto-antibodies. As far the hyperthyroidism is concerned a predominant role of humoral response is attributed to the auto immune process in its pathogenesis. Nevertheless in both the diseases a great majority of the patients are positive for TPOAb.⁸

Various studies on subclinical hypothyroidism have brought forth a positive correlation between TPOAb positivity and increased TSH levels in subclinical subjects.^{5,13,14,15,16} In a more convincing study on functional aspects of TPO Ab, Silva et al analysed TPO Ab IgG subtypes in sera from patients with overt hypothyroidism as well as those with subclinical condition. It was shown that the proportion of IgG1 was higher than IgG4 in cases with overt hypothyroidism. At variance to this the proportion of

IgG1 was substantially less in subclinical group resulting in a low IgG1/IgG4. The authors suggested the subclinical cases with higher proportion of IgG1 are likely to progress to overt condition.¹⁶

It is known that IgG1 sub-class has not only complement fixing activity but is also known to mediate death of follicular cells through antibody dependent cell-mediated cytotoxicity (ADCC) by binding to the FC receptors on NK⁺ cells.¹⁷ In contrast to this IgG4 sub-class lacks complement fixing activity and has poor affinity for FC receptors.¹⁸

In another study on immunoglobulin G subclasses of TPOAb in Euthyroid, Subclinical and overt hypothyroidism groups, Xie et al attempted to relate proportions of subclasses with different thyroid functional status.¹⁹ They showed that sera from hypothyroid patients the prevalence of IgG2 was significantly higher than that of sub-clinical hypothyroid cases and the later was significantly higher than that of the euthyroid group. The pro-inflammatory cytokine Interferon-gamma produced by CD4⁺ cells in these patients in the H group may induce the isotype switching to IgG2 in plasma cells producing TPOAb. In this regard in an earlier study we demonstrated IFN-gamma over producer genotype was associated with risk of hypothyroidism, and interestingly low producer genotype was associated with predisposition to hyperthyroidism.²⁰

Based on these observations it is suggested that genotype analysis of IFN- γ may be included in TPOAb prevalence studies in order to predict with greater reliability those subclinical cases who are likely to progress to over hypothyroidism.

So far as the hyperthyroidism is concerned a pathogenic role of TPO Ab in the disease process is uncertain because of minimal damage if any to follicular cells.²¹ Ironically there are no reports available on IgG subclasses on TPOAb in hyperthyroid cases an information that could have been helpful in inferring a possible pathogenic role of these autoantibodies. It is suggested here that hyperthyroidism being an autoimmune thyroid condition with the predominant role of antibody response, TPOAb IgG subclasses can be analysed in relation to IL-10 genotypes. In view of reports which indicate that switching to IgG4 synthesis by plasma cells appears to be modulated through IL-10; over producer genotype individuals may produce higher proportion of IgG4 TPOAb.^{22,23,24}

A paradigm shift in investigation on functional aspects of Anti-TPO antibodies would be to include genotype analysis for SNPs in the gene coding for IL-10/IFN- γ in order to predict with greater reliability progression to overt hypothyroidism.

The choice of SNPs which have an impact on gene expression of IFN- γ /IL-10 coding genes may lead to more fruitful results in delineating precise differences between overt and subclinical cases of hypothyroidism.

Conclusion

It is concluded that higher sera positivity was observed for TPO compare to TG. These variations may be due to differences in antigenic characteristics of these two thyroid antigens.

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