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A study on relationship between thyroid peroxidase antibodies (Anti-TPO antibodies) and thyroid dysfunction patients

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ABSTRACT

Background: Thyroid peroxidase (TPO) enzyme is required for the synthesis of thyroid hormones. Any alteration in TPO results in a deranged thyroid profile. Antibodies against TPO serve as useful markers for the detection of auto-immune thyroid diseases. Thus, present study was conducted to analyse the relation of anti-TPO antibodies with thyroid dysfunction.

Materials and Methods: After obtaining an ethical approval, this cross-sectional study was carried out for a period of 2 years. Adult patients (≥18 years old) presenting with symptoms related to thyroid dysfunction and referred for analysis of T3, T4, TSH and anti-TPO antibodies were included in the study. Over night fasting blood samples were collected, labelled and stored. Anti-TPO antibody test for human IgG was done using Enzyme Linked Immunosorbent Assay (ELISA) kit. Statistical analysis was carried out using SPSS (version 26.0). Chi square test was used to compare the data and p-value <0.05 was considered as statistically significant.

Results: Total 100 patients were analyzed. Maximum 32 patients were found in 21-30 year age group. Mean age for females was 33.7 ± 1.20 years. Male: female ratio was 1: 2.12. Hypothyroid was noted in maximum 73 patients with 49 females and 24 males. Positive anti-TPO antibody patients were 44 out of which 33 were females. Almost 35 out of 44 patients were anti-TPO antibody positive and hypothyroid with majority 27 females. Maximum12 out of 27 hypothyroid and anti-TPO antibody positive females belonged to 21-30 year age group.

Conclusion: Anti-TPO antibodies were found to be associated with hypothyroid dysfunction. It was noted more among females of reproductive age group.

1. Introduction

Thyroid peroxidase (TPO) enzyme is required for the synthesis of thyroid hormones. It regulates the amount of hormone to be released as well as thyrotropin receptor. Any alterations might result in a deranged thyroid profile. In few cases, auto-antibodies may also be formed against TPO. Measuring the levels of circulating antibodies against TPO as well as Thyroglobulin (TG) helps in the detection of auto-immune thyroid diseases easily. Unnikrishnan et al. mentioned that about 42 million people in India were estimated to be suffering from thyroid diseases.[1] A population study had suggested that the prevalence of anti-TPO and anti-TG antibodies was 9.5% and 8.5% respectively.[2] However, it is reasonable to measure only TPO antibodies since antibodies against TG are uncommon.

Auto-immune thyroid diseases present clinically either as hyperthyroidism or hypothyroidism due to over-production of hormone or destruction of the glandular follicles respectively. But laboratory analysis show that >90% Hashimoto's thyroiditis and 40%-70% Grave's disease cases possess auto-antibodies irrespective of the functional status of thyroid gland.[3,4] TPO antibodies attack the complexes that are present in the thyroid gland. Thus, auto-antibodies serve as useful markers for auto-immune thyroid diseases. As the auto-immune process reduces

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thyroid function gradually, there is a compensatory phase where normal thyroid hormone levels are maintained by a rise in TSH. Later, TSH levels rise further and symptoms become apparent presenting as clinical hypothyroidism. In such cases, there is always a risk of patient's condition progressing to overt hypothyroidism. In the recent years, it has also been hypothesized that puberty goiter in adolescents and multi-nodular goiter in adults are due to autoimmune thyroiditis.[5]

Prevalence of thyroid antibodies is higher in women than in men. As per Whickham survey, the annual risk of developing hypothyroidism in anti-TPO antibodies positive women with normal thyrotropin levels was 2.1%.[6] Prevalence in high TSH group was 18.6% versus 3% in low TSH group.[7] In India very few studies had been conducted to find out the relation of anti-TPO antibodies with thyroid dysfunction. Hence, present study was carried out to analyze the relation of anti-TPO antibodies with thyroid dysfunction.

2. Materials and Methods

This cross-sectional study was conducted after obtaining an approval from Institutional Ethics Committee for a period of 2 years at a Tertiary Care Teaching Hospital of India. All adult patients (≥18 years old) visiting to super speciality endocrine clinic with the symptoms related to thyroid dysfunction and referred for analysis of T3, T4, TSH and anti-TPO antibodies were included in the study. Those patients who refused to participate in the study were excluded out. After taking written informed consent from study patients, data such as patient's age; gender; symptoms; T3, T4, TSH values, TPO positive and negative; were collected on pre-designed proforma.

Overnight fasting blood samples were collected, labelled and stored. T3, T4 and TSH were processed by using patient's serum sample weekly twice. It was done using an automated quantitative test used for immune-enzymatic determination using Enzyme Linked Fluorescent Assay (ELFA) technique. An ELISA (Enzyme Linked Immunosorbent Assay) kit with a semi-quantitative in-vitro assay for human IgG anti-TPO antibodies in plasma was used. Samples were then used to perform anti-TPO test by ELISA twice weekly. They were stored at-20°centigrade temperature in deep freezers available at Central Research Centre.

Diluted patient samples were incubated for 30 minutes at room temperature. Reagent wells were washed thrice with 400 microliters working strength ash buffer. In case of positive samples, IgG antibodies (also IgA and IgM) would bind to the antigens. A second incubation was carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) to detect these bound antibodies.[8] Enzyme conjugate, which was capable of promoting a colour reaction, had to be incubated and washed in the same way too. Substrate was incubated for 15 minutes after pipetting 100 microliters of chromogen whereas100 microliters of stop solution was used to stop the reaction. Photometric measurement of the colour intensity was made at a wavelength of 450 nm within 30 minutes of adding stop solution with a reference wavelength of 620-650 nm.

Normal value of T3, T4, and TSH were 1.3-3.1 ng/ml, 5.13-14 ug/dl and 0.27-4.2 uIU/ml respectively. Anti TPO positive and negative results were evaluated by calculating ratio using formula (Euroimmun (Medizinische Labordiagnostika AG):

Extinction of control or patients sample/ Extinction of calibrator 2

Ratio < 1.0 was considered negative and ratio ≥ 1.0 was considered positive.

After entering data in Microsoft Excel Office 365, statistical analysis was carried out using Microsoft Excel Office and SPSS (version 26.0). Chi Square test was used to compare the data and p-value <0.05 was considered as statistically significant.

3. Results

Total 100 patients were studied and analyzed. Mean age of study population was 38.11 ± 2.54 years whereas mean ages of males and females were 47.46 ± 1.56 and 33.7 ± 1.20 years respectively. Majority 32 patients belonged to 21-30 year age group followed by 24 patients in 31-40 year age group (Figure 1).

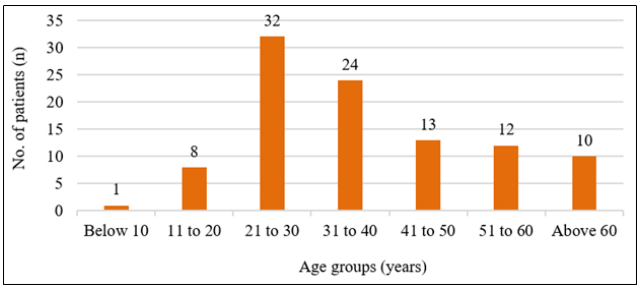

Fig. 1: Age distribution of patients (N=100)

Male: female ratio was 1: 2.12 with 68 (68%) females and 32 (32%) males. Among 100 patients; 15, 73 and 12 patients were Euthyroid, Hypothyroid and Hyperthyroid respectively. Out of 73 Hypothyroid patients, 24 were males and 49 were females (Table 1).

Out of 100 patients, 44 patients were anti-TPO antibody positive and 56 were anti-TPO antibody negative. Among 32 males, anti-TPO antibody positive and negative results were noted in 11 (34.37%) and 21(65.62%) males respectively. Anti-TPO antibody positive and negative females were 33 (48.52%) and 35 (51.47%) out of 68 females respectively. Hypothyroid was noted in 35 out of 44 anti-TPO antibody positive patients and majority 27 were females (Table 2).

Table 1: Gender wise patients with thyroid dysfunction

Gender	Thyroid dysfunction			Total no. of patients (N = 100)
	Euthyroid (n=15)	Hypothyroid (n=73)	Hyperthyroid (n=12)	
Males	07	24	01	32
Females	08	49	11	68

Table 2: Anti-TPO antibody status and thyroid dysfunction

Thyroid dysfunction	Anti-TPO antibody Positive (n = 44)		Anti-TPO antibody Negative (n = 56)	
	Males (n=11)	Females (n=33)	Males (n=21)	Females (n=35)
Euthyroid	02	04	05	04
Hypothyroid	08	27	16	22
Hyperthyroid	01	02	00	09
p-value		<0.05		

Highest 14 followed by 11 patients belonged to 21-30 and 31-40 years age groups among 35 hypothyroid patients with positive anti-TPO antibody status respectively (Table 3).

Maximum 12 out of 27 females found in 21-30 years age group were hypothyroid females with anti-TPO antibody positive status (Table 4).

4. Discussion

In order to diagnose thyroid dysfunction, laboratory investigations should be accompanied with clinical co-relation. Among various laboratory tests available, detection of anti-TPO antibodies is one important measurement. This is because anti-TPO antibodies appear mostly with the lymphocyte infiltration in the thyroid gland. As mentioned earlier, anti-TG antibodies are less important as compared to anti-TPO antibody measurement. Shinto et al concluded that anti-TPO antibody is more sensitive than anti-TG antibody in diagnosing autoimmune thyroiditis (98.1% v/s 61.8%; p value < 0.05).[5]

Autoimmune thyroid diseases were found more in females than in males in present study and mean age of female patients was 33.7 years. Adult females of child-bearing age were more prone to developing auto-immune thyroid diseases. This is in accordance with Unnikrishnan et al. study where there was a higher prevalence of anti-TPO antibodies in females ($p<0.05$).[9] Tipu et al also found that anti-TPO antibodies were more often raised in females as compared to males ($p<0.001$) and their mean ranks were also higher ($p<0.001$).[10]

In present study, 44% patients were anti-TPO antibody positive. Daneshpazhooh et al. concluded that increased anti-TPO antibody levels were found in18% patients.[11] In a report from Australia, 21% had positive anti-thyroid antibody.[12] In Greece study, the prevalence of anti-TPO antibody was 24.1%.[13]

The present study showed anti-TPO antibody positive result in approximately 50% females. This result was statistically significant. Similar higher distribution was noted among females in various other studies.[14–17] Such female dominance can be due to higher number of female patients included in study as well as higher prevalence of thyroid dysfunction in females.[1]

With respect to thyroid dysfunction, maximum study population showed hypothyroid dysfunction. Among female patients, more than 50% were hypothyroid as compared to males. These results were statistically significant. Thus, Hypothyroid as a thyroid dysfunction was found in higher number of anti-TPO antibody positive patients. It was also more common in the age-group of 21-40 years in both genders. Similar findings were observed by Ghoraishian et al. where they studied relationship of anti-TPO with T3, T4, and TSH in 2425 individuals and found these to be significantly deranged in antibody positive group.[18] Guillermo et al. also stated that hypothyroidism was more common in females (41%) than in males (19%) and in patients with positive anti-TPO antibodies.[19] In present study, anti-TPO antibodies were found morein females of child bearing age. Similar findings were observed in various studies.[1,5–7,14,20]

Around 6% euthyroid patients were anti-TPO positive in present study. Such subjects were at a risk for progression to sub-clinical and overt autoimmune hypothyroidism. A study showed decrease in TPO antibodies and improvement in quality-of-life (QoL) in T4-treated hypothyroid patients with selenium supplementation.[21]

In spite of a high prevalence of recognised thyroid disease in the population, a considerable number do have undiagnosed thyroid dysfunction with positive anti-TPO antibody results and show high TSH levels.[6,7,22] NHANES III measured serum TSH, total serum T4, anti-TPO antibodies and anti-TG antibodies from a sample of 17,353 people aged $\geq$12 years. They were representing the geographic and ethnic distribution of the U.S. population. A large proportion of the U.S. population unknowingly have laboratory evidence of thyroid disease, which supports the usefulness of screening for early detection.[23] Thus, the diagnosis of thyroid autoimmunity could generally be based on measurement of anti-TPO antibodies with additional

Table 3: Thyroid dysfunction and anti-TPO antibody status according to age-groups

Age groups (years)	Anti-TPO antibody Positive (n = 44)			Anti-TPO antibody Negative (n = 56)		
	Euthyroid (n = 06)	Hypothyroid (n = 35)	Hyperthyroid (n = 03)	Euthyroid (n = 09)	Hypothyroid (n = 38)	Hyperthyroid (n = 09)
<10	-	-	-	-	01	-
11-20	-	02	-	-	04	02
21-30	03	14	02	01	08	04
31-40	02	11	01	01	07	02
41-50	01	04	-	01	06	01
51-60	-	04	-	01	07	-
>60	-	-	-	05	05	-
p-value				<0.05		

Table 4: Thyroid dysfunction and anti-TPO antibody status according to age group and gender wise

Age groups (years)	Anti-TPO antibody Positive (n = 44)						Anti-TPO antibody Negative (n = 56)					
	Euthyroid (n=06)		Hypothyroid (n=35)		Hyperthyroid (n=03)		Euthyroid (n = 09)		Hypothyroid (n = 38)		Hyperthyroid (n = 09)	
	M (n=2)	F (n=4)	M (n=8)	F (n=27)	M (n=1)	F (n=2)	M (n=5)	F (n=4)	M (n=16)	F (n=22)	M (n=0)	F (n=9)
<10	-	-	-	-	-	-	-	-	-	01	-	-
11-20	-	-	01	01	-	-	-	-	01	03	-	02
21-30	-	03	02	12	01	01	-	01	03	05	-	04
31-40	01	01	03	08	-	01	-	01	-	07	-	02
41-50	01	-	02	02	-	-	-	01	02	04	-	01
51-60	-	-	-	04	-	-	01	-	07	-	-	-
>60	-	-	-	-	-	-	04	01	03	02	-	-
p-value							<0.05					

M = Males; F = Females

measurement of anti-TG antibodies in special cases.

Lacking histopathological co-relation of thyroid gland tissue to confirm hypothyroidism was a limitation in present study. Other parameters like serum cholesterol level could also be correlated with anti-TPO antibody positive patients. This could add information to existing literature. Hence it was observed that hypothyroidism was the most frequent thyroid dysfunction in patients with positive anti-TPO antibody status. It can also be stated that the negative anti-TPO antibody results may or may not be associated with thyroid dysfunctions.

5. Conclusion

The present study concludes that anti-TPO antibodies were commonly associated with hypothyroid dysfunction. It was noted more among females of reproductive age group. Thus, such patients should be screened for early detection of thyroid diseases.

6. Sources of Funding

Nil.

7. Conflict of Interest

Nil.

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