ORIGINAL ARTICLE

FREQUENCY DISTRIBUTION OF ANTI-THYROID PEROXIDASE AND ANTI-THYROGLOBULIN ANTIBODIES IN RELATION TO TSH, T3 AND T4 LEVELS

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Objective: Anti-thyroid peroxidase (anti-TPO) and Anti-thyroglobulin antibodies (anti-TG) are members of thyroid auto-antibodies that are considered important markers of Autoimmune Thyroid Disease. Our retrospective observational study assesses the frequency distribution of serum levels of Anti thyroid peroxidase (anti-TPO), anti thyroglobulin (anti-Tg), thyroid hormones T3, T4 and Thyroid Stimulating Hormone (TSH). **Methods:** Both male (n=60) and female (n=58) individuals were selected and their serum levels of TSH, T4, T3, anti-TG and anti-TPO antibodies were examined using Electrochemiluminescence technology in Cobas e411. **Results:** Anti-TPO was considerably high in both male and female hypothyroid patients compared to hyperthyroid patients. Sixty-three percent females had elevated anti-TPO levels compared to 42% of male patients. Anti-TG was elevated in 74% of normal female patients as compared to hyper- or hypothyroid patients. In males, 41% of hypothyroid patients showed elevated anti-TG levels compared to hyperthyroid or normal patients. **Conclusion:** Anti-TPO and anti-TG are proficient markers for assessing the patients with suspicion of autoimmune thyroid disease in addition to its ability to characterize prevalence of both clinical and sub clinical thyroid diseases.

Keywords: anti-TPO, anti-TG, T3, T4, hyperthyroidism

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INTRODUCTION

Thyroid dysfunction includes sub-clinical hyper and hypothyroidism along with autoimmune thyroid disease. These thyroid anomalies are widespread in developed and underdeveloped countries of the world.[1-6] About 2–4% women and 1% men are affected with autoimmune thyroid disease (AITD).[6–8] In hypothyroidism, iodine supplementation has proved to be beneficial, but continuous iodine exposure may cause goiter, hypo or hyper thyroidism and thyroid autoimmunity.[9] Hashimoto's thyroiditis and Graves disease are well known consequences of AITD.[10] Other conditions due to AITD include atrophic autoimmune hypothyroidism, postpartum thyroiditis and thyroid associated orbitopathy.[7] For the diagnosis of AITD, several diagnostic parameters, such as anti thyroid antibodies, are now used routinely which include, anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-Tg), and anti-thyroid stimulating hormone receptor (anti-TSH-R) antibodies.[1,2,6–8] More commonly, Anti-Tg and anti-TPO are being used, in combination with thyroid hormones in suspected individuals, to evaluate the thyroid status and to treat them.[6,11] Anti-thyroid antibodies have also been found in other autoimmune diseases like Systemic Lupus Erythematosus, Vitiligo and also in Autoimmune Polyglandular Syndrome Type 3a.[12–15] Type I Diabetes, which is itself an autoimmune disease, is associated with thyroid autoimmunity.[16] In addition, gestational diabetics are also found to have thyroid autoimmunity.[16]

The present research aims to study anti-Tg and anti-TPO antibodies along with serum T3 and T4 levels to judge the clinical significance.

MATERIAL AND METHODS

This was a retrospective observational study aimed to measure the thyroid antibodies anti-Tg and anti-TPO along with thyroid function tests in patients suspected of having thyroid dysfunction. The duration of the study was six months from January 2016 to June 2016. A total of 118 (60 male and 58 female) adult subjects were included in the study. All individuals who were confirmed cases of thyroid dysfunction or who were suspected of having thyroid disease were included in the study. Age, gender, any other disease and pregnancy data was collected and correlated. Patients with history of liver or renal disease, interferon therapy or β blocker use were excluded from the study.

Six millilitres blood was collected in red top tubes containing activated silica particles which act as clot activator and allowed to clot. Serum was separated and stored at -20 °C. Dilutions and aliquots were prepared where needed. Electrochemiluminescence (ECL) technology was used to analyze anti-Tg, anti-TPO antibodies, tri-iodothyronine (T3), tetra-iodothyronine (T4) and thyroid stimulating hormone (TSH) on Cobas e411 immunoassay analyzer (Roche Diagnostics). The cut-off values of Anti-Tg and anti-TPO were <115 IU/ml and <34 IU/ml respectively, whereas the reference intervals for T3, T4 and TSH

were 0.8–2.00 ηg/ml, 5.1–14.1 µg/dl and 0.27–4.2 µIU/ml respectively. Anti-TPO, T3 and T4 were analysed by competitive ECL immunoassay and Anti-Tg and TSH using sandwich ECL immunoassay.

Data were analyzed using SPSS-13. Student's paired *t*-test, and regression correlation analysis R^2 among thyroid hormones and thyroid antibodies were performed and $p<0.05$ was taken as significant. The results were presented as Mean±SD.

RESULTS

Out of a total of 60 males, 36 had normal, and 24 had elevated anti-TPO levels. In 58 females, 28 had normal and 30 had elevated anti-TPO levels. For anti-Tg in males, 38 had normal and 22 had elevated levels whereas in females, 31 had normal and 27 had elevated levels (Table-1).

The frequency distribution of TSH with anti-TPO in females showed elevated anti-TPO with low TSH in 20%, whereas 63% of the females had elevated anti-TPO with elevated TSH levels whereas 17% of normal TSH patients had elevated anti-TPO antibodies. In males, 33% had low TSH with elevated anti-TPO, 42% had elevated TSH and anti-TPO and 25% of normal TSH had elevated anti-TPO antibodies (Table-2). The frequency distribution of anti-TPO with T4 showed elevated anti-TPO in 47%, 33% and 20% of females with low, high and normal T4 respectively. Elevated anti-TPO was seen with 37%, 37% and 26% of males with low, high and normal T4 levels (Table-2). The frequency distribution of anti-TPO with T3 levels in the different genders showed 44% of females had low T3 and elevated anti-TPO, 33% had elevated levels of bothT3 and anti-TPO and 23% of women with normal T3 had elevated anti-TPO levels. In the case of males, 42% had low T3 and elevated anti-TPO, 33% had elevated levels of both T3 and anti-TPO and 25% had normal T3 with high anti-TPO levels (Table-2).

When anti-Tg levels were compared with TSH in the different sexes, it was found that 19% of females had low TSH with elevated anti-Tg, 7% had elevated TSH and anti-Tg and 74% of women with normal TSH had elevated anti-Tg antibodies. In the case of males, 32% had low TSH and high anti-Tg, 41% had elevated TSH as well as anti-Tg and 27% of males with normal TSH had high anti-Tg (Table 3). Anti-Tg with different T4 levels showed in females, 11%, 22% and 67% with low, elevated and normal T4 levels respectively. 45%, 23% and 32% of males showed abnormal anti-Tg with low, elevated and normal T4 levels (Table-3). When anti-Tg was compared with T3 levels, 11%, 19% and 70% of women had low, elevated and normal T3 respectively, with high values of anti-Tg. In the case of males, 41%, 32% and 27% had low, elevated and normal T3 respectively with high anti-Tg antibodies (Table-3).

Table-1: Gender wise distribution/frequency in relation to Anti TPO and Anti-Tg antibody levels [n(%)]

	Gender	Normal	Elevated	Total
Anti-TPO	Males	36(60)	24(40)	60
	Females	28(48)	30(52)	58
Anti-Tg	Males	38(63)	22(37)	60
	Females	31(53)	27(47)	58

Table-2: Frequency distribution of TSH, T4 and T3 in subjects in relation to Anti-TPO antibody levels [n(%)]

	Anti-TPO			
	Males		Females	
Parameters	**Normal (n=36)**	**Elevated (n=24)**	**Normal (n=28)**	**Elevated (n=30)**
TSH levels				
Low	1(11)	8(89)	6(50)	6(50)
Elevated	4(29)	10(71)	2(10)	19(90)
Normal	31(84)	6(16)	20(80)	5(20)
T4 levels				
Low	10(53)	9(47)	4(22)	14(78)
Elevated	8(47)	9(53)	8(44)	10(56)
Normal	18(75)	6(25)	16(73)	6(27)
T3 levels				
Low	9(47)	10(53)	3(19)	13(81)
Elevated	8(50)	8(50)	7(41)	10(59)
Normal	19(76)	6(24)	18(72)	7(28)

Table-3: Frequency distribution of TSH, T4 and T3 in males and females in relation to Anti-Tg antibody levels [n(%)]

	Anti-Tg levels			
	Males		Females	
Parameters	**Normal (n=38)**	**Elevated (n=22)**	**Normal (n=31)**	**Elevated (n=27)**
TSH levels				
Low	1(13)	7(87)	7(58)	5(42)
Elevated	4(31)	9(69)	19(90)	2(10)
Normal	33(85)	6(15)	5(20)	20(80)
T4 levels				
Low	10(50)	10(50)	13(81)	3(19)
Elevated	7(58)	5(42)	9(60)	6(40)
Normal	21(75)	7(25)	9(33)	18(67)
T3 levels				
Low	10(53)	9(47)	15(83)	3(17)
Elevated	8(53)	7(47)	8(62)	5(38)
Normal	20(77)	6(23)	8(30)	19(70)

DISCUSSION

In countries where iodine deficiency is not present, autoimmunity is thought to be the main cause of thyroid diseases, from hyperthyroidism to hypothyroidism.[17] Elevated levels of anti-Tg and anti-TPO antibodies are mainly associated with thyroid autoimmune diseases and thyroid cancers but low concentrations are also found in normal individuals.[17] Thyroid antibody is secreted in a Mendelian dominant manner and it is seen more in young women and relatives of autoimmune thyroid disease (AITD) patients.[17] In our study, we found elevated levels of anti-TPO in 17% females and 25% of males with normal TSH levels (Table-2). This is in consistence with the finding of Bhattacharjee *et al.*[17]

It is known that in the normal population, 10% show anti-TPO, whereas 30% of the elderly also exhibit anti-TPO antibodies.[18] In our study, 17% of females and 25% of males had elevated anti-TPO with normal TSH levels. These findings may be consistent with normal individuals. Anti-TPO is also seen in sub-clinical hypothyroidism.[4,19] Similarly, in a study based on the Busselton Thyroid Study, thyroid anti-bodies (anti-Tg and anti-TPO were found to be elevated in 12.4% of subjects without a history of thyroid disease. Raised anti-TPO was found in 10.7% and elevated anti-Tg in 5.5% of the population.[20] There was normal T4 along with elevated anti-TPO antibody in our study in 20% and 26% of females and males respectively, showing subclinical thyroid disease (Table-2).

Originally called thyroid microsomal antigen, TPO is present on the apical region of the thyroid follicular cells and is involved in cell mediated cytotoxicity. Therefore, anti-TPO antibody titer shows the degree of lymphocytic infiltration of the thyroid gland which is helpful in diagnosing the activity of Hashimoto's Thyroiditis.[21] In our study, elevated anti-TPO with low TSH was observed in 20% females and 33% males, and with high TSH was seen in 63% females and 42% males respectively (Table-2). The frequency of raised anti-TPO with low T4 was 47% females and 37% males and elevated T4 was 33% females and 37% males (Table-2). Comparing anti-TPO with T3 we found almost the same variables. Elevated anti-TPO with low T3 was found in 44% females and 42% males, raised T3 in 33% females and 33% males respectively (Table-2). Males show an almost equal tendency of hyper or hypo thyroidism with the presence of raised anti-TPO whereas there are a greater number of females with hypothyroidism related with anti-TPO antibodies.

In the Wickham survey performed in the United Kingdom in the 1970s, the prevalence of anti-Tg was about 2% and anti-TPO was about 6.8% in the sample of 2,799 subjects. The frequency increased with age in females but not in males.[22] In 2002, the National Health and Nutrition Examination Survey (NHANES) data showed anti-TPO in 13% and anti-Tg in 11% of the population of 17,353 subjects, using more sensitive assays. There was an increase in thyroid antibodies with age in females and anti-TPO was found more in Caucasians as compared to African Americans.[23] Legakis *et al*[4] found a greater prevalence of anti-Tg and anti-TPO in females as compared to males along with high TSH levels after the age of 50 years. In our study, 52% females and 40% males showed elevated anti-TPO and 47% females and 37% males showed elevated anti-Tg. (Table-1).

In the past, 15.4% of the selected population showed anti-Tg positivity. In a recent study there was a positive correlation of TSH and T4 with anti-Tg antibodies.[6] In our study, we found elevated anti-Tg with normal TSH in 74% of females and 27% males showing that in normal or subclinical thyroid disease, anti-Tg is found to be elevated. (Table 3). When anti-Tg was compared with T4, we found that in females, elevated anti-Tg with normal T4 was seen in 67%. In males, increased anti-Tg was seen with low T4 levels that is hypothyroidism in 45% of patients (Table-3). Other studies also show elevated anti-Tg antibodies in Hashimoto's Thyroiditis and Graves' disease. Both anti-Tg and anti-TPO antibodies are associated with Hasimoto's Thyroiditis so there is an association of anti-thyroid antibodies and thyroid cancer.[10]

CONCLUSION

Anti-TPO is considerably high in both male and female hypothyroid patients as compared to hyperthyroid patients. When we compare female and male hypothyroid patients, 63% females showed elevated anti-TPO levels as compared to 42% of male patients. Anti-Tg was found to be elevated in 74% of normal female patients as compared to hyper- or hypothyroid patients. In males, 41% of hypothyroid patients showed elevated anti-Tg levels as compared to hyperthyroid or normal patients.

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