Significance of thyroid peroxidase antibodies (anti-TPO antibodies) in patients undergoing thyroid function tests

Bijaya Gautam[1]*, Satish Poudel[2], Shanti Pokhrel[3]

[1]Department of Biochemistry, Gandaki Medical College Teaching Hospital and Research Center, Pokhara, Nepal, [2]Medical Laboratory Technology, Pokhara University, Pokhara, Nepal, [3]Department of Emergency Medicine, Gandaki Medical College Teaching Hospital and Research Center, Pokhara, Nepal

ABSTRACT

Introduction: Thyroid peroxidase (TPO) enzyme is a poorly glycosylated enzyme responsible for the synthesis of the thyroid hormones. TPO antibodies appear where there is tissue destruction in thyroid follicles, thereby making it a good marker for detection of autoimmune thyroid disorders. Thus, this study was conducted to analyze the significance of anti-TPO antibodies in patients undergoing a thyroid function test. **Methods:** This is a hospital-based cross-sectional study conducted on 235 patients who had undergone thyroid function tests (TFTs) along with anti-TPO. Overnight fasting samples were collected, labeled and stored. The serum level of fT3, fT4, TSH and anti-TPO antibodies were measured using CLIA method from Diasorin Liaison instrumentation. **Results:** Out of total patients, Male: female ratio of patient was 1:3.35 which showed female preponderance over male. Maximum number of patients belongs to 21 to 30 years age group (87/37.2%). Total 87 patients had thyroid related disorders. Among this, 74 were females, 26 patients had subclinical hypothyroidism (25 were females), 30 had clinical hypothyroidism (26 were females), 4 had subclinical hyperthyroidism (all females) and 27 had clinical hyperthyroidism (19 were females). Positive anti-TPO antibody patients were 73, out of which 59 were female. TSH had significant positive correlation, while T4 and T3 had negative significant correlation with anti-TPO. **Conclusions:** Assessment of anti-TPO antibodies is valuable while assessing thyroid function tests, as it can help in early diagnosis as well as to establish autoimmune pathogenesis of thyroid disorders.

Keywords: Anti-TPO, autoimmune thyroid disorder, hypothyroidism, thyroid function test.

***Correspondence:**
Dr. Bijaya Gautam
Department of Biochemistry
Gandaki Medical College Teaching Hospital and Research Center, Nepal
Email: gautmbj@gmail.com
ORCID iD: https://orcid.org/0009-0009-7291-3582

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INTRODUCTION

Autoimmune thyroid disorder (AITDs) is a multiple group of inflammatory thyroid diseases. It is an organ-specific autoimmune disease. It includes Graves' disease, Hashimoto's thyroiditis (HT), chronic autoimmune thyroiditis, autoimmune hypothyroidism, among which Graves' disease and Hashimoto's thyroiditis are the most common.[1] AITDs follows the overproduction of thyroid hormone (hyperthyroidism) or destruction of the follicular cells (hypothyroidism).[2] AITDs occurs due to the appearance of anti-thyroid peroxidase (TPO), anti- Thyroglobulin (Tg), and anti-thyroid-stimulating hormone receptor antibodies in the serum.[3] The risk factor associated to the pathogenesis of AITDs includes exogenous factors as high iodine, infections, radiations, smoking and endogenous factors as sex and genetic disposition.[4] Thyroid-stimulating antibodies are responsible for Graves' hyperthyroidism, which bind and activate the thyrotropin receptor on thyroid cells. It is presented as graves' ophthalmopathy in the eyes and localized dermopathy or myxedema in the skin as particular symptoms of Graves' disease and other symptoms of hyperthyroidism like weight loss, weakness, dyspnea, palpitation, increased hunger and thirst, sweating,

sensitivity to heat, tremor, irritability.[5] The laboratory finding in serum shows higher Anti-TPO antibodies, excess anti-thyroglobulin antibody, increased clearance of iodide from the plasma and decreased retention of iodide in the thyroid.[6] Hashimoto's thyroiditis is presented with diffuse goiter with lymphocytic infiltration and the presence of thyroid-specific autoantibodies. Among the variant of HT, IgG4 thyroiditis is widely recognized.[7] It manifests as the symptoms of hypothyroidism which include weight gain, constipation, sensitive to cold, depression, slow movement, muscle aches and weakness, brittle hair and nails, carpal tunnel syndrome with increased TSH level.[6]

For the diagnosis of AITDs, anti-thyroid autoantibodies such as anti-TPO, anti-thyroglobulin and anti-TSH receptor autoantibodies plays an important role.[8] TPO enzyme is a poorly glycosylated enzyme responsible for the synthesis of the thyroid hormone for oxidation of iodine and iodination of tyrosyl residues of the thyroglobulin molecule.[9] In hypothyroidism such as Hashimoto's and atrophic thyroiditis, TPO antibodies appears where there is the tissue destruction in thyroid follicles.[10] Hypothyroidism is present as subclinical and overt hypothyroidism where the measurement of anti-TPO antibodies plays a crucial role in diagnosis of AITDs for the initiation of treatment so that it does not follow overt hypothyroidism.[11] Anti-thyroglobulin antibodies are directed against the thyroid precursor, thyroglobulin.[1] In Graves' disease and Hashimoto's thyroiditis, the prevalence of anti-TPO and anti-TG is high whereas anti-TSHR antibodies is found to be more common in Graves' disease which signifies the specific production than other antibodies.[12] Among others autoantibodies, Anti-TPO antibodies is the major and most common antibodies present in the AITDs diseases which appears in high titers.[10] Greater number of patients with thyroiditis have high serum anti-TPO than anti-thyroglobulin antibody.[13] The prevalence of anti-TPO antibodies is 25.93% and 74.07% in male and female respectively.[10]

Among the Nepalese population, the most common and most emerging public health problem is Thyroid disorder. The risk factors of thyroid disorder are genetic predisposition, pregnancy, viral infections and dietary iodine intake. Thyroid disorder can be classified as hypothyroidism and hyperthyroidism.[14] Hypothyroidism is the state where the thyroid gland is unable to produce the thyroid hormone sufficiently, whereas hyperthyroidism is the state where the thyroid gland produces enough thyroid hormone.[15] Hypothyroidism and hyperthyroidism are further sub classified into subclinical and overt hypothyroidism and hyperthyroidism respectively.[4] The prevalence of hyperthyroidism and hypothyroidism in the eastern part of Nepal was found to be 13.68% and 17.19% respectively.[16] The prevalence of thyroid disorder was found to be 17.42%.[14] Fine needle aspiration cytology (FNAC), which is considered as minimally invasive, cost-effective, easy to perform, can also aid in the diagnosis of thyroid disorder and its malignancy. In evaluation of thyroid disorders, FNAC has found to be of high diagnostic accuracy and high sensitivity in diagnosis of thyroid malignancy.[17]

The use of FNAC requires high skill and professionalism in order to diagnose thyroid disorders. So, evaluation of serum autoantibodies, Anti-TPO, is commonly used. There is minimal study on the antibody status in thyroid dysfunction patients. The objective of this study is to correlate the Anti-TPO level to thyroid hormone and provide the possible diagnosis of thyroid disorder before it follows a severe form of disease.

METHODS

This quantitative hospital based cross-sectional study was conducted from April 5, 2023 to September 4, 2023 among the patients visiting the endocrinology department of Gandaki Medical College Teaching Hospital and Research Center, Pokhara. The ethical clearance was obtained from the Institutional Review Committee, Gandaki Medical College (Ref. No. 254/079/080). A written informed consent was taken from all the participants prior to data collection. A convenient sampling technique was used to enroll the participants in the study. Based on a previous study, at a 95% confidence interval and 17.42% prevalence, a sample size of 214 was calculated.[14] Taking 10% as non-response rate, the final sample size was 235.

All subjects coming for thyroid function tests and Anti-TPO antibody were included in the study. Patients with systemic lupus erythematous, hepatitis C, chronic urticaria and those refusing to be the part of study were excluded from the study.

Overnight fasting venous blood samples (5 ml) were drawn aseptically from the patients. The blood sample was collected in plain a vial of tubes without anticoagulant, allowed to clot for 10 to 15 minutes. After clotting, the vial was centrifuged at 3000 rpm for 10 to 15 minutes to separate out the serum. In case of delay, the vial was placed in the refrigerator at -20^0 Celsius. The serum level of free T3 (fT3), free T4 (fT4), TSH and Anti-TPO antibodies was measured using CLIA method from Diasorin Liaison instrumentation. A reference range of analytes from reagent manufacturer was used for data interpretation.

According to it, fT3 (2.3 to 4.2 pg/ml), fT4 (0.89 to 1.76 ng/dl), TSH (0.55 to 4.78 µIU/ml) and Anti-TPO (0 to 30 IU/ml) was considered normal. In diagnosis, if fT3 and fT4 values greater than normal range but decreased TSH was considered clinical hyperthyroidism, if fT3 and fT4 values within normal range but decreased TSH, it was considered subclinical hyperthyroidism. If fT3 and fT4 values are less than normal range but increased TSH, it was considered clinical hypothyroidism, and if fT3 and fT4 values were within normal range but increased TSH, was considered subclinical hypothyroidism.

The obtained data was entered into a Microsoft Excel sheet and the statistical analysis was done using a statistical package on social sciences (SPSS) version 22.0. Pearson's correlation was used for correlation and a p-value <0.05 was considered as statistically significant.

RESULTS

Table 1 shows the percentage of age groups among study group. Total 235 patients were included in this study. Among them, 181 were females and 54 were male. Male: female ratio of patient was 1:3.35.

Table 1: Percentage of age groups among study group

Age category	Male (n=54)	Female (n=181)	Total	%
<20	3	16	19	8.08%
21-30	14	73	87	37.2%
31-40	13	38	51	21.7%
41-50	8	18	26	11.06%
51-60	7	25	32	13.61%
61-70	6	9	15	6.38%
>71	3	2	5	2.12%
Total	54	181	235	100%

Mean age for females was 35.38±14.16 and male was 41.75±16.58. Maximum 87(37.2%) patient were found in 21 to 30 age group. Among 235 patients, 87 patients had thyroid related disorders.

Table 2: Number of patients with thyroid disorder types

Thyroid Disorder Type	Total Patients	Female Patients
Total with Thyroid Disorder	87	74
Subclinical Hypothyroidism	26	25
Clinical Hypothyroidism	30	26
Subclinical Hyperthyroidism	4	4
Clinical Hyperthyroidism	27	19
Total	87	74

Table 2 shows the number of patients with the thyroid disorder types. Among this, 74 were female, 26 patients had subclinical hypothyroidism (25 were females), 30 had clinical hypothyroidism (26 were females), 4 had subclinical hyperthyroidism (all females) & 27 had clinical hyperthyroidism (19 were females).

Table 3: Mean value of TFTs with different types of thyroid disorders

Thyroid Disorder Type	Mean fT3	Mean fT4	Mean TSH
Clinical Hypothyroidism	1.94pg/ml	0.65ng/dl	23.45µIU/ml
Subclinical Hypothyroidism	2.92pg/ml	1.05ng/dl	8.22µIU/ml
Clinical Hyperthyroidism	6.8pg/ml	5.22ng/dl	0.11µIU/ml
Subclinical Hyperthyroidism	2.95pg/ml	1.23ng/dl	0.25µIU/ml

Table 4: Anti-TPO and the mean value with thyroid disorder

Test	Positive with thyroid disorder	Positive with hypothyroidism
Anti-TPO	58 (417.59 IU/ml)	25 (134.45 IU/ml)

Positive anti-TPO antibody patients were 73 overall, out of which 62 (84.9%) were female. Around 58(66.6%) of patients with thyroid disorder were anti-TPO positive. 82% of hypothyroid patient were anti-TPO positive.

Table 5 shows Correlation between Anti-TPO and the thyroid hormones within study group and Figure 1 shows correlation between Anti-TPO and TSH. TSH had significant positive correlation (r=0.411, p=<0.001), FT4 had negative significant correlation (r=-0.058, p=<0.001) and FT3 had negative significant correlation (r=-0.70, p=<0.001) with Anti-TPO using Pearson's correlations.

Table 5: Correlation between Anti-TPO and the thyroid hormones within study group

Thyroid hormones	R	p-value
fT3	-0.70	<0.001
fT4	-0.058	<0.001
fTSH	0.411	<0.001

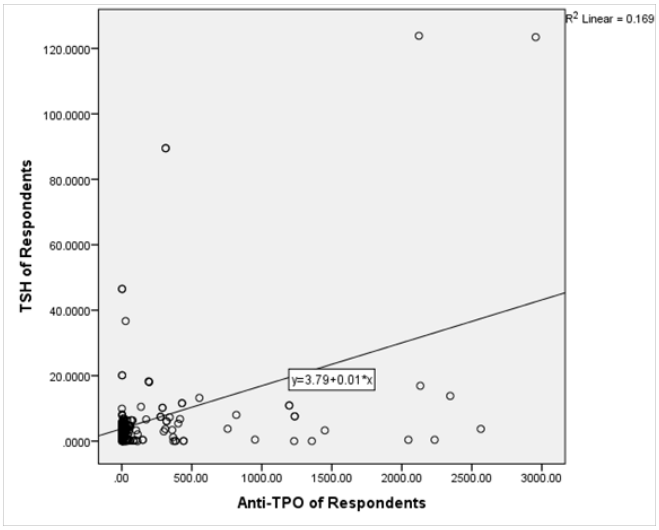

Figure 1: Correlation between Anti-TPO and TSH

DISCUSSION

Autoimmune thyroiditis is closely associated with activity of anti-TPO antibodies. Anti-TPO antibodies are

considered having etiologic and diagnostic value for assessment of thyroid dysfunction and pathogenesis of hypothyroidism.[10] Significant titers of these antibodies can reflect etiopathogenesis of thyroid dysfunction. Thyroid failures are associated with rapid increase in anti-TPO titre. To evaluate significance of anti-TPO testing in thyroid disease patient, present study has been proposed.

In this study, 77% of patient visiting to hospital for thyroid checkup were females. Yadav et al. also reflected that majority (76.80 %) patients coming for thyroid assessment over the duration of two years (1504 cases) were females.[14] In our study, the male to female ratio was 1:3.35, which is similar to the finding of Yadav et al.[14] Also, we found that female subjects were having thyroid problems more than male subjects, which is in concordance with the study of Yadav et al.[14]

This study found that, majority of patient with thyroid disorder were found in 21 to 30 year age group (37.2%). This age group is the working age group with busy schedule. So they might not be aware of regular thyroid function tests. This could be the reason for having thyroid disorder in majority number of this age group. Similarly, study of Ashwin et al. also suggested presence of majority of thyroid disease in same age group (32%).[2] Also, our study showed that 64.3% of patient with thyroid disease had hypothyroidism, which is similar finding by Ashwin et al.

We found that hypothyroidism is predominant thyroid disease in our population (64.3% of total thyroid disease). Hypothyroidism is mainly linked with iodine deficiency and Nepal is considered as an endemic zone of iodine deficiency. Dietary habit of consuming goitrogens as well as predominance of hilly areas that often undergo landslides that washes iodine from the soil may exacerbate hypothyroidism due to iodine deficiency.

About 62(84.9%) out of 73 anti-TPO positive patients were females in current study, which is similar finding to the study conducted by Sachin et al.[10] Again 56 patients had hypothyroidism, of which 51 were females and anti-TPO positive were 46(82%) in this study. This also confirms that females are affected more by autoimmune thyroid disease.

From this study, it is concluded that the prevalence of anti-TPO is more in females as compared to males which confirms that women are more susceptible to autoimmune thyroid disease than male. This result is concurrent with the study done by Canaris et al. which showed that women are 2 to 4 times more susceptible to autoimmune thyroid disease than males.[18] Similarly, the study conducted by Mohanty et al. reflected that 45 among the 61 subclinical hypothyroid patients had elevated anti-TPO (73.78%).[19] This study is also like our study. Swain et al. has also reported that most of the patients with autoimmune thyroid disease were women.[20]

In our study, TSH had significant positive correlation (r=0.411, p=<0.001), FT4 had negative significant correlation (r=-0.058, p=<0.001) and FT3 had negative significant correlation (r=-0.70, p=<0.001) with Anti-TPO using Pearson's correlations. There is significant correlation between hypothyroidism and Anti-TPO antibody. The similar result was obtained from Ghoraishian et al. which reported significant correlation between TSH and FT4 concentration and elevated Anti-TPO antibody was demonstrated.[21] Similarly, the study done in Greece reported significant association between sub-clinical hypothyroidism and positive anti-thyroid antibodies.[22]

Siriwardhane et al. described that there is early appearance of thyroid autoantibody, anti-TPO, prior to the onset of the thyroid hormone disruption, hence the addition of anti-TPO in conjunction with traditional thyroid markers like TSH, T3 & T4 would aid to reduce long term morbidity and associated health concerns.[4] Measurement of TSH, T3 and T4 along with anti-TPO benefits as a predictive marker in diagnosing euthyroid cases who might be at risk for potential thyroid dysfunction. But, it should be taken in consideration that anti-thyroid antibodies may be positive in significant cases of elderly.[1]

CONCLUSIONS

Assessment of anti- TPO antibodies has significant importance in assessing the hypothyroidism which could be due to the autoimmune pathogenesis. Anti-TPO antibodies titer is relatively high in females than males. Hence, Anti-TPO helps in early diagnosis and prognosis for patients with hypothyroidism.

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AUTHORS' CONTRIBUTION

BG designed the research, collected data, performed statistical analysis, and prepared the first draft of the manuscript, SP and SP explained and interpreted the

data and contributed to preparing the final draft of the manuscript. All authors read and approved the manuscript.

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