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Prevalence of Anti-Thyroid Peroxidase Antibodies in Patients with Thyroid Disorders

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Abstract

Background

Autoimmune thyroid disorders (AITDs) are among the most common organ-specific autoimmune diseases and represent a major cause of thyroid dysfunction worldwide. Their prevalence has been increasing in both developed and developing countries, with a higher occurrence among females. Anti-thyroid peroxidase (anti-TPO) antibodies are widely recognized as important biomarkers for the diagnosis and assessment of autoimmune thyroid diseases.

Objective

This study aimed to evaluate the prevalence of thyroid hormone dysfunction and determine the frequency of anti-TPO antibody positivity in individuals with thyroid-related disorders, as well as to assess the association between anti-TPO titers and thyroid hormone parameters.

Methods

A prospective cross-sectional observational study was conducted between February 2025 and September 2025 in the Department of Biochemistry, Department of Laboratory Medicine, AMCH. A total of 162 individuals with suspected thyroid disorders were included. Serum levels of thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), and anti-TPO antibodies were measured using chemiluminescence immunoassay (CLIA) on the Siemens ADVIA Centaur® XP platform. Statistical analysis was performed to evaluate associations between anti-TPO titers, thyroid hormone levels, age, and sex.

Results

Among the 162 participants, 54.3% were aged 20–39 years. Females constituted 86.4% of the study population, while males accounted for 13.6%. Hypothyroidism was identified in 47.5% of individuals and hyperthyroidism in 21.6%. Positive anti-TPO antibody titers were detected in 50% of the participants, with females representing 96% of positive cases. Anti-TPO titers showed significant associations with serum TSH ($p = 0.009$), serum fT3 ($p = 0.037$), and sex ($p < 0.001$). No significant association was observed with serum fT4 ($p = 0.071$) or age ($p = 0.582$).

Conclusion

Hypothyroidism was the most prevalent thyroid disorder, particularly among individuals aged 20–39 years, and showed a marked female predominance. The significant association between anti-TPO

antibodies and thyroid hormone parameters highlights the clinical importance of anti-TPO evaluation in diagnosing and monitoring autoimmune thyroid disorders.

INTRODUCTION

Thyroid disorders represent one of the most prevalent endocrine conditions worldwide and may manifest as either hypo- or hyper-function of the thyroid gland. These dysfunctions can arise from multiple causes, including congenital abnormalities, iodine deficiency, pregnancy, viral infections, radiotherapy, surgery, infiltrative diseases, and autoimmune mechanisms.[1,2] The incidence of thyroid disease attributable to iatrogenic factors and iodine imbalance is influenced by environmental and clinical determinants.[3] However, the underlying mechanisms of autoimmune thyroid disease remain incompletely understood, although genetic susceptibility plays a crucial role in conditions such as Graves' disease and autoimmune hypothyroidism.[4]

Autoimmunity results from the loss of immune tolerance, leading to an abnormal immune response against self-antigens. The characteristic features of autoimmune diseases include the presence of autoreactive T lymphocytes, circulating autoantibodies, and chronic inflammation.[5] In such conditions, autoreactive lymphocytes can induce tissue damage through antibody production against host tissues or through cytotoxic effector T-cell responses directed against self-peptides. Environmental and genetic factors interact to initiate autoimmune responses, and several susceptibility genes, including those associated with the major histocompatibility complex (MHC), have been linked to increased predisposition to autoimmune disorders.[6,7]

Autoimmune thyroid disease (AITD) is the most common organ-specific autoimmune disorder, predominantly affecting middle-aged women. Globally, approximately 2–4% of women and up to 1% of men are affected, with prevalence increasing with age.[8] The global burden of AITD has shown a progressive increase in recent decades. Epidemiological studies report an overall prevalence of approximately 12%, with a marked female predominance and higher occurrence in Caucasian populations.[9] AITD encompasses a spectrum of disorders including Graves' disease, Hashimoto's thyroiditis, atrophic autoimmune hypothyroidism, postpartum thyroiditis, and thyroid-associated orbitopathy. Among these, Hashimoto's thyroiditis and Graves' disease represent the most common clinical forms and share several immunological features. These disorders may evolve from one form to another during the course of the autoimmune process.[10]

The pathogenesis of AITD is multifactorial, involving complex interactions between genetic predisposition and environmental triggers. Although the precise etiology remains unclear, genetic susceptibility combined with environmental exposures is believed to initiate and propagate autoimmune responses. Excess iodine intake, iodine supplementation in previously deficient regions, radiation exposure, and environmental pollutants have all been implicated in triggering thyroid autoimmunity.[11,12] Several susceptibility genes have been identified, including the HLA-DR locus and non-MHC genes such as CTLA-4, CD40, PTPN22, thyroglobulin, and the TSH receptor gene.[13] Environmental triggers such as

iodine intake, medications, infections, smoking, and psychological stress further contribute to disease development through gene–environment interactions.[14]

Patients with autoimmune thyroiditis frequently exhibit circulating antibodies against thyroid-specific antigens, including thyroglobulin (Tg), thyroid peroxidase (TPO), sodium-iodide symporter proteins, and thyroid-stimulating hormone (TSH) receptors.[15] Among these antigens, thyroid peroxidase, thyroglobulin, and TSH receptors are considered the most clinically relevant.[16] The presence of autoantibodies against these antigens constitutes a hallmark of AITD and plays an important role in the pathogenesis of both hypothyroidism and hyperthyroidism.[17]

Thyroid peroxidase (TPO) is a membrane-bound glycoprotein enzyme located in thyroid follicular cells that catalyzes iodination and coupling reactions necessary for the synthesis of thyroid hormones, triiodothyronine (T3) and thyroxine (T4).[18] Multiple T- and B-cell epitopes are present within the TPO molecule, leading to the development of autoantibodies directed against this enzyme.[19] Anti-TPO antibodies are predominantly of the IgG class, particularly IgG1 and IgG4 subclasses.[20] These antibodies can induce thyroid tissue damage through complement activation and antibody-dependent cellular cytotoxicity, contributing to the pathogenesis of autoimmune thyroid disorders.[21]

Measurement of anti-TPO antibodies is considered one of the most reliable laboratory markers for the diagnosis of autoimmune thyroid diseases. Anti-TPO antibodies are detected in approximately 80–90% of patients with Hashimoto's thyroiditis and 65–75% of patients with Graves' disease, although they may also be present in a smaller proportion of individuals with nodular goiter, thyroid carcinoma, or even in apparently healthy individuals.[22] Serum anti-TPO antibody levels are positively correlated with the severity and activity of chronic autoimmune thyroiditis.[23]

Detection of these antibodies therefore plays a crucial role not only in establishing the autoimmune etiology of thyroid dysfunction but also in epidemiological studies and prognostic evaluation.[24][25] Anti-TPO positivity has been shown to increase the risk of progression from subclinical to overt hypothyroidism,[26] emphasizing its importance in early diagnosis and clinical management.[27] Consequently, evaluation of serum anti-TPO antibody levels alongside thyroid hormone parameters may provide valuable insights into the autoimmune mechanisms underlying thyroid dysfunction.

Given the increasing burden of thyroid disorders and the limited epidemiological data regarding autoimmune thyroid disease in certain populations, assessing the prevalence of anti-TPO antibodies among patients with thyroid dysfunction may help differentiate autoimmune from non-autoimmune causes and improve clinical management strategies.[28] Therefore, the present study aims to evaluate the prevalence of anti-TPO antibodies in patients with thyroid dysfunction and to examine their association with thyroid hormone levels.

The primary objective of this study was to determine the prevalence of **anti–thyroid peroxidase (anti-TPO) antibodies** in the serum of patients with thyroid dysfunction. Additionally, the study aimed to

differentiate autoimmune from non-autoimmune causes of thyroid dysfunction, assess the prevalence of anti-TPO antibodies across different age groups, and evaluate their distribution in relation to sex.

The study also sought to determine the presence of anti-TPO antibodies in a community-based population of patients predominantly presenting with symptoms suggestive of thyroid dysfunction. Furthermore, the research aimed to investigate whether the prevalence of anti-TPO antibodies differs significantly in patients with upper-normal thyroid-stimulating hormone (TSH) levels compared with those having TSH values within the lower normal range.

In addition, the study addressed several key research questions, including whether autoimmunity represents a significant cause of hypothyroidism, whether autoimmune thyroiditis demonstrates a sex predilection, whether there is a significant association between autoimmune thyroiditis and advancing age, and whether serum TSH levels show a significant correlation with circulating anti-TPO antibody levels.

Conceptual Framework

The variables considered in this study included thyroid autoimmunity as the dependent variable, while age, sex, dietary iodine intake, topography, environmental stress, and genetic predisposition were treated as independent variables that may influence the occurrence of autoimmune thyroid disorders. For the purpose of this study, several operational definitions were applied. Autoimmunity refers to the loss of normal immune homeostasis resulting in an abnormal immune response directed against the body's own tissues. Hypothyroidism was defined as an elevated thyroid-stimulating hormone (TSH) level accompanied by decreased serum thyroid hormone levels (T3, T4, or both).[29] Subclinical hypothyroidism was defined as elevated TSH levels with normal circulating thyroid hormone levels. Hyperthyroidism was defined by elevated T3 levels with suppressed TSH levels, while subclinical hyperthyroidism referred to diminished TSH levels with normal thyroid hormone concentrations.[30] Euthyroid sick syndrome was defined as reduced T3 or T4 levels with normal TSH levels. Euthyroid status indicated normal concentrations of T3, T4, and TSH. Goiter was defined as a diffuse enlargement of the thyroid gland.[31]

METHODOLOGY

Study Design and Type

This study was designed as a **prospective cross-sectional observational study** incorporating both qualitative and quantitative analytical components.

Study Site and Population

The study was conducted in the **Department of Biochemistry, Arogyam Medical College and Hospital (AMCH), Roorkee, Haridwar, Uttarakhand, India**. The study population comprised patients attending the **Outpatient Departments (OPD) of Internal Medicine and Otorhinolaryngology (ENT)** at AMCH who presented with clinical symptoms suggestive of thyroid dysfunction during the study period.

Study Duration

Data collection was carried out over a period of **eight months from February 2025 to September 2025**.

Sampling Technique and Sample Size

A **purposive (judgmental) non-probability sampling technique** was employed for participant selection. A total of **162 patient samples** were included in the study.

Sample Collection and Processing

Approximately **5 mL of venous blood** was collected from each participant under aseptic conditions using a **clot activator tube (yellow-capped vial)**. Standard universal precautions for phlebotomy were followed. After collection, the samples were transported to the Department of Biochemistry and centrifuged to obtain serum, which was subsequently used for biochemical analysis.[32]

Laboratory Analysis

Serum levels of **thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), and anti-thyroid peroxidase (anti-TPO) antibodies** were measured using **chemiluminescence immunoassay (CLIA)** on the **Siemens ADVIA Centaur® XP immunoassay system**.

Measurement of Serum TSH

Serum TSH levels were determined using a **two-site sandwich immunoassay based on direct chemiluminescent technology**. The assay employs a monoclonal mouse anti-TSH antibody labeled with acridinium ester and a polyclonal sheep anti-TSH antibody coupled to paramagnetic particles. The amount of emitted chemiluminescent signal (relative light units, RLUs) is directly proportional to the concentration of TSH present in the sample.[33]

The reference range for serum TSH was **0.35–5.50 mIU/L**, with assay linearity ranging from **0.01 to 150 mIU/L**.[33]

Measurement of Serum fT3

Serum fT3 levels were measured using a **competitive chemiluminescent immunoassay**. In this assay, free T3 in the sample competes with a T3 analog bound to paramagnetic particles for binding to acridinium-labeled monoclonal anti-T3 antibodies. The emitted signal shows an inverse relationship with the concentration of fT3 in the sample.[34]

The reference range for serum fT3 was **2.3–4.2 pg/mL**, with assay linearity of **0.2–20 pg/mL**.[34]

Measurement of Serum fT4

Serum fT4 concentrations were measured using a **competitive chemiluminescent immunoassay** in which free thyroxine in the patient sample competes with labeled thyroxine for a limited number of binding sites on biotinylated anti-T4 antibodies attached to paramagnetic particles. The detected chemiluminescent signal is inversely proportional to the fT4 concentration in the sample.[35]

The reference range for serum fT4 was **0.89–1.76 ng/dL**, with assay linearity between **0.1 and 12.0 ng/dL**.[35]

Measurement of Serum Anti-TPO Antibodies

Anti-TPO antibody levels were determined using a **competitive chemiluminescent immunoassay**. In this method, anti-TPO antibodies present in the patient sample compete with monoclonal mouse anti-TPO antibodies bound to paramagnetic particles for binding to labeled TPO antigen. The emitted chemiluminescent signal demonstrates an inverse relationship with anti-TPO antibody concentration.[36]

The reference range for anti-TPO antibodies was **< 60 U/mL**, with values ≥ 60 U/mL considered **positive for thyroid autoimmunity**.[36]

Criteria for Case Selection

Inclusion Criteria

Individuals with confirmed thyroid disorders, those undergoing treatment, newly diagnosed cases, and patients presenting with overt or subclinical thyroid abnormalities were included in the study. Demographic data including **age, sex, occupation, locality, pregnancy status, and associated comorbidities** were recorded and analyzed where applicable.

Exclusion Criteria

Patients with a history of **major surgery, malignancy, hepatic or renal disease, interferon therapy, or beta-blocker use** were excluded from the study.

Validity and Reliability

All laboratory procedures were conducted under the supervision of a qualified **pathologist**. Standardized laboratory protocols were followed, and all reagents, instruments, and analytical procedures were calibrated and validated to ensure reliability and reproducibility of results.

Data Management and Collection

Demographic information and relevant clinical data were collected using a **structured questionnaire**, and laboratory results were systematically recorded for statistical analysis.

Data analysis

Data was analyzed with the help of SPSS statistical software version 20.0. The Chi square test at 5% level of significance was used to determine the significant association of anti-TPO with fT_3, fT_4 and TSH. Two-tailed test was used for analysis of significance because the research hypothesis was bi-directional. The information obtained was presented as tables, pie chart, line and diagram.

RESULTS

A total of **162 participants** were included in the study. The socio-demographic characteristics of the study population are presented in **Table 4.1**. The majority of participants (54.3%) belonged to the **20–39 years** age group, followed by **40–59 years (34.6%)**, while **4.9%** and **6.2%** were within the **0–19 years** and **60–79 years** age groups, respectively. The **mean age of the study population was 37.34 ± 14.08 years**, with an age range of **1 to 71 years**. Females constituted the majority of the study population (**86.4%**), whereas males accounted for **13.6%**.

The distribution of thyroid dysfunction across different age groups is shown in **Table 4.2**. Overall, **hypothyroidism was the most prevalent thyroid disorder (47.5%)**, followed by **euthyroid status (30.9%)** and **hyperthyroidism (21.6%)**. The **20–39 years age group demonstrated the highest frequency of thyroid dysfunction**, with **44 individuals diagnosed with hypothyroidism and 21 with hyperthyroidism**. Among individuals aged **40–59 years**, **26 were hypothyroid and 10 were hyperthyroid**, whereas in the **60–79 years age group**, **six cases of hypothyroidism and three cases of hyperthyroidism** were observed. These findings suggest an **increasing trend in the occurrence of thyroid dysfunction with advancing age**.

Gender-wise distribution of thyroid dysfunction is presented in **Table 4.3**. Thyroid dysfunction demonstrated a **marked female predominance**, with a **female-to-male ratio of approximately 6:1**. Among the **140 female participants**, **70 were hypothyroid**, **32 were hyperthyroid**, and **38 were euthyroid**. In contrast, among **22 male participants**, **7 were hypothyroid**, **3 were hyperthyroid**, and **12 were euthyroid**.

The prevalence of **anti–thyroid peroxidase (anti-TPO) antibodies** in the study population is illustrated in **Figure 4.3**. Elevated anti-TPO antibody levels (**>60 U/mL**) were detected in **81 participants (50%)**, indicating a substantial proportion of cases with underlying autoimmune thyroid etiology.

Age-wise distribution of anti-TPO antibody positivity is shown in **Table 4.4**. The highest prevalence of anti-TPO positivity was observed in the **20–39 years age group**, where **47 of 88 individuals (29%)** demonstrated elevated antibody titers. Among participants aged **40–59 years**, **25 individuals (15.4%)** showed anti-TPO positivity, while **six individuals (3.7%)** in the **60–79 years age group** were positive for

anti-TPO antibodies. Although the prevalence of anti-TPO positivity appeared to increase with age, this trend was **not statistically significant**.

Gender-based distribution of anti-TPO antibodies is presented in **Table 4.5**. A strong **female predominance** was observed among anti-TPO positive cases. Of the **81 participants with elevated anti-TPO antibody levels**, **78 (48.1%) were females**, while only **3 (1.9%) were males**, highlighting the higher susceptibility of females to autoimmune thyroid disorders.

The relationship between anti-TPO antibody positivity and thyroid functional status is illustrated in **Table 4.6**. Among the **77 individuals diagnosed with hypothyroidism**, **45 (27.8%) demonstrated elevated anti-TPO antibody titers**, indicating a significant autoimmune component. Similarly, **20 of 35 hyperthyroid individuals** and **16 of 50 euthyroid individuals** also showed anti-TPO positivity. These findings suggest that **anti-TPO antibody positivity is more frequently associated with hypothyroidism**, although it may also occur in other thyroid functional states.

The association between serum anti-TPO antibody levels and selected clinical variables is summarized in **Table 4.7**. A **statistically significant association** was observed between **serum anti-TPO antibody levels and TSH concentrations ($\chi^2 = 9.389$, $p = 0.009$)**. Similarly, **serum fT3 levels showed a significant association with anti-TPO antibody levels ($\chi^2 = 6.583$, $p = 0.037$)**. However, the association between **serum fT4 levels and anti-TPO antibodies was not statistically significant ($\chi^2 = 5.299$, $p = 0.071$)**.

A **strong statistically significant association** was also observed between **sex and anti-TPO antibody levels ($\chi^2 = 13.465$, $p < 0.001$)**, indicating a markedly higher prevalence of autoimmune thyroid disease among females. In contrast, although anti-TPO positivity showed an increasing trend with age, **no statistically significant association was found between age and anti-TPO antibody levels ($\chi^2 = 1.952$, $p = 0.582$)**.

Overall, the findings indicate that **autoimmune thyroid disease is highly prevalent in the study population, particularly among females and individuals with hypothyroidism**, and that **anti-TPO antibodies are significantly associated with TSH and fT3 levels**.

TABLE 4.1

Socio-Demographic Characteristics of Respondents

n=162		
Variables	**Frequency**	**Percentage (%)**
Age (in years)		
0-19	08	4.9
20-39	88	54.3
40-59	56	34.6
60-79	10	6.2
Mean ±S.D: 37.34± 14.08 Minimum: 1 Maximum: 71		
Gender		
Male	22	13.6
Female	140	86.4

Table 4.1 shows socio-demographic characteristics of respondents. Fifty four percent (54.3%) of respondents were in between 20-39 years of age. The mean age of study population was 37.34 years and standard deviation was 14.08 years.

TABLE 4.2

Prevalence of Thyroid Dysfunctions in Different Age Groups

n=162				
Variables	**Clinical conditions**			**Total**
	Hypothyroid n (%)	**Euthyroid n (%)**	**Hyperthyroid n (%)**	**n (%)**
Age (in years)				
0-19	1 (0.6)	6 (3.7)	1 (0.6)	8 (4.9)
20-39	44 (27.1)	23 (14.2)	21 (13.0)	88 (54.3)
40-59	26 (16.1)	20 (12.3)	10 (6.2)	56 (34.6)
60-79	6 (3.7)	1 (0.6)	3 (1.9)	10 (6.2)
Total n (%)	**77 (47.5%)**	**50 (30.9%)**	**35 (21.6%)**	**162 (100%)**

FIGURE 4.1: Prevalence of Thyroid Dysfunctions in Different Age Groups

Figure 4.1 shows the prevalence of thyroid dysfunctions in different age groups. Age group of 20-39 years showed highest frequency of thyroid dysfunction. Of 88 individuals in between 20-39 years, 44

were hypothyroid and 21 were hyperthyroid. Among 56 subjects aged 40-59 years, 26 were hypothyroid and 10 were hyperthyroid. Of 10 individuals in between 60-79 years, 6 were hypothyroid and 3 were hyperthyroid. Thus, the incidence of thyroid dysfunction showed an increasing trend with age.

TABLE 4.3

Distribution of Thyroid Dysfunction According To Gender

n=162			
Variables	**Gender**		**Total**
	Male	**Female**	**n (%)**
	n (%)	**n (%)**	
Clinical condition			
Hypothyroid	7 (4.3)	70 (43.2)	77 (47.5)
Euthyroid	12 (7.4)	38 (23.5)	50 (30.9)
Hyperthyroid	3 (1.9)	32 (19.7)	35 (21.6)
Total	**22 (13.6%)**	**140 (86.4%)**	**162 (100%)**

FIGURE 4.2: Distribution of Thyroid Dysfunctions According to Gender

Figure 4.2 shows distribution of thyroid dysfunction in accordance to gender. Thyroid dysfunction was more prevalent in females than in males. Prevalence of thyroid dysfunction showed a female preponderance with the ratio of 6:1. Among 140 females incorporated in the study, 70 were hypothyroid; 32 were hyperthyroid and 38 were euthyroid. Of 22 males, 7 were hypothyroid; 3 were hyperthyroid and 12 were euthyroid.

FIGURE 4.3 : Prevalence of Anti-TPO Antibody in Study Population

Figure 4.3 shows the prevalence of Anti-TPO antibody in the study population. Anti-TPO antibody was found to be prevalent in fifty percent (50%) of study population. Out of 162 cases, 81 of them showed raised anti-TPO titre (>60 U/ml) suggesting an underlying autoimmune etiology.

TABLE 4.4 Prevalence of Anti-TPO Antibody in Different Age Group

n=162			
Variables	**Serum Anti-TPO level**		**Total**
	Positive (>60 U/ml) n (%)	**Negative (<60 U/ml) n (%)**	**n (%)**
Age (in years)			
0-19	3 (1.9)	5 (3)	8 (4.9)
20-39	47 (29)	41 (25.3)	88 (54.3)
40-59	25 (15.4)	31 (19.2)	56 (34.6)
60-79	6 (3.7)	4 (2.5)	10 (6.2)
Total	**81 (50%)**	**81 (50%)**	**162 (100%)**

FIGURE 4.4 Prevalence of Anti-TPO Antibody in Different Age Groups

Figure 4.4 shows the distribution of anti-TPO antibody in different age groups. Highest no. of individuals with positive anti-TPO antibody titer belonged to 20-39 years of age. Of 88 individuals belonging to 20-39 years, 47 were positive for anti-TPO antibody. Among 56 individuals in between 40-59 years, 25 demonstrated positive anti-TPO titer. Also, of 10 individuals within 60-79 years, 6 were positive for anti-TPO antibody. Prevalence of positive anti-TPO titer showed an increasing trend with age.

TABLE 4.5 : Prevalence of Anti-TPO Antibody in Accordance to Gender

n=162			
Variables	**Gender**		**Total**
	Male n (%)	**Female n (%)**	**n (%)**
Serum Anti-TPO antibody level			
Positive (>60 U/ml)	3 (1.9)	78 (48.1)	81 (50%)
Negative (<60 U/ml)	19 (11.7)	62 (38.3)	81 (50%)
Total	**22 (13.6%)**	**140 (86.4%)**	**162 (100%)**

FIGURE 4.5 : Gender-wise Distribution of Anti-TPO Antibody

Figure 4.5 shows the distribution of anti-TPO antibody among male and female gender. Prevalence of positive anti-TPO titer showed a marked predilection towards females. Of 140 female participants, 78 demonstrated elevated level of anti-TPO antibody in serum. While only 3 of 22 males were positive for anti-TPO antibody.

TABLE 4.6: Prevalence of Anti-TPO Antibody in Thyroid Dysfunction

n=162			
Variables	**Serum Anti-TPO antibody level**		**Total**
	Positive (>60 U/ml) n (%)	**Negative (<60 U/ml) n (%)**	**n (%)**
Clinical conditions			
Hypothyroid	45 (27.8)	32 (19.7)	77 (47.5)
Euthyroid	16 (9.9)	34 (21.0)	50 (30.9)
Hyperthyroid	20 (12.3)	15 (9.3)	35 (21.6)
Total	**81 (50%)**	**81 (50%)**	**162 (100%)**

FIGURE 4.6 : Prevalence of Anti-TPO Antibody in Thyroid Dysfunctions

Figure 4.6 shows the prevalence of anti-TPO antibody in thyroid dysfunctions. Out of 81 positive cases, 45 of them were hypothyroid, 16 were euthyroid and 20 were hyperthyroid. Also, among 77 hypothyroid subjects, 45 had demonstrable elevated antibody titer (>60 U/ml). Among 35 subjects with hyperthyroidism, 20 were anti-TPO positive and 16 of 50 euthyroid cases also tested positive for anti-TPO antibody. Thus, Anti-TPO positivity is more commonly associated with hypothyroidism.

TABLE 4.7: Association of Serum Anti-TPO Antibody with Selected Variables

n=162				
Variables	**Serum Anti-TPO level**		**Chi square value**	**p-value**
	Positive (n=81)	**Negative (n=81)**	**(χ^2)**	
TSH				
Low	20 (57.1%)	15 (42.9%)	9.389	0.009
Normal	16 (32.0%)	34 (68.0%)		
High	45 (58.4%)	32 (41.6%)		
Free T_4				
Low	27 (62.8%)	16 (37.2%)		
Normal	45 (43.3%)	59 (56.7%)	5.299	0.071
High	9 (60.0%)	6 (40.0%)		
Free T_3				
Low	22 (66.7%)	11 (33.3%)		
Normal	48 (43.2%)	63 (56.8%)	6.583	0.037
High	11 (61.1%)	7 (38.9%)		
Sex				
Female	78 (55.7%)	62 (44.3%)		
Male	3 (13.6%)	19 (86.4%)	13.465	<0.001
Age				
0-19 years	3 (37.5%)	5 (62.5%)		
20-39 years	47 (53.4%)	41 (46.6%)		
40-59 years	25 (44.6%)	31 (55.4%)	1.952	0.582
60-79 years	6 (60.0%)	4 (40.0%)		

Note: p-value <0.05; statistically significant.

Table 4.7 demonstrates the association of Serum Anti-TPO antibody with selected variables that were included in the study. In patients with elevated TSH, 41.6% had normal anti-TPO but 58.4% had abnormally high anti-TPO and the differences in between patients who had normal anti-TPO and patients with high anti-TPO were significant (p=0.009). Also, in patients with depressed TSH levels, 42.9% had normal anti-TPO but 57.1% had abnormally high anti-TPO and the difference in between patients with normal anti-TPO and those with high anti-TPO were significant (p=0.009)

DISCUSSION

The present study evaluated the prevalence of thyroid dysfunction and the association of **anti–thyroid peroxidase (anti-TPO) antibodies** with thyroid hormone parameters among patients presenting with suspected thyroid disorders. A total of **162 participants** were included in the study, with the majority belonging to the **20–39 years age group (54.3%)**. The mean age of the study population was **37.34 ± 14.08 years**, indicating that thyroid dysfunction was more frequently observed in the young and middle-aged population. Furthermore, a marked **female predominance (86.4%)** was observed compared with males (13.6%), reflecting the well-established higher susceptibility of women to thyroid disorders.

Hypothyroidism was the most prevalent thyroid dysfunction in the present study, affecting **47.5% of participants**, whereas **hyperthyroidism was observed in 21.6%** of the individuals. The highest frequency of thyroid dysfunction was observed in the **20–39 years age group**, where **50% of individuals were hypothyroid and 23.8% were hyperthyroid**. Similarly, among individuals aged **40–59 years**, **46.4% were hypothyroid and 17.8% were hyperthyroid**. A comparable pattern was observed in the **60–79 years age group**, where **60% of individuals were hypothyroid and 30% were hyperthyroid**. These findings suggest that the **incidence of thyroid dysfunction tends to increase with advancing age**. Similar observations were reported by **Cyriac et al.**, who found the highest prevalence of hypothyroidism (48.6%) in individuals aged 18–30 years[43], and by **Saha et al.**, who reported a prevalence of 40.5% in the 36–45 years age group[47].

The present study also demonstrated a strong **female predominance in thyroid dysfunction**, with a female-to-male ratio of approximately **6:1**. Among the study population, **43.2% of females were hypothyroid compared with 4.3% of males**, whereas **19.7% of females and 1.9% of males were hyperthyroid**. These findings are consistent with previous studies that have reported a significantly higher prevalence of thyroid disorders among females. Saha et al.[47] and Cyriac et al.[42] also reported a similar female predominance, while **Jeena et al.** documented that approximately **75% of hypothyroid patients were females**[46]. The higher prevalence among females may be attributed to hormonal and immunological factors that predispose women to autoimmune disorders.

In the present study, **anti-TPO antibodies were detected in 50% of the study population**, indicating a substantial contribution of autoimmune mechanisms in thyroid dysfunction. Comparable findings were reported by **Kontiainen et al.**, who documented an anti-TPO prevalence of 47%[49]. Higher prevalence rates have been reported in certain populations, such as 66% in the study by Jayashankar et al.[44], whereas **Ghoriashian et al.** reported a comparatively lower prevalence of 35.69%[40]. These variations may be attributed to differences in study population characteristics, geographic factors, and diagnostic methodologies.

Age-wise analysis of anti-TPO positivity revealed that the **highest proportion of antibody-positive individuals (58%) belonged to the 20–39 years age group**, followed by the **40–59 years age group (30%)**. Similar findings were reported by **Ghoriashian et al.**, who observed that **58.42% of patients with elevated**

anti-TPO antibodies were aged between 20 and 39 years[40]. Likewise, **Cyriac et al.** reported the highest prevalence of anti-TPO antibodies in the 31–40 years age group (41.1%)[42].

A marked **female predominance in anti-TPO positivity** was also observed in the present study. Among individuals with elevated anti-TPO titers, **96% were females**, whereas only **4% were males**. Of the **140 female participants**, **78 (55.7%)** demonstrated elevated anti-TPO antibody levels compared with **3 (13.6%) among males**. These findings are consistent with previous reports indicating that autoimmune thyroid diseases predominantly affect women. **Swain et al.** reported that approximately **95% of patients with autoimmune thyroid disease are females**, most commonly between **30 and 50 years of age**. Similarly, **Ghoriashian et al.** reported that **89.14% of anti-TPO positive cases were females**[40]. The higher prevalence of autoimmune thyroid disease among females has also been documented by **Canaris et al.**, who reported that such disorders occur **2–4 times more frequently in women than in men**[8].

Analysis of anti-TPO positivity according to thyroid functional status revealed that **55% of antibody-positive individuals were hypothyroid**, whereas **25% were hyperthyroid and 20% were euthyroid**. Among **77 hypothyroid patients**, **45 (58.4%)** demonstrated elevated anti-TPO antibody titers. These findings are consistent with previous studies reporting a strong association between hypothyroidism and anti-TPO positivity. **Jeena et al.** reported that **60% of hypothyroid patients had elevated anti-TPO titers**[46], while **Kontiainen et al.** reported a prevalence of 61%[49]. Similarly, **Ghoriashian et al.** observed anti-TPO positivity in **64.45% of hypothyroid individuals**[40], and **Cyriac et al.** reported an even higher prevalence of 71.4%[43]. **Mohanty et al.** also reported a prevalence of **76%** among hypothyroid patients. These findings collectively suggest that **autoimmune mechanisms play a significant role in the pathogenesis of hypothyroidism**.

In the present study, **57.1% of hyperthyroid patients** and **32% of euthyroid individuals** demonstrated anti-TPO positivity. However, **Kontiainen et al.** reported lower prevalence rates, with anti-TPO antibodies detected in **26% of hyperthyroid patients and 12% of euthyroid individuals**[49]. Similarly, **Jeena et al.** reported anti-TPO positivity in **25% of euthyroid subjects**[46]. These findings indicate that although anti-TPO antibodies are more commonly associated with hypothyroidism, they may also be detected in other thyroid functional states.

Statistical analysis revealed a **significant association between serum anti-TPO antibody levels and serum TSH concentrations (p = 0.009)**. This observation is supported by previous studies. **Vaseghani et al.** demonstrated that anti-TPO antibody titers correspond closely with TSH levels[50], while **Cyriac et al.** reported a significant positive association between TSH and anti-TPO levels ($p = 0.003$)[45]. Similarly, **Ghoriashian et al.** reported a highly significant association between elevated TSH levels and anti-TPO positivity ($p < 0.0001$)[40]. Long-term follow-up studies by **Vanderpump et al.** have shown that increasing serum TSH levels above **3 mIU/L** are associated with a higher risk of developing overt hypothyroidism[8]. Additionally, **Bjuro et al.** demonstrated that the prevalence of elevated TSH levels was nearly **ten times higher in individuals with positive anti-TPO antibodies** during a 20-year follow-up study[37].

A **significant association was also observed between serum anti-TPO antibody levels and serum fT3 concentrations (p = 0.037)**. Similar findings have been reported by Ghoriashian et al.[40], **Dayal et al.** (p = 0.005)[48], and **Legakis et al.** (p < 0.001)[39]. However, **no statistically significant association was observed between anti-TPO antibody levels and serum fT4 concentrations (p = 0.071)**. Comparable results were reported by Dayal et al.[48] and Al-Juburi et al.[41].

Furthermore, **sex showed a strong statistically significant association with anti-TPO antibody levels (p < 0.001)**, confirming the greater susceptibility of females to autoimmune thyroid disease. In contrast, **age did not demonstrate a statistically significant association with anti-TPO antibody positivity (p = 0.582)**, despite an observed increasing trend with advancing age. Similar findings have been reported by **Legakis et al.**, who observed a significant association between sex and anti-TPO levels but not with age[39]. Likewise, **Bjuro et al.** also demonstrated a significant relationship between sex and anti-TPO antibody levels[37].

Overall, the findings of the present study highlight the **high prevalence of autoimmune thyroid disease, particularly among females and individuals with hypothyroidism**, and emphasize the clinical importance of **anti-TPO antibody testing in the evaluation of thyroid dysfunction**.

CONCLUSION

The present study evaluated the prevalence of thyroid dysfunction and its association with **anti–thyroid peroxidase (anti-TPO) antibodies** and thyroid hormone parameters (TSH, fT3, and fT4) in a cohort of 162 individuals. Hypothyroidism emerged as the most common thyroid abnormality among the study participants and demonstrated a marked **female predominance**. A relatively high prevalence of anti-TPO antibody positivity was observed in the study population, indicating a substantial contribution of **autoimmune mechanisms in the etiology of thyroid disorders**.

The findings further revealed that both thyroid dysfunction and anti-TPO antibody positivity were most frequently observed among individuals aged **20–39 years**. The marked predominance of anti-TPO positivity among females suggests that autoimmune processes play an important etiological role in thyroid disease, particularly among women. Statistical analysis demonstrated a **significant association between serum anti-TPO antibody levels and serum TSH, serum fT3, and sex of the participants**, whereas no statistically significant association was observed between anti-TPO antibody levels and **serum fT4 or age**.

Overall, the results highlight the **clinical importance of evaluating both thyroid hormone levels and anti-TPO antibodies** in the diagnosis and assessment of thyroid dysfunction. Early identification of autoimmune thyroid disease through anti-TPO antibody testing may help clinicians establish the underlying etiology, predict disease progression, and implement appropriate therapeutic interventions. Further large-scale and molecular studies are recommended to better understand the mechanisms underlying autoimmune thyroid disorders and their risk factors in the general population.

Declarations

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Conflict of Interest

The authors declare **no conflict of interest**.

Ethical Statement

The study was conducted in accordance with ethical standards for biomedical research involving human participants. **Verbal informed consent** was obtained from all participants prior to inclusion in the study. Ethical approval for the research was granted by the **Institutional Ethics Committee (IEC) of Arogyam Medical College and Hospital (AMCH), Roorkee, India**. All procedures performed in this study were carried out in compliance with institutional ethical guidelines and applicable regulations governing human research.

References

1. Vanderpump MP, Tunbridge WM (2002) Epidemiology and prevention of clinical and subclinical hypothyroidism. Thyroid 12(10):839–847
2. Kumar P, Kumar CM (2009) Clark's clinical medicine. Saunders Elsevier, Edinburgh
3. Tunbridge WM, Caldwell G (1991) The epidemiology of thyroid diseases. Werner Ingbar's thyroid 6:578–588
4. Jacobson EM, Tomer Y (2007) The genetic basis of thyroid autoimmunity. Thyroid 17(10):949–961
5. Sinha AA, Lopez MT, McDevitt HO (1990) Autoimmune diseases: the failure of self tolerance. Science 248(4961):1380
6. Metcalfe KA, Hitman GA, Rowe RE, Hawa M, Huang X, Stewart T, Leslie RD (2001) Concordance for type 1 diabetes in identical twins is affected by insulin genotype. Diabetes Care 24(5):838–842
7. Brix TH, Kyvik KO, Hegedüs L (2000) A population-based study of chronic autoimmune hypothyroidism in Danish twins. J Clin Endocrinol Metabolism 85(2):536–539

8. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC (2000) The Colorado thyroid disease prevalence study. Arch Intern Med 160(4):526–534
9. Bülow Pedersen I, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Laurberg P (2002) Large differences in incidences of overt hyper-and hypothyroidism associated with a small difference in iodine intake: a prospective comparative register-based population survey. J Clin Endocrinol Metabolism 87(10):4462–4469
10. Jameson JL, Weetman AP (2001) Disorders of the thyroid gland. Harrisons principles Intern Med 2:2060–2083
11. Pearce EN, Gerber AR, Gootnick DB, Khan LK, Li R, Pino S, Braverman LE (2002) Effects of chronic iodine excess in a cohort of long-term American workers in West Africa. J Clin Endocrinol Metabolism 87(12):5499–5502
12. Zoeller TR (2010) Environmental chemicals targeting thyroid. Horm (Athens) 9(1):28–40
13. Huber A, Menconi F, Corathers S, Jacobson EM, Tomer Y (2008) Joint genetic susceptibility to type 1 diabetes and autoimmune thyroiditis: from epidemiology to mechanisms. Endocr Rev 29(6):697–725
14. Tomer Y, Huber A (2009) The etiology of autoimmune thyroid disease: a story of genes and environment. J Autoimmun 32(3):231–239
15. Brown RS (2013) Autoimmune thyroiditis in childhood. J Clin Res Pediatr Endocrinol 5(Suppl 1):45
16. Sinclair D (2006) Clinical and laboratory aspects of thyroid autoantibodies. Ann Clin Biochem 43(3):173–183
17. Marcocci CL, Chiovato LU (2000) Thyroid-directed antibodies. Werner and Ingbar's the thyroid: a fundamental and clinical text, 8th edn. Lippincott Williams & Wilkins, Philadelphia, pp 414–431
18. McLACHLAN SM, Rapoport B (1992) The molecular biology of thyroid peroxidase: cloning, expression and role as autoantigen in autoimmune thyroid disease. Endocr Rev 13(2):192–206
19. Mclntosh R, Watson P, Weetman A (1998) Somatic hypermutation in antoimmune thyroid disease. Immunol Rev 162(1):219–231
20. Silva LM, Chavez J, Canalli MH, Zanetti CR (2003) Determination of IgG subclasses and avidity of antithyroid peroxidase antibodies in patients with subclinical hypothyroidism–a comparison with patients with overt hypothyroidism. Hormone Res Paediatrics 59(3):118–124
21. Raspe E, Costagliola S, Ruf J, Mariotti S, Dumont JE, Ludgate M (1995) Identification of the thyroid Na+/I– cotransporter as a potential autoantigen in thyroid autoimmune disease. Eur J Endocrinol 132(4):399–405
22. Mariotti S, Anelli S, Ruf J, Bechi R, Czarnocka B, Lombardi A, Carayon P, Pinchera A (1987) Comparison of serum thyroid microsomal and thyroid peroxidase autoantibodies in thyroid diseases. J Clin Endocrinol Metabolism 65(5):987–993
23. YOSHIDA H, AMINO N, YAGAWA K, UEMURA K, SATOH M, MIYAI K, KUMAHARA Y (1978) Association of serum antithyroid antibodies with lymphocytic infiltration of the thyroid gland:

studies of seventy autopsied cases. J Clin Endocrinol Metabolism 46(6):859–862

24. Chiovato LU, Bassi PA, Santini FE, Mammoli CL, Lapi PA, Carayon PI, Pinchera AL (1993) Antibodies producing complement-mediated thyroid cytotoxicity in patients with atrophic or goitrous autoimmune thyroiditis. J Clin Endocrinol Metabolism 77(6):1700–1705
25. Braverman LE (1990) Iodine induced thyroid disease. Acta Med Austriaca 17:29
26. Desai MP (1997) Disorders of thyroid gland in India. Indian J Pediatr 64(1):11–20
27. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA (1977) The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol 7(6):481–493
28. Demers LM, Spencer C (2006) The thyroid: pathophysiology and thyroid function testing. TEITZ Textbook of Clinical Chemistry and Molecular Diagnostics. Elsevier, a division of Reed Elsevier India Pvt Ltd., New Delhi, pp 2053–2095
29. Magner J (2014) Historical Note: Many Steps Led to the 'Discovery'of Thyroid-Stimulating Hormone. Eur thyroid J 3(2):95–100
30. Thyroid History Timeline – American Thyroid Association www thyroid org Retrieved 13 November (2016)
31. Niazi, Khan A, Kalra S, Irfan A, Islam A Thyroidology over the ages. Indian J Endocrinol Metabol (2016-11-13); 15 (Suppl2): S121–S126
32. World Health Organization WHO guidelines on drawing blood: best practices in phlebotomy
33. Centaur ADVIA, Centaur ADVIA XP, and, Centaur ADVIA, Systems XPT TSH; 10629907_EN Rev P, 2014–2008
34. Centaur ADVIA, Centaur ADVIA XP, and, Centaur ADVIA, Systems XPT FT3; 10629863_EN Rev. M, 2015-01
35. Centaur ADVIA, Centaur ADVIA XP, and, Centaur ADVIA, Systems XPT FT4; 10629864_EN Rev. H, 2015-02
36. Centaur ADVIA, Centaur ADVIA XP, and, Centaur ADVIA, Systems XPT aTPO; 10630967_EN Rev B, 2014–2008
37. Bjoro T, Holmen J, Kruger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L, Brochmann H (2000) Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol 143(5):639–647
38. Vanderpump MP, Tunbrldge WM, French J, Appleton D, Bates D, Clark F, Evans JG, Hasan DM, Rodgers H, Tunbridge F, Young ET (1995) The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol 43(1):55–68
39. Legakis I, Manousaki M, Detsi S, Nikita D (2013) Thyroid function and prevalence of anti-thyroperoxidase (TPO) and anti-thyroglobulin (Tg) antibodies in outpatients hospital setting in an area with sufficient iodine intake: influences of age and sex. Acta Medica Iranica 51(1):25–34

40. Ghoraishian SM, Moghaddam SH, Afkhami M (2006) Relationship between anti-thyroid peroxidase antibody and thyroid function tests. World J Med Sci 1:44–47
41. Al-Juburi S, Taresh HR, Mahmood AA, Al-Fatlawi RB, THE RELATIONSHIP BETWEEN ANTI-THYROIDAL PEROXIDISE ANTIBODIES (2015) AND THYROID HORMONES (T3, T4 AND THYROID STIMULATING HORMONE TSH) AMONG PATIENTS WITH AUTOIMMUNE THYROID DISEASE. Eur Sci J ESJ. ;11(6)
42. Ali HH, Alam JM, Hussain A, Naureen S (2015) Correlation of Thyroid Antibodies (Anti-Thyroid Peroxidase and Anti-Thyroglobulin) with Pituitary and Thyroid Hormones in Selected Population Diagnosed with Various Thyroid Diseases. Middle-East J Sci Res 23(9):2069–2073
43. Cyriac T, Chellappa P, Sinnet P, Anish I (2015) Prevalence of hypothyroidism and its association with anti-thyroid peroxidase antibody among adult sea food consuming population attending a tertiary health care centre in Kerala. Int J Biomed Adv Res 6(9):648–655
44. Jayashankar CA, Avinash S, Shashidharan B, Sarathi V, Shruthi KR, Nikethan D, Harshavardhan J (2017) The prevalence of anti-thyroid peroxidase antibodies in subclinica and clinical hypothyroid patients. Int J Res Med Sci 3(12):3564–3566
45. Arakeri S, Vasu G (2016) Correlation of anti-thyroid peroxidase antibody levels with status of thyroid function among the tribal population residing in hilly area. J Diagn Pathol Oncol 1(2):21–23
46. Jeena EJ, Malathi M, Sudeep K (2013) A hospital-based study of anti-TPO titer in patients with thyroid disease. Muller J Med Sci Res 4(2):74
47. Saha PK, Baur B, Gupta S (2007) Thyroid stimulating hormone measurement as the confirmatory diagnosis of hypothyroidism: A study from a tertiary-care teaching hospital, Kolkatta. Indian J Community Med 32(2):139
48. Dayal D, Naganur SH, Siakia BK, Singh B (2015) Thyroid dysfunction and autoantibodies in first degree relatives of North Indian children with autoimmune thyroiditis. Thyroid Res Pract 12(3):96
49. Kontiainen S, Melamies L, Miettinen A, Weber T (1994) Thyroid autoantibodies in serum samples with abnormal TSH levels. APMIS 102(7–12):716–720
50. Vasheghani M, Jalali R, Dabbaghmanesh MH, Sadeghalvad A, Omrani GR (2011) Thyroid autoimmunity role in the evolution of endemic goiter in rural area, Fars, Iran. Arch Iran Med 14(3):164

Figures

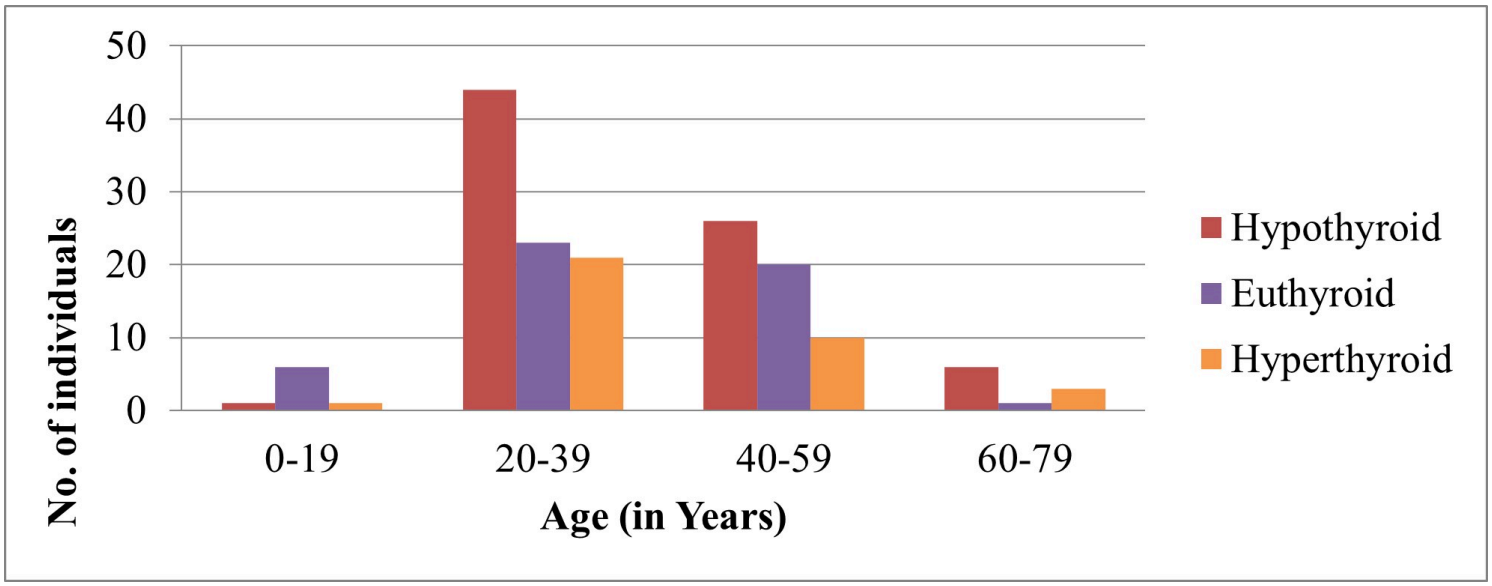


Figure 1

FIGURE 4.1: Prevalence of Thyroid Dysfunctions in Different Age Groups

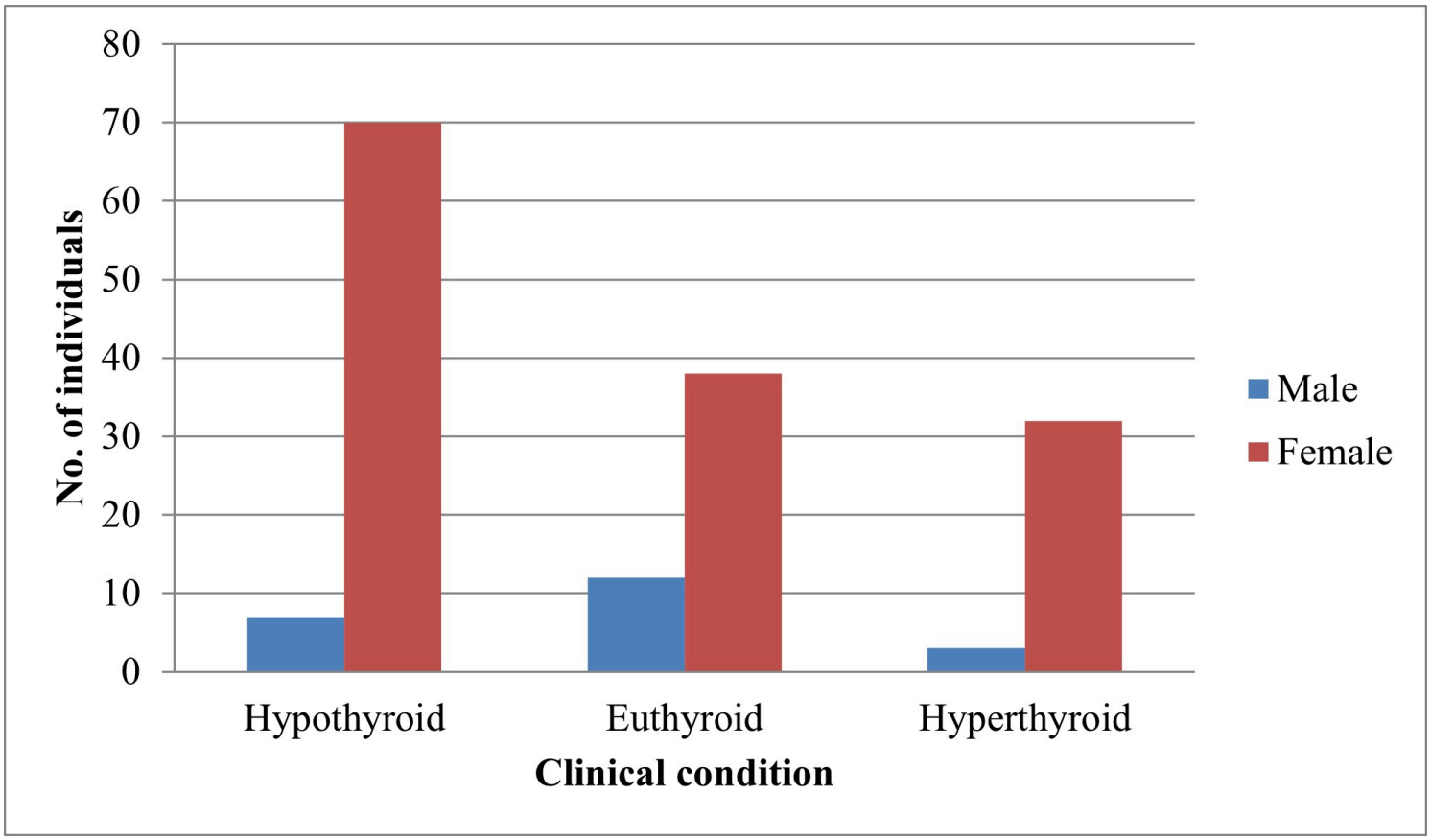


Figure 2

FIGURE 4.2: Distribution of Thyroid Dysfunctions According to Gender

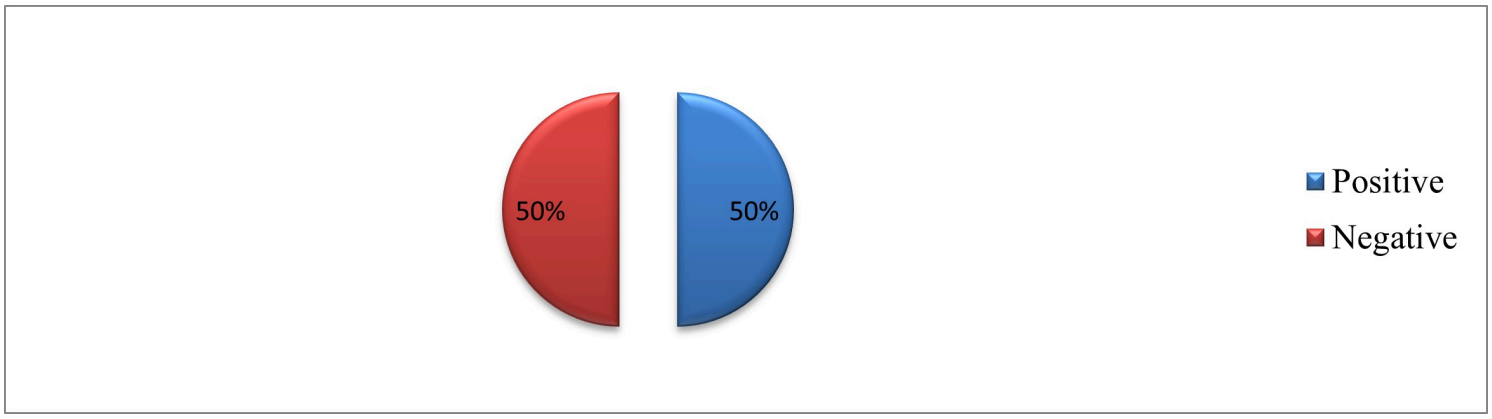


Figure 3

FIGURE 4.3 : Prevalence of Anti-TPO Antibody in Study Population

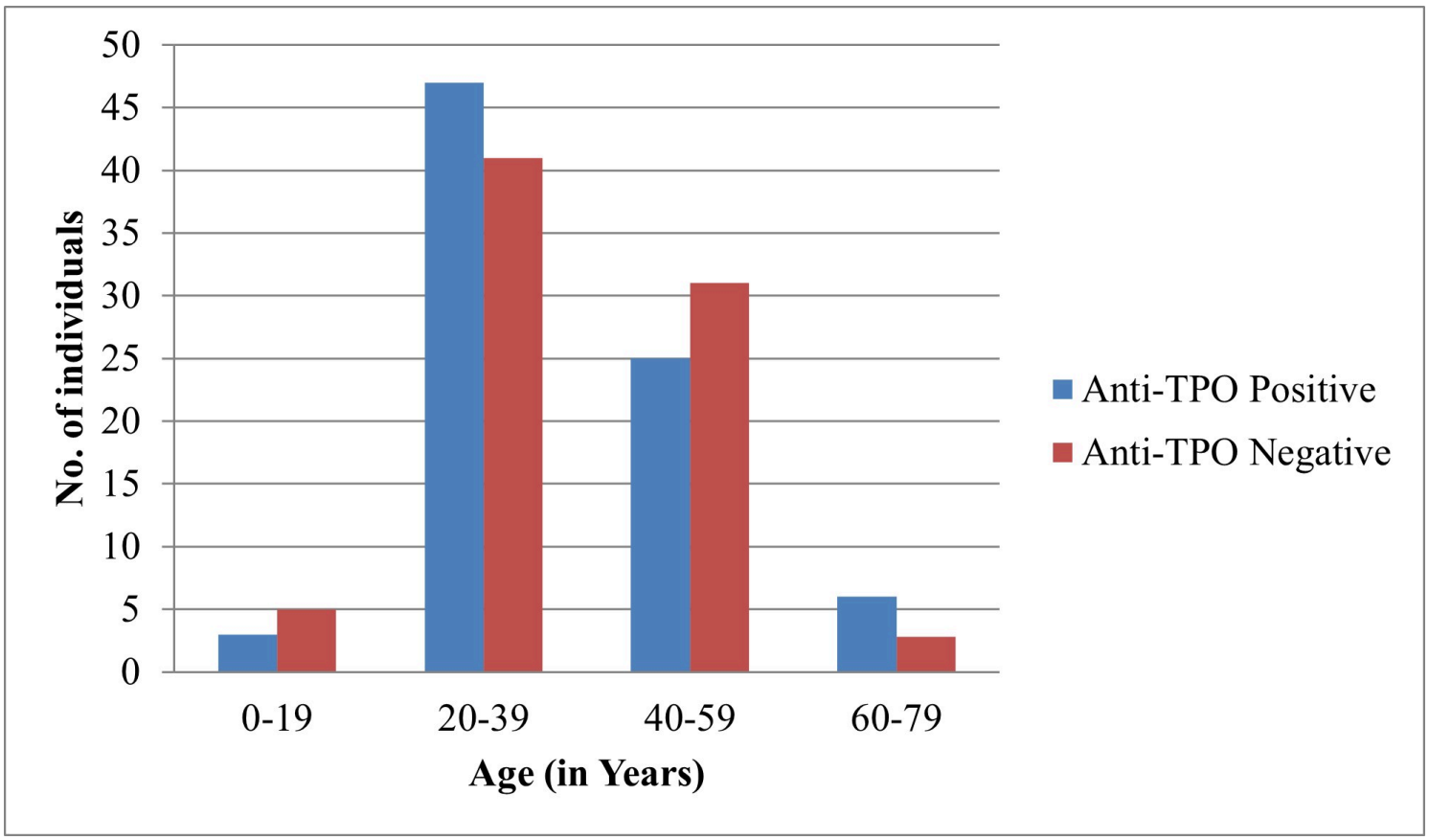


Figure 4

FIGURE 4.4 Prevalence of Anti-TPO Antibody in Different Age Groups

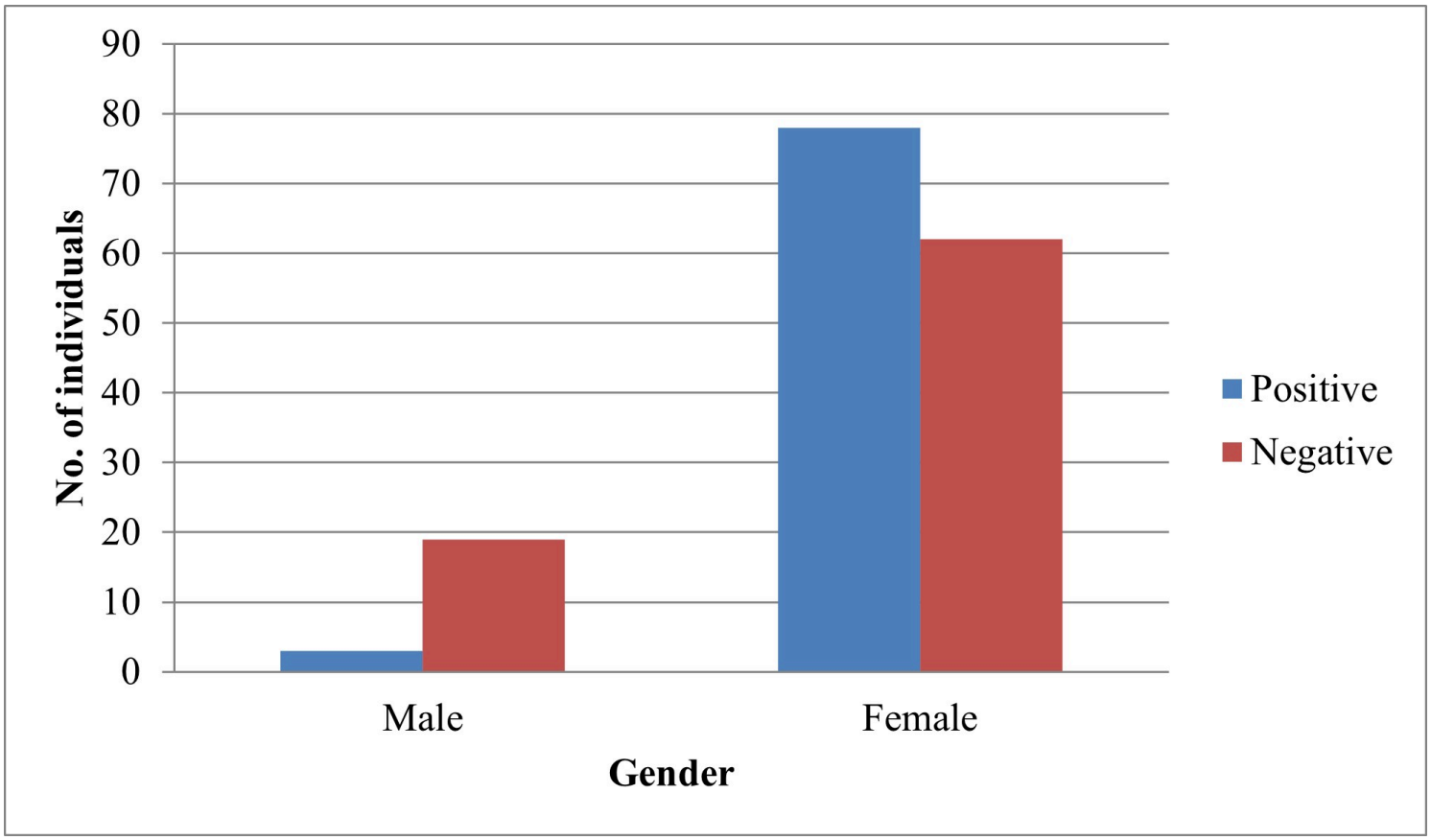


Figure 5

FIGURE 4.5 : Gender-wise Distribution of Anti-TPO Antibody

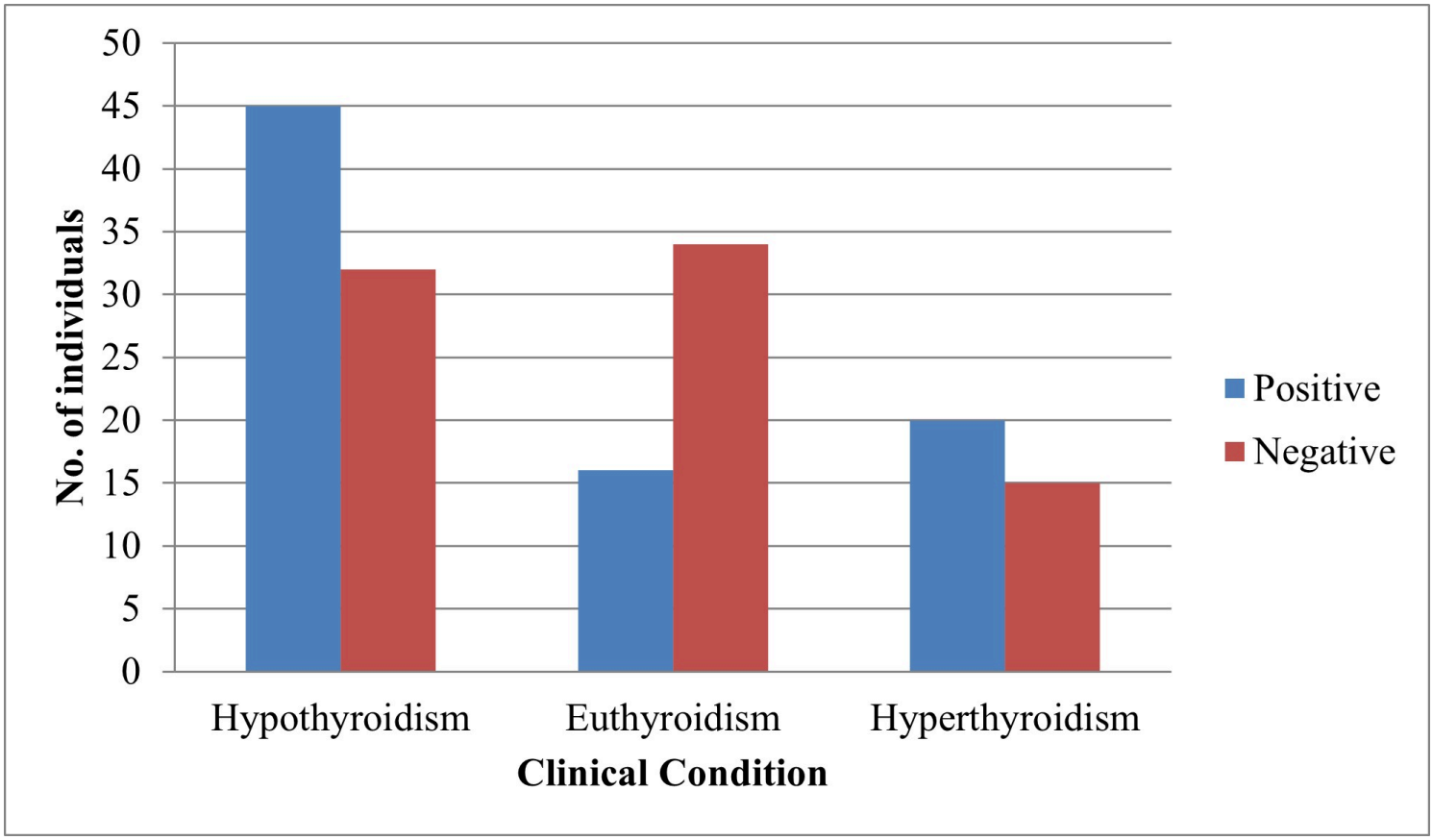


Figure 6

FIGURE 4.6 : Prevalence of Anti-TPO Antibody in Thyroid Dysfunctions