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Original Article

Biochemistry Section

The Association of Thyroid Autoimmunity and Lipid Profile in Hypothyroidism: A Cross-sectional Study

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ABSTRACT

Introduction: Thyroid disorders, particularly hypothyroidism, are the most common thyroid disorders in India, affecting one in ten adults. Autoimmunity may play a significant role in the progression of hyperlipidaemia, which is one of the recurrent issues associated with thyroid disorders. Although serum lipids have been proven to control immunological and inflammatory responses, little is known about their relationship to thyroid autoimmunity.

Aim: To investigate the association between Thyroid Peroxidase Antibody (anti-TPO) and serum lipid parameters among patients diagnosed with hypothyroidism.

Materials and Methods: A hospital-based cross-sectional study was conducted at Parul Sevashram Hospital in Vadodara, Gujarat from November 2022 to May 2023. A total of 103 patients with hypothyroidism were included in the study. All patients underwent estimation of thyroid function parameters such as triiodothyronine (T3), thyroxine (T4), and Thyroid-Stimulating Hormone (TSH), as well as anti-TPO levels and selected lipid profile parameters such as Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoproteins (HDL), and Low-Density Lipoproteins (LDL). The association between anti-TPO and lipid levels was further analysed. Statistical analysis was performed using the Chi-square test.

Results: Among the 103 hypothyroid patients, 83 (80.6%) were females, while only 20 (19.4%) were males. Positive serum anti-TPO antibodies were found in 79 (76.7%) patients, whereas negative Anti-TPO cases accounted for 24 (23.3%). The total serum cholesterol, with a mean of 226.88±37.44 mg/dL, and serum LDL, with 145.31±34.60 mg/dL, were significantly increased (p-value <0.01), whereas the serum TG with a mean of 181.06±52.13 mg/dL, and serum HDL, with 44.61±7.87 mg/dL, were found to be non significant (p-value >0.05).

Conclusion: Hypothyroid patients positive for anti-TPO antibodies are at risk of experiencing disrupted lipid levels. There was a significant association between positive anti-TPO status and TC and LDL in the present study.

Keywords: Hypercholesterolaemia, Hyperlipidaemia, Immunological response, Thyroid autoantibodies, Thyroid-stimulating hormone

INTRODUCTION

Thyroid dysfunction appears to be the most prevalent endocrine condition, affecting a significant portion of the population [1]. Insufficient thyroid hormone or inadequate activity in target tissue production leads to hypothyroidism [2]. Hypothyroidism becomes more common as people age. However, since the population distribution of TSH concentration gradually increases with age, the reference range for TSH also increases accordingly [3,4]. Thyroid hormones have an impact on all major systems and organs, and maintaining healthy levels is essential for proper functioning. Approximately 21% of the population suffers from thyroid disorders, with women being more susceptible than males [3-6].

Autoimmune diseases exhibit a marked incidence in the population, and among them, Autoimmune Thyroid Disorder (AITD) emerges as one of the most prevalent forms [7]. Patients with AITD frequently experience enhanced levels of serum thyroid autoantibodies such as anti-TPO-Ab, anti-TG-Ab, and TSHR-Ab [7]. The fundamental mechanisms underlying the progression of thyroid autoimmunity are likely attributed to a combined TPO and TG-specific cytotoxic immune response [8]. Hashimoto's thyroiditis is a prevalent autoimmune disease, with a significant prevalence in women [9]. There are a few other cases in which autoimmune antibodies are detected, such as Graves' disease. Additionally, TPO antibodies can also cause hyperlipidaemia, which is currently a major concern [10]. According to a critical review on the management of dyslipidaemia, approximately 25-30% of urban and 15-20% of rural subjects are suffering from hyperlipidaemia [11].

Several studies have indicated that TPO-Ab positivity, either alone or in combination with elevated TSH, is crucial for the occurrence of thyroid disease [12-14]. Thyroid disorders cause significant impairments in lipoprotein composition and transport because thyroid hormones alter the activity of numerous crucial enzymes in lipid metabolism. Hypercholesterolaemia and higher LDL levels are evident in cases of hypothyroidism. The causes of hyperlipidaemia in hypothyroidism include decreased liver LDL excretion and decreased LDL receptor count, leading to in hyperlipidaemia [15,16].

There have been a few articles focusing on how thyroid autoantibodies affect lipid parameters [17,18], and the association between hyperlipidaemia and thyroid-related disorders is now well-established. However, whether thyroid autoimmunity in people with hypothyroidism also causes hyperlipidaemia is still debated. Keeping this in view, authors hypothesised that there could be a significant influence of anti-TPO antibodies on the lipid profile. Therefore, the current investigation focuses on detecting TPO antibodies in hypothyroid patients to assess the presence of autoimmune thyroiditis and its association with their lipid profile.

MATERIALS AND METHODS

A hospital-based, cross-sectional study was conducted at Parul Sevashram Hospital, Vadodara, Gujarat, India from November 2022 to May 2023. The study subjects were individually counseled about the study, and an informed consent form was obtained from each patient. The present study was approved by the Ethics Committee of Parul University–Institutional Ethics Committee for

Human Research (PU-IECHR), Vadodara, with IEC approval no. (PUIECHR/PIMSR/00/081734/5310).

Inclusion criteria: Patients aged >18 years, irrespective of gender, with prespecified variables such as T3, T4, and TSH confirming hypothyroidism were included in the study.

Exclusion criteria: Patients under the age of 18, those with hyperthyroidism, myocardial infarction, congestive heart failure, smokers, alcoholics, diabetics, post-thyroid surgery patients, patients with a history of neck radiotherapy, and pregnant women were excluded from the study.

Sample size: A total of 103 patients, including both males and females, were analysed for the study. The sample size was determined using a non probability, convenient sampling method with a 95% confidence level, using an online statistical tool called OpenEpi.

Data collection: Data related to the patients' age and gender were collected from all the study participants. A 3 mL venous blood sample was drawn from each participant using a sterile venipuncture technique in plain (red) vials without anticoagulant after a 12-hour fasting period. The samples were then centrifuged for 15 minutes at 2500 rpm to separate serum and blood. The serum samples were processed for lipid profile, thyroid profile, and TPO antibody analysis.

The thyroid profile (T3, T4, and TSH) was measured using the Maglumi 800 Immuno Assay Analyser by the Chemiluminescent Immunoassay (CLIA) method. The reference ranges were provided by the kit: T3 (0.87-1.78 ng/mL), T4 (6.03-12.23 µg/dL), TSH (0.38-5.33 µIU/mL). Hormone levels (T3, T4) below the normal range and elevated TSH indicated hypothyroidism.

Lipid estimations (TG, TC, HDL, and LDL) were performed using an automated analyser (Fully automatic Erba EM 360, clinical chemistry analyser) with an enzymatic (Erba) kit method. LDL values were determined using Friedewald's formula: LDL=Total Cholesterol–{HDL+(Triglyceride/5)}. According to the National Cholesterol Education Program Adult Treatment Panel 3 Guidelines for serum lipid, TC value ≥200 mg/dL indicated high cholesterol, LDL level ≥130 mg/dL indicated high LDL, TG value ≥150 mg/dL indicated hypertriglyceridemia, and HDL value <40 mg/dL indicated low HDL [19].

TPO antibody was analysed using the chemiluminescent-based ADVIA Centaur® anti-TPO assay, with a normal value of <60.00 U/mL. Participants with a value of ≥60.00 U/mL were considered positive for the anti-TPO antibody. Finally, the prevalence of TPO antibodies and the association between TPO antibodies and the lipid profile were calculated in the 103 patients.

STATISTICAL ANALYSIS

Data entry was performed using Microsoft Excel 2021. Descriptive statistics and frequency statistics were used to present the data for categorical variables. A two-sample independent t-test was conducted to compare mean values between the positive and negative anti-TPO groups. Categorical variables were presented as Mean±SD. The data were analysed using the Statistical Package for the Social Sciences (SPSS) software package, version 26.0. To determine the significance in categorical data, a Chi-square test was performed. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 103 individuals, including 83 (80.6%) females and 20 (19.4%) males, were examined to detect anti-TPO antibodies and assess serum lipid profiles. In this study, 14 males and 65 females were found to be positive for anti-TPO antibodies. The individuals were divided into three age groups: 18 to 35, 36 to 45, and 46 to 60 years. Among the 24 patients who tested negative for anti-TPO antibodies, 12 (50%) were in the 18-35 age group, 9 (37.5%) were in the 36-45 age group, and 3 (12.5%) were in the 46-60 age group. On the other hand, among the 79 individuals who tested positive for anti-TPO antibodies, 48 (60.7%) were in the 18-35 age group, 21 (26.6%) were in the 36-45 age group, and 10 (12.7%) were in the 46-60 age group. The highest number of patients was observed in the 18-35 age group according to percentage distribution [Table/Fig-1].

Parameters	Positive Anti-TPO Antibody (n=79) anti-TPO ≥60 U/mL n (%)	Negative Anti-TPO Antibody (n=24) anti-TPO ≤60 U/mL n (%)	Total n (%)
Gender			
Male	14	6	20 (19.4)
Female	65	18	83 (80.6)
Total	79 (76.7)	24 (23.3)	103 (100.0)
Age			
18-35*	48 (60.7)	12 (50.0)	60
36-45	21 (26.6)	9 (37.5)	30
46-60	10 (12.7)	3 (12.5)	13
Total	79	24	103 (100.0)

[Table/Fig-1]: Gender and age distribution among patients with positive and negative anti-TPO antibody.
Suggested that a maximum number of patients were observed in the age group of 18-35 years for Positive anti-TPO followed by 36-45 years of age

The study showed that TC level in the negative anti-TPO group was 190.84±26.83 mg/dL, whereas in the positive anti-TPO group, it was 226.88±37.44 mg/dL. The mean TG level was lower in the negative anti-TPO antibody group (151.64±31.97 mg/dL) compared to the positive anti-TPO antibody group (181.06±52.13 mg/dL). Serum LDL was lower in the negative anti-TPO antibody cases, with a mean value of 111.12±28.59 mg/dL, compared to the positive anti-TPO antibody group, with a mean value of 145.31±34.60 mg/dL. Serum HDL was higher in the negative anti-TPO antibody group, with a mean of 49.20±8.65 mg/dL, compared to the positive anti-TPO antibody group, with a mean of 44.61±7.87 mg/dL [Table/Fig-2].

Variables	Anti-TPO Positive (n=79)	Anti-TPO Negative (n=24)	p-value
Age (years)	35.19±9.41	36.33±10.66	0.615
TC (mg/dL)	226.88±37.44	190.84±26.83	<0.001
TG (mg/dL)	181.06±52.13	151.64±31.97	0.01
LDL (mg/dL)	145.31±34.60	111.12±28.59	<0.001
HDL (mg/dL)	44.61±7.87	49.20±8.65	0.016

[Table/Fig-2]: Comparison of variables between anti-TPO positive and anti-TPO negative.
Anti-TPO: Anti thyroid peroxidase antibody; TC: Total cholesterol; TG: Triglycerides; LDL: Low-density lipoprotein; HDL: High-density lipoprotein. Two-Sample Independent t-test was performed from OpenEpi, Version 3

The study showed that hypercholesterolaemia was observed in 5 (20.8%) cases in the negative anti-TPO group, compared to 52 (65.8%) cases in the positive anti-TPO group (p-value <0.01). Hypertriglyceridemia was found in 12 (50%) cases in the negative anti-TPO antibody group, compared to 53 (67.1%) cases in the positive anti-TPO antibody group (p-value=0.12). Serum LDL was high in 4 (16.7%) cases of negative anti-TPO antibody, whereas 50 (63.3%) cases in the positive anti-TPO antibody group showed high LDL levels (p-value <0.01). Serum HDL was high in 2 (8.3%) cases in the negative anti-TPO antibody group, while 21 (26.6%) cases in the positive anti-TPO antibody group had low HDL levels (p-value >0.05). The study revealed that serum TC and LDL levels were highly significant (p-value <0.01), whereas serum TG and HDL levels were statistically insignificant (p-value >0.05) [Table/Fig-3].

DISCUSSION

The current study aims to evaluate the association between anti-thyroid antibodies and lipid profiles, revealing an association with dyslipidaemia. Previous studies had emphasised the role of thyroid hormone or TSH in lipid metabolism. Hypothyroidism is often linked to elevated levels of serum TC, TG, and LDL, as thyroid hormone

Lipid profile (mg/dL)	Value	TPO positive (n=79) n (%)	TPO negative (n=24) n (%)	p-value
TC	<200	27 (34.2)	19 (79.2)	<0.01**
	≥200	52 (65.8)	5 (20.8)	
TG	<150	26 (32.9)	12 (50.0)	0.129
	≥150	53 (67.1)	12 (50.0)	
LDL	<130	29 (36.7)	20 (83.3)	<0.01**
	≥130	50 (63.3)	4 (16.7)	
HDL	<40	21 (26.6)	2 (8.3)	0.60
	≥40	58 (73.4)	22 (91.7)	

[Table/Fig-3]: Lipid profile in Anti-TPO positive and Anti-TPO negative cases.
Chi-square test applied to see the association between the Lipid parameters in two groups.
TC: Total cholesterol; TG: Triglyceride; LDL: Low-density lipoprotein; HDL: High-density lipoprotein
**A p-value <0.05 is statistically significant

influences lipid metabolism. Similar findings have been observed in previous studies, which have shown elevated TC and LDL cholesterol levels, along with normal or elevated HDL levels, in individuals with hypothyroidism [20]. Similar observations have been reported in other studies as well [21,22]. However, few researchers have emphasised the direct or indirect involvement of TPO antibodies in lipid metabolism in hypothyroidism. Cengiz H et al., demonstrated a positive correlation between anti-TPO antibody and TC, TG, and LDL cholesterol levels [15]. Kumar M et al., reached a similar conclusion, reporting an increased incidence of dyslipidaemia in patients with elevated anti-TPO antibody levels [23]. Present study aimed to determine the prevalence of serum anti-TPO antibodies and their association with lipid profiles in patients with hypothyroidism.

Autoimmune hypothyroidism is characterised by a decrease in thyroid hormones (T3, T4) and an increase in TSH [24,25]. Antibodies against TPO, Thyroglobulin (Tg), and TSH receptors (TSHR) are commonly found in patients with autoimmune thyroid diseases [7]. In present study, authors had focused only on TPO antibodies for the association. This study indicates the presence of TPO antibodies in nearly three-fourths of hypothyroid patients. Similar to present study findings, other investigations also suggest the prevalence of serum anti-TPO antibodies as a cause of hypothyroidism due to autoimmunity [20,26].

Present study found that women were more likely to have thyroid dysfunction compared to men, especially among those under 35 years of age. This finding was consistent with studies from around the world. In present study, there was a female predominance, with 80.6% of the total study population being women, while only 19.4% were men. This suggests that hypothyroidism is more common in females than males. Similar observations have been reported in a study by Srivastava VK and Singh H where females constituted 86% of the total study population [27]. Other study has also reported a higher proportion of female patients compared to males [28]. According to Shreshta PS et al., out of a total of 205 patients positive for anti-TPO antibodies, 83.4% were women and 16.6% were men. They also concluded that women are more prone to developing anti-TPO antibodies compared to men [28]. Similar observations have been reported in other studies as well [29,30].

In India, the prevalence of anti-TPO positivity appears to be steadily increasing. Research conducted in the Delhi-NCR region between 2007 and 2010 found that 13.3% of adults tested positive for TPO antibodies. By 2013, this percentage had risen to 22.01% [31,32]. In present study, prevalence of 76.7% was observed for TPO antibodies as a major marker of autoimmune thyroid disease in hypothyroidism. Mohanty S et al., proclaimed that 74% of study subjects had elevated anti-TPO levels [33]. However, in a study by Pramodh K, the prevalence was reported to be 56.1% [34]. It is interesting to note that the total number of females with TPO antibodies was significantly higher (82.3%) compared to males (17.7%) in present study. The prevalence of positive serum anti-TPO antibodies was highest in the age group of 18 to 35 years (60.7%), followed by the 36 to 45 years age group (26.6%), while it was much lower (12.7%) in the 46 to 60 years age group. Other findings also suggest a higher occurrence in the age group of 21-40 years in both genders, which includes the aforementioned 18-35 years age group [25,35,36].

In a study on women with thyroid dysfunction, Jaseem T et al., discovered that anti-TPO antibodies were significantly associated with hyperlipidaemia [37]. Similar findings were observed by Chen Y et al., who found a positive association between thyroid autoimmunity, hyperlipidaemia, and metabolic syndrome, especially in women [38]. In present study, the association between lipid profiles and anti-TPO antibodies was examined and found that positive anti-TPO antibodies was examined were significantly associated with elevated serum cholesterol and LDL levels (p-value <0.05). TPO-positive cases showed a predominance of TC and LDL, along with significantly lower levels of HDL. These findings are supported by another study by Topaloglu O et al., which reported a positive correlation between TPO antibody levels and TC and LDL cholesterol levels [39].

In the present study, no significant rise in TG levels with the increase in anti-TPO positive cases was observed. However, in a study by Surendranath SP et al., TG was significantly associated with anti-TPO antibodies in the group with thyroid autoantibodies [18]. Similarly, non significant variations in HDL and TG levels was found, which was consistent with the findings of Pramodh K [34]. Topaloglu O et al., also reported a negative correlation between TPO antibody levels and HDL cholesterol levels in euthyroid premenopausal women [39]. Based on these results, it is possible to suggest a relationship between anti-TPO antibodies and lipid levels. Present study indicates that TPO antibodies may indirectly participate in lipid metabolism and trigger hyperlipidaemia in hypothyroid patients. Thyroid autoimmunity can influence the lipid profile even in the absence of thyroid dysfunction [40-42]. This could be explained by increased inflammation, which may cause endothelial dysfunction and promote atherosclerosis [43,44]. The chronic inflammation may be due to increased levels of Interferon gamma (IFN-γ) and Tumour Necrosis Factor alpha (TNF-α), which could in turn lead to obesity and hyperlipidaemia even without elevated TSH levels [45]. However, further investigation is necessary to fully understand the mechanisms underlying this association and develop targeted treatments for patients with elevated anti-TPO antibodies.

Limitation(s)

In present study, only tests for TPO antibodies was conducted and did not utilise any other antibodies for the association. The main limitation of this study was the small sample size. Additionally, patient groups with overt and subclinical hypothyroidism was not compared.

CONCLUSION(S)

The prevalence of anti-TPO antibodies in hypothyroid patients was found to be 76.7%, with a higher proportion of females. There was a significant association between positive anti-TPO antibodies and TC and LDL levels. Present study clearly suggests that anti-TPO antibodies are one of the leading causes that play a significant role in lipid metabolism in individuals with hypothyroidism, possibly due to increased immune responses. However, in future studies, it would be beneficial to also test for TG and TSHR antibodies for more accurate results. Identifying patients with autoimmune hypothyroidism who are at high risk for developing dyslipidaemia or hyperlipidaemia can help healthcare providers implement early intervention strategies to prevent cardiovascular disease and improve patient outcomes.

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- Was informed consent obtained from the subjects involved in the study? Yes
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