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Original Research Article

A Study of Association of Serum Anti-TPO and TNF-Alpha in Hypothyroid Subjects

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Abstract

Background: The thyroid is a ductless endocrine gland that performs crucial functions in the body's metabolism as well as maturation and growth. Thyroid hormones control the metabolism of cells, which is the rate of their activity. Thyroid hormones regulate the rate of oxygen consumption. Although thyroid hormones have a similar effect and affect the proper functioning of all body cells, their effects are particularly noticeable in certain tissues and on certain functions.. Hypothyroidism is brought on by a decreased thyroid hormone production. Thyroperoxidase also known as thyroid peroxidase (TPO) or iodide peroxidase, catalyses the oxidation of iodide to produce iodine atoms, which are then added to tyrosine residues on thyroglobulin to produce thyroxine or triiodothyronine, or thyroid hormones. Tumor necrosis factor alpha (TNF-α) acts as a factor that causes the necrosis of tumors, but it has additional important functions as a pathological component of autoimmune diseases.

Method: The present study was conducted in the Department of Biochemistry in collaboration with the Department of Medicine in Rohilkhand Medical College and Hospital, Bareilly among 150 adults between the age group 20-55 years attending the hospital selected by a systematic random sampling method. Laboratory and anthropometric parameters were evaluated using standard protocols. Informed written consent was obtained from the subjects and the ethical approval of the institution was obtained before the start of the study. Taking all aseptic precautions, 5 ml of blood was drawn by venipuncture from a peripheral vein, with a disposable syringe. Samples for thyroid profile (TSH, FT3, FT4), TNF-alpha & Anti- TPO were collected in a plain vial. The blood thus collected in clean dry glass tubes was allowed to stand for 30 minutes at room temperature for the retraction of clots. This was then centrifuged at 3000 r.p.m. to separate the serum for 10 minutes. The serum was stored at 4-8℃ in the refrigerator for further analysis. To avoid haemolysis of samples appropriate care was taken. Estimation of levels TSH, FT3, FT4, TNF-alpha & Anti- TPO of both groups was carried out and the data was analysed using SPSS version 23. Descriptive data were given as mean ± standard deviation (SD). Descriptive data were

given as mean ± standard deviation (SD). Comparisons between controls and patient's groups were performed using independent t-test and p values < 0.05 were considered statistically significant. Pearson correlation coefficients (r) were calculated to quantify the relationship between TSH and other variables.

Result: ANTI-TPO & TNF-α both have statistically significant association with hypothyroid disorder as p-value is 0.001. We have also observed positive correlation of anti-TPO antibody and negative correlation of TNF-alpha with TSH in hypothyroidism.

Conclusion: Out of all the investigations we have concluded the estimation of anti-TPO and TNF-alpha will helpful inflammatory marker to diagnose hypothyroidism and thyroid dysfunctions.

Keywords: Hypothyroidism, TNF-alpha, Anti-TPO.

Introduction

The thyroid is situated in the neck, between the C5 and T1 vertebrae, directly beneath the larynx, where it folds all around anterior trachea[1]. Adult thyroids typically weigh 20–30 g, measure 5 centimetres in height and breadth, and are somewhat heavier in women [1]. The thyroid gland exerts a major control on the physiology of the whole body, and it raises fundamental questions about molecular physiological processes. In addition, increased proliferation of thyroid cells is associated with several pathologies and may involve different mechanisms. Thyroid hormones, which are secreted by the thyroid gland, regulate the body's metabolic rate (metabolic rate). Thyroid hormones affect metabolic rate by boosting the production of proteins in nearly every bodily tissue and by increasing the quantity of oxygen that cells take in [2].

Thyroid hormones, also known as thyroxine (T4) and triiodothyronine (T3), are crucial for basal metabolism and the operation of practically all human tissues and systems. A sensitive negative feedback loop, in which thyroid stimulating hormone (TSH) helps to stimulate the formulation and release of thyroid hormones, which in turn adversely give feedback to the anterior pituitary and hypothalamus to limit further TSH release, typically keeps thyroid stimulating hormone (TSH) release in addition to T4 and T3 within relatively narrow limits [3].

Reduced thyroid hormone release is what causes hypothyroidism. In children, it causes cretinism, while in adults, it causes myxoedema. There are two types of hypothyroidism: primary and secondary. Primary hypothyroidism is caused by an intrinsic thyroid gland abnormality, whereas secondary hypothyroidism is caused by a pituitary or hypothalamus problem [4].

Hypothyroidism is a common condition worldwide with a severe impact on health. It is a lifelong chronic condition whereby the thyroid hormone is produced in insufficient amounts causing multiple symptoms, such as slow movements, slow metabolism, cold intolerance, fatigue, hoarseness, weight gain, and constipation. There could also be defects in cognitive function, such as decreased attention and poor memory. Hypothyroidism is also linked to a wide range of psychiatric disorders, especially depression and paranoid disorders. Hypothyroidism is more prevalent in women than in men, and the disease is strongly associated with increasing age [5].

In the United States, data from the National Health and Nutrition Examination Survey indicate that the prevalence of hypothyroidism (both overt and subclinical) is 4.6% [6].

The global burden of hypothyroidism is significant. In the developed world, hypothyroidism prevalence is approximately 4-5% and that of subclinical hypothyroidism is approximately 4-15% [6,7].

In India, hypothyroidism is common. Iodine deficiency is the commonest cause of goitre and hypothyroidism in India. Since India adopted the universal salt iodination programme in 1983, a decline in goitre prevalence has been observed in several parts of the country, which were previously endemic [8,9].

Hypothyroidism may result from thyroiditis, an inflammation of the thyroid. Thyroid hormone may seep into the bloodstream as a result of this inflammation. Neurotransmitters (your joyful mood and focus hormones) can be affected by inflammation, and healthy levels of neurotransmitters are required to stimulate TSH. This is probably one of the reasons thyroid people experience depression more frequently.

Autoimmune hypothyroidism is caused by thyroid-specific antibodies against thyroid peroxidase (anti-TPO), The enzyme involved in thyroid hormone synthesis, and against thyroglobulin (anti-Tg), the backbone against which thyroid hormones are synthesised and stored. The interaction of these antibodies with TPO and Tg causes a lymphocyte response and subsequent thyroid fibrosis and a decrease in thyroid hormone production [10].

Our thyroid may become dysfunctional as a result of inflammation, which can negatively impact TSH secretion. Thyroid function will undoubtedly suffer if TSH levels are abnormal since the brain releases TSH, which instructs the thyroid to function. Iodine is among the elements that often come up in connection with the thyroid [11].

A catalyst that is frequently discovered in the thyroid organ is called thyroid peroxidase (TPO). TPO plays a crucial role in the production of thyroid chemicals. Autoantibodies against TPO in the blood can be identified using the hostile to TPO test. The presence of antibodies against thyroid peroxidase (anti-TPO) and thyroglobulin (anti-TG) in the serum is directly correlated with the recognition and diagnosis of immune system thyroid disorders (AITDs) [12].

Thyroid peroxidase is a weakly glycosylated, membrane-bound enzyme responsible for iodine (I_2) oxidation and iodination of tyrosyl residues in Tg molecules. Thyroid anti-peroxidase antibodies (TPO-Abs) potentiate pro-inflammatory cytokine responses. Anti-TPO antibodies are derivatives of oxidative stress, characterized by reduced antioxidant capacity, extensive glycosylation, and oxygen metabolites in the blood [13].

Anti-TPO antibodies are more prevalent and more suggestive of thyroid dysfunction. Reduced antioxidant potential, advanced glycosylation products, and oxygen metabolites in blood are signs of oxidative stress caused by anti-TPO antibodies [10].

In hypothyroidism, anti TPO antibodies are particularly useful in determining when to start treatment and how long it should last. A higher incidence of overt hypothyroidism in people with subclinical hypothyroidism is linked to the existence of anti-TPO antibodies [14].

Anti-thyroid antibodies, immune cells, and cytokines may all play a part in the aetiology of autoimmune thyroid disease. The identification of immune cells and anti-thyroid antibodies within the thyroid gland, as well as the measurement of cytokine levels in peripheral blood, provide evidence of their potential roles in the onset of autoimmune thyroid diseases [15].

It was firmly established that antagonist of TPO is an important demonstration tool regarding immune response thyroid issues, conventional thyroid disease, evident situations, and evaluation of medication progress and clinical consequences. A believable indicator of pathogenetic thinking is anti-TPO. Fundamentally, many studies suggested that high attacker of Tg levels and enemy of TPO linkages were related [16]. Low amounts of auto-antibodies, including anti-thyroid peroxidase antibodies (TPO-Ab), which are known to contribute to autoimmune thyroid disease, have been found in the periphery blood of healthy people [17].

A cytokine known as tumour necrosis factor alpha (TNF-) was first referred to as a circulating mechanism cytokine in 1975. At initially, it was believed that TNF-α production was exclusive to immune cells like lymphocytes and activated macrophages. Further research, however, revealed that epithelial and endothelial cells also produce it [18].

TNF-α is a pleiotropic inflammatory cytokine produced and secreted mainly by monocytic cells, lymphocytes and other cell types such as thyroid epithelial cells and fibroblasts within the thyroid gland [19].

They function at various stages of B-cell development and, with the right stimulus, can be synthesised by B cells themselves. Due to a nucleotide's polymorphism in the cytokine's boosting region, TNF- α and IL-6, which were actively elevated during the acute stage of critical illness, reduced the transcription of mRNA required for the synthesis of numerous proteins in the hepatocyte [20].

The cytokine tumour necrosis factor-alpha (TNF- α) has a wide range of immunological and metabolic functions. TNF-alpha and its receptor have been linked to the cytotoxic processes that define thyroid loss in autoimmune thyroid disease. TNF-alpha receptors have been found in thyroid follicular cells. Serum TNF-alpha levels have been found to be higher in Graves' disease patients, and injection of TNF-alpha to individuals has been demonstrated to cause hormonal changes like those seen in the nonthyroidal sickness syndrome [21].

Various studies showed that anti-TPO values are high in hypothyroid individuals in comparison to euthyroid individuals. Similarly, some studies reported a high occurrence of TNF-α in hypothyroidism. But contradictory results were seen in some studies. However, there is currently no consensus because of unresolved issues about an effective and sensitive prediction tool. Therefore, the present study was undertaken to access the association of serum anti-TPO and TNF-α on hypothyroid individuals and correlate them with levels of TSH. Numerous studies have indicated the futility of this approach as a significant number of affected women with thyroid dysfunction will fail to be identified.

Method and Materials

This cross-sectional study was conducted in Department of Biochemistry in collaboration with Department of Medicine in Rohilkhand Medical College and Hospital, Bareilly among 150 adults between the age group 20-55 years attending the hospital selected by a systematic random sampling method.

A standard protocol was used to assess laboratory and anthropometric parameters. Written informed consent was obtained from the subjects and institutional ethical approval was obtained before starting the study. Taking all aseptic precautions, 5 ml of blood was drawn by venipuncture from a peripheral vein, with a disposable syringe. Samples for thyroid profile (TSH, FT3, FT4) were collected in a plain vial. The blood thus collected in clean dry glass tubes was allowed to stand for 30 minutes at room temperature

for the retraction of clot. This was then centrifuged at 3000 r.p.m. for 10 minutes to separate the serum. The serum was stored at 4-8℃ in the refrigerator for further analysis. Care was taken to avoid homolysis of samples. All the obtained serum samples were used for the estimation of FT3, FT4, TSH, Anti- TPO & TNF-alpha by ELISA (Enzyme Linked Immunosorbent Assay) with standard protocol by ERBA Lisa Scan EM - TRANSASIA BIO-MEDICALS LIMITED) (Sr. No-116409)

Inclusion criteria:

Clinically and biochemically analysed hypothyroid and hyperthyroid patients of the two genders old enough 20-55 years, without any set of experiences of thyroxine and any antithyroid medications who visited over the most recent 3 months from both the In-patient and the Out-patient branch of Medicine and Biochemistry, at Rohilkhand Medical College and Hospital, Bareilly, were included in the study.

Exclusion criteria:

1. Pregnant women
2. Alcoholism - The Dietary Guidelines for Americans suggests that "moderate drinking is viewed as polishing off "up to 1 beverage each day for ladies and up to 2 beverages each day for men" for grown-ups of legitimate drinking age [22].
3. Current Smokers - As per National Survey on Drug Use and Health (NSDUH), a national wellbeing overview supported by the Substance Abuse and Mental Health Services Administration (SAMHSA), on October 19, 2011, current smokers are characterized as somebody who has smoked in excess of 100 cigarettes during their life and has smoked over the most recent 28 days [23].
4. Any individual having history of Diabetes mellitus, Coronary artery disease, Rheumatoid arthritis, Metabolic disorders, other systemic disease (liver disease, renal disease etc.), Post thyroidectomy patients and individuals on heavy drugs, steroids and individuals suffering from cancer-related disorders were excluded.

Ethical approval: Institutional ethical clearance from the Institutional Ethical Committee (IEC) of Rohilkhand Medical College & Hospital was taken prior to the study (BIU/REG/PhD/411—02/07/2022).

Informed consent: Informed consent was obtained from all the participants.

Statistical analysis: In order to examine the relationship between thyroid parameters and anthropometric indices, Statistical analysis was conducted using SPSS statistics 23.0 software, ANOVA, t-test and post-hoc test along with Pearson's correlation with $P \leq 0.05$ as the level of significance. The chi-squared test was used to test and describe the relationship between two categorized variables. Binomial logistic regression was used to test the predictors of binary outcome variables.

Result In our study, out of 150 individuals, 50 were hypothyroid subjects, 50 were hyperthyroid subjects and 50 were euthyroid individuals. It was observed that in our study, among hyperthyroid patients, the maximum study participants 34.0% belonged to 20-30 years and minimum 18.0% belonged to 41-50 years. Among euthyroid patients; the maximum study participants 42.0% belonged to 20-30 years and minimum 14.0% belonged to 41-65 years. Among hypothyroid patients, the maximum study participants 38.0% belonged to 31-40 years and minimum 10.0% belonged to 51-65 years.

There were more females than males in the present study. Among 50 hyperthyroid patients, there were 24.0% males and 76.0% females. Among 50 hypothyroid patients, there were 26.0% males and 74.0% females.

There was a statistically significant association of Anti-TPO was seen in hypothyroid individuals as p value < 0.001 when compared to euthyroid individuals. The mean Anti-TPO value of hypothyroid individuals was 11.3 ± 5.1 and euthyroid individual Anti-TPO value was 0.46 ± 0.33. Among hypothyroid individuals, 98% individuals were having Anti-TPO values > 1.99 which was maximum and only 2% were having Anti-TPO value between 0.01 – 0.228 which was minimum.

There was also statistically significant association of TNF-α was seen in hypothyroidism as p value < 0.001 when compared to euthyroid individuals. The mean TNF-α level of hypothyroid individuals was 23.3 ± 10.5 and euthyroid individual TNF- α level was 10.31 ± 3.73. Among hypothyroid individuals, 80% individuals were having TNF-α values >15.6 which was maximum and 20% were having TNF-α value <15.6 which was minimum.

Table 1: Distribution of study participants according to age.

AGE	Study Parameters					
	Hyperthyroid		Euthyroid		Hypothyroid	
	Count	%	Count	%	Count	%
20-30	17	34.0%	21	42.0%	15	30.0%
31-40	13	26.0%	15	30.0%	19	38.0%
41-50	9	18.0%	7	14.0%	11	22.0%
51-65	11	22.0%	7	14.0%	5	10.0%
Total	50	100.0%	50	100.0%	50	100.0%

Age had no statistically significant association with hypothyroid and when compared with euthyroid subjects.

Among hypothyroid patients, the maximum study participants 38.0% belonged to 31-40 years and minimum 10.0% belonged to 51-65 years. Among euthyroid patients; the maximum study participants 42.0% belonged to 20-30 years and minimum 14.0% belonged to 41-65 years.

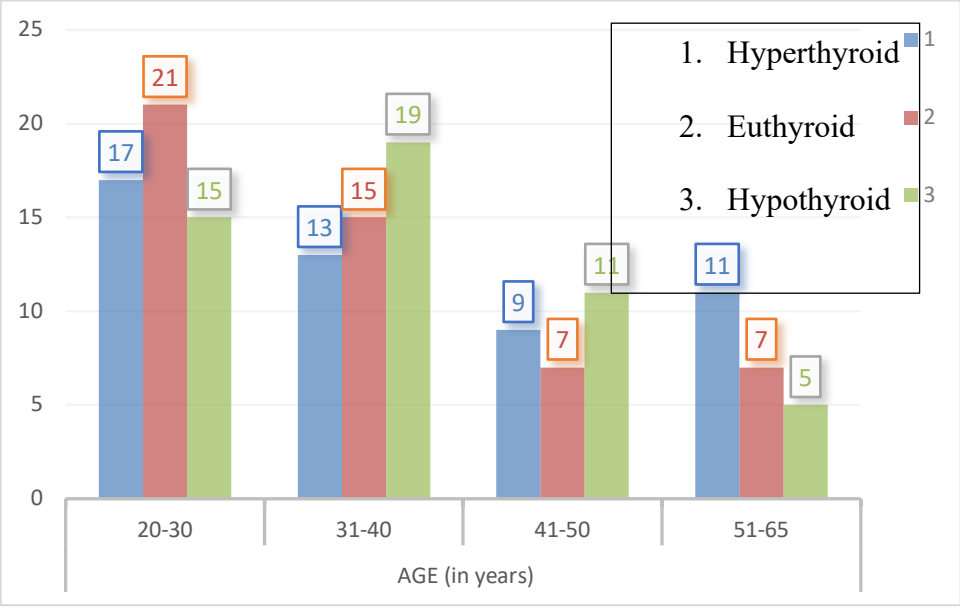


Figure 1: Distribution of study participants according to age.

Table 2: Distribution of study participants according to gender.

SEX	Study Parameters					
	Hyperthyroid		Euthyroid		Hypothyroid	
	Count	%	Count	%	Count	%
Male	12	24.0%	13	26.0%	13	26.0%
Female	38	76.0%	37	74.0%	37	74.0%
Total	50	100.0%	50	100.0%	50	100.0%

Among 50 hyperthyroid patients, there were 24.0% males and 76.0% females. Among euthyroid patients, there were 26.0% males and 74.0% females. Among hypothyroid patients, there were 26.0% males and 74.0% females. There were more females than males in the present study.

Table 3: Distribution of study participants according to ANTI TPO among Hypothyroid and Euthyroid.

Anti-TPO (0.0 – 0.228) ng/ml	Hypothyroidism		Euthyroidism	
	No Of Patients	%	No Of Patients	%
0.01 – 0.228	1	2 %	17	34 %
0.229 – 1.99	0	0 %	33	66 %
> 1.99	49	98 %	0	0 %
p-value	0.000			

ANTI TPO had statistically significant association with hypothyroid disorder as P value < 0.001 when compared with euthyroid subjects.

Among hypothyroid individuals, 98% individuals were having Anti-TPO values > 1.99 which was maximum and only 2% were having Anti-TPO value between 0.01 – 0.228 which was minimum.

Table 4: Distribution of study participants according to TNF-α among Hypothyroid and Euthyroid.

TNF-α (<15.6 pg/ml)	Hypothyroidism		Euthyroidism	
	No Of Patients	%	No Of Patients	%
< 15.6	10	20 %	44	88 %
> 15.6	40	80 %	6	12 %
p-value	0.000			

TNF-α has statistically significant association with hypothyroid as p-value is < 0.001 when compared with euthyroid subjects.

Among hypothyroid individuals, 80% individuals were having TNF-α values >15.6 which was maximum and 20% were having TNF-α value <15.6 which was minimum.

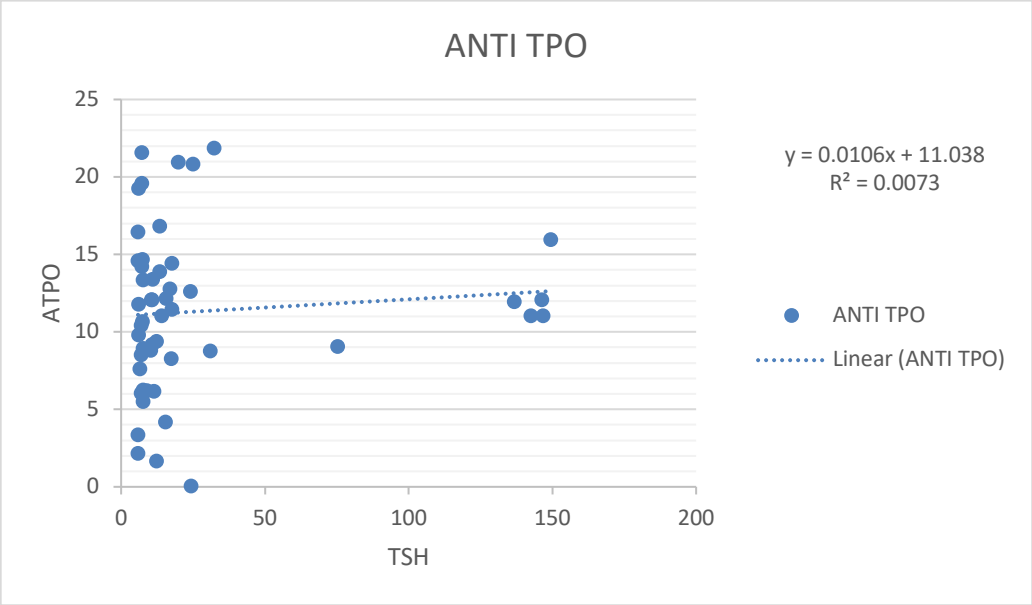
Table 5: Comparison of Biochemical markers in different groups.

Biochemical Markers	Hypothyroidism	Euthyroidism
	Mean ± SD	Mean ± SD
FT3 (pg/ml)	0.9 ± 1.2	4.32 ± 1.10
FT4 (ng/ml)	2.8 ± 0.4	1.76 ± 1.46
TSH (µIU/l)	26.7 ± 40.8	2.57 ± 1.01
ANTI-TPO (ng/ml)	11.3 ± 5.1**	0.46 ± 0.33**
TNF-α (pg/ml)	23.3 ± 10.5**	10.31 ± 3.73**

** = Statistically significant

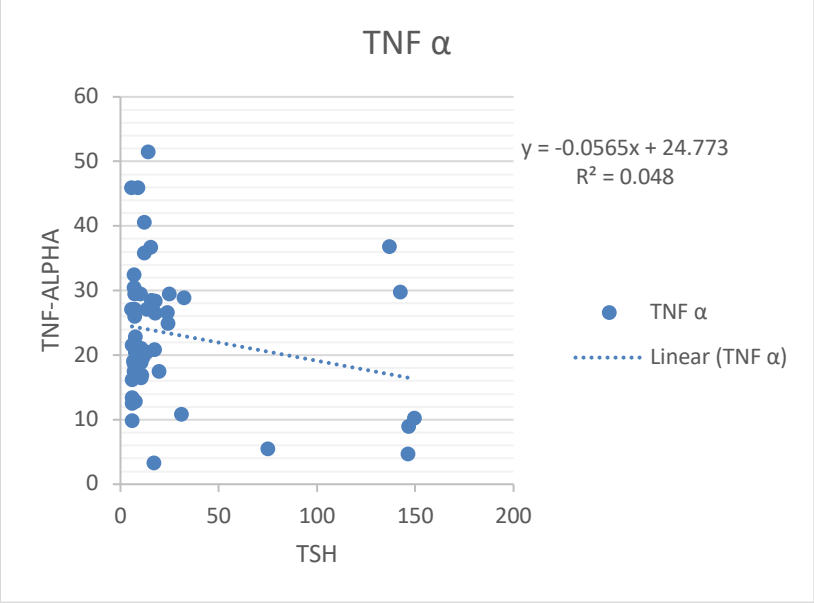
The above table 5 is showing the biochemical marker's comparison of studied subjects in different groups. The mean Anti-TPO value of hypothyroid individuals was 11.3 ± 5.1 and euthyroid individuals mean Anti-TPO value was 0.46 ± 0.33 which was statically significant when compared with euthyroid subjects.

The mean TNF-α level of hypothyroid individuals was 23.3 ± 10.5 and euthyroid individuals the mean TNF- α level was 10.31 ± 3.73 which was also statically significant when compared with euthyroid subjects.



Graph 1: Correlation of TSH to anti-TPO in hypothyroidism.

Chart 1 is showing the positive correlation of TSH with anti TPO in hypothyroidism.



Graph 2: Correlation of TSH to TNF-alpha in hypothyroidism.

Chart 2 is showing the negative correlation of TSH with TNF-alpha in hypothyroidism.

Discussion

The thyroid hormone is widely known for regulating several body processes, including growth and metabolism. A self-regulating circuit known as the hypothalamic-pituitary-thyroid axis is made up of the thyroid gland, anterior pituitary gland, and hypothalamus. Iodine deficiency to iodine abundance in India has been fully achieved. By rendering the thyroid gland more vulnerable to injury from the body's own immune system, iodine supplementation is thought to be a contributing factor to an increase in autoimmune thyroid dysfunction and hypothyroidism. Thyroid hormones circulate coupled to the transport proteins and are lipophilic. Only a little portion of the thyroid hormone (free T4)—about 0.2%—is unbound and functional.

In our study, Anti-TPO & TNF-α were statistically significant and found to be high in hypothyroid individuals which is statically significant when compared with euthyroid subjects

In our study, the mean of serum anti TPO was 11.3 ± 5.1 in hypothyroid group, and 0.46 ± 0.33 in euthyroid group. The mean TNF-α level of hypothyroid individuals was 23.3 ± 10.5 and euthyroid individuals the mean TNF- α level was 10.31 ± 3.73. Shimizu *et al*. (2020) were found FT3 level of euthyroidism was 3.2±0.3, FT4 level was 1.2 ±0.2 and serum TSH level was 1.49 μIU/l with ANTI- TPO value was 9.0 ng/ml in euthyroidism, which was statistically significant (p-value 0.015).[24] According to his study, one of the known causes of an autoimmune thyroid condition that leads to hypothyroidism is TPO-Ab. Levels of TPO-Ab titre may be positively correlated with atherosclerosis by showing inadequate thyroid function since hypothyroidism has been linked to the advancement of atherosclerosis. They discovered more proof that, among the general euthyroid population with a normal TSH range, the TPO-Ab titre is positively related with atherosclerosis even with a normal range of TPO-Ab titre (TPO-Ab negative). This data suggests that aberrant thyroid function is not the reason of the atherosclerosis risk for higher amounts of TPO-Ab within the normal range [24].

According to research of Manish Jantikar A (2020), the majority of the study's participants had hypothyroid dysfunction in terms of thyroid malfunction. Compared to male patients, more than 50% of female patients had hypothyroidism. These outcomes had statistical significance. Thus, a greater frequency of individuals with anti-TPO antibody positivity had hypothyroidism as a thyroid malfunction. In both sexes, it was more prevalent in the age range of 21 to 40. Similar discussion was found in a study of Ghoraishian (2006) that, the connections between anti-TPO with T3, T4 and TSH in [24,25] people and discovered that these were considerably aberrant in the antibody positive group. In the previous investigation, around 6% of euthyroid patients had anti-TPO positivity. Such participants ran the risk of developing overt and subclinical autoimmune hypothyroidism. A study found that selenium supplementation reduced TPO antibodies and improved quality of life (QoL) in hypothyroid individuals receiving T4 treatment [25]. Even though there is a significant prevalence of thyroid illness, many people also have undiagnosed thyroid problems with positive anti-TPO antibody tests and high TSH levels.

In our study we have observed the value of TNF-alpha was 23.3 ± 10.5 in hypothyroidism, 18.2 ± 10.47 in hyperthyroidism and 10.31 ± 3.73in euthyroidism. Choudhury P et. al; (2019) were found the level of TNF-alpha was 407+150.78 in hypothyroidism and 57+17.53 in euthyroidism [26,27] Gluvic et. a; (2022)

were found higher statistically significant level of TNF-alpha in Hypothyroidism and normal level in euthyroidism. In the study of Chunping Sun et. al; (2021), Interleukin-2 (IL-2) and interleukin-6 (IL-6) as well as tumour necrosis factor alpha (TNF-alpha) levels were higher assessed in thyroid dysfunction [28]. Kolesnikova O et. al; were found higher level of TNF-alpha was 8.60 ± 0.54 with TSH value was <10. 0 µIU/l and 11.93 ± 0.92 with TSH value was >10.0 µIU/L in thyroid dysfunction [29]. Godinjak A et. al; were demonstrated, hypothyroidism raises the risk of cardiovascular disease in postmenopausal women and is related with higher levels of CRP, homocysteine, and TNF-α [30]. According to the research of Ioana Roman I et. al; were found negative correlation of TNF-alpha to TSH in euthyroid group [31]. Parimal S. Tayde et. al; were found TNF-alpha level was 146.72 pg/ml to be higher in hypothyroidism and 44.12 pg/ml in euthyroidism [32].

Limitations

Considering that the study is cross-sectional in nature, it could be used as a basis to draw causal conclusions. Lack of fund, time & manpower prevented the inclusion of a large study group & other sensitive biochemical markers of hypothyroidism and hyperthyroidism.

Conclusion

Out of all the investigations we have concluded the estimation of anti TPO and TNF-alpha will helpful inflammatory marker to diagnose hypothyroidism and thyroid dysfunctions. Furthermore, it is cleared from our study that females are more prone to inflammation and thyroid dysfunction in comparison to males. This study will help the clinicians to formulate novel diagnostic and therapeutic protocol for appropriate management of their patients. Our findings indicate that the higher values of TPO-Ab along with higher values of TNF-α are caused by hypothyroidism and thyroid function abnormality. Moreover, we recommend routine screening for autoimmune thyroid disorders among patients attending clinic for regular check-up.

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