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Testing the Tormenting Trio-A Study of Thyroperoxidase(TPO) Activity, Serum Ferritin and Thyroid Diseases among Pregnant Women

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Abstract

***Background:** Limited literature has explored the connection between Hypothyroidism and Iron deficiency anaemia. Some studies have noted that Iron deficiency can reduce thyroid function by reducing Thyroid peroxidase (TPO) enzyme activity[1-4]. None of the studies have been done to evaluate TPO enzyme activity in Hypothyroidism and Iron deficiency anaemia patients except few studies done in rodents[4]. Hence, we tried to do a qualitative and quantitative analysis of TPO enzyme activity among iron deficient hypothyroid, iron deficient euthyroid, iron normal hypothyroid and iron normal euthyroid pregnant women.*

***Methods:** This was a cross-sectional study conducted among 500 pregnant women at JSS Hospital, a tertiary care hospital in Mysuru. Following strict aseptic protocols, approximately 5 mL of venous blood was collected and analysed for levels of Haemoglobin (Hb), Thyroid Stimulating Hormone (TSH), Triiodothyronine (T3), Thyroxine (T4), Anti-Thyroid Peroxidase (Anti-TPO) antibody and Serum Ferritin. Then pregnant women were divided into 4 groups-iron deficient hypothyroid, iron deficient euthyroid, normal iron hypothyroid and normal iron euthyroid pregnant women. Quantitative analysis of TPO enzyme was done by luminometric method for all pregnant women and was compared between above groups.*

***Results:** Out of 500 women, 9 were excluded because of pre analytical error, hence there were 491 pregnant women in the study. Among 491 pregnant women,156 (31.77%) were hypothyroid and 7 (1.42%) had thyrotoxicosis. The results showed that 55% of euthyroid subjects and 61% of hypothyroid subjects had normal levels of serum ferritin, while 44.7% of euthyroid subjects and 38% of hypothyroid subjects had lower levels of serum ferritin. The results did not show any correlation between the serum ferritin levels and thyroid status. Comparative analysis of TPO enzyme activity among iron sufficient and iron deficient euthyroid showed elevated TPO enzyme activity in iron deficient euthyroid subjects. However, among the iron sufficient and iron deficient hypothyroid subjects, the TPO activity was similar. Similarly, comparison between iron deficient euthyroid and hypothyroid also did not show any significant changes in the TPO enzyme activity.*

***Conclusion:** Contrary to expectations, TPO enzyme activity was not reduced in iron-deficient hypothyroid subjects. Further studies are warranted to elucidate the intricate relationship between TPO enzyme activity, serum ferritin levels, and thyroid status among pregnant women, as well as its potential impact on the overall growth and development of the foetus.*

***Key words:** Iron deficiency anaemia, hypothyroidism, ferritin, thyroid peroxidase, thyroid stimulating hormone.*

Introduction

Thyroid diseases and iron deficiency anaemia are very common diseases affecting pregnant women worldwide. Despite anaemia occuring frequently with thyroid diseases, it is often underestimated and their relationship is not well understood[1]. Various reasons including decreased erythropoietin production, vitamin B12, folate and concomitant iron deficiency due to decreased absorption from the gut can lead to anaemia among hypothyroid subjects[1].

Thyroperoxidase (TPO) is an essential enzyme responsible for catalyzing the iodination of tyrosine residues within thyroglobulin, thereby facilitating the formation of monoiodotyrosine and diiodotyrosine, which subsequently undergo coupling to produce T3 and T4, namely thyroxine or triiodothyronine[2]. The TPO gene is situated on chromosome 2p25, spanning 17 exons. Transcription of the TPO gene yields a 3kb mRNA, which translates into a 110 kDa glycosylated hemo-protein, known as the TPO enzyme[3]. Initially referred to as thyroid peroxidases (ThOX), this enzyme plays a pivotal role in thyroid hormone synthesis[3].

Iron is an important component of the TPO enzyme and plays a major role in the synthesis and metabolism of thyroid hormones[4]. Deficiency of iron is known to decrease the efficacy of TPO enzyme and affect thyroid hormone metabolism[5]. Maternal and foetal complications occur when pregnant women suffer from these diseases[6,7].

Despite the well-documented relationship between iron

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levels and TPO enzyme activity, no studies, to date, have investigated the activity of TPO in individuals with hypothyroidism and iron deficiency anaemia. Recent research, primarily conducted in rodent models, has aimed to address this gap. Therefore, the current study endeavours to assess TPO enzyme activity among pregnant women categorised into iron-deficient hypothyroid, iron-deficient euthyroid, iron-sufficient hypothyroid, and iron-sufficient euthyroid groups. To the best of our knowledge, this study represents the first attempt to report TPO activity in both euthyroid and hypothyroid human subjects.

Material and Methods

The study was a cross-sectional investigation conducted between January 2018 to June 2020 at JSS Hospital, a tertiary care teaching and research hospital attached to JSS Medical College at Mysuru, Karnataka. Inclusion criteria-pregnant women aged 18 - 45 years (n = 500) in first trimester of pregnancy were recruited into this study. Informed written consent was taken from all women. Ethics clearance was obtained from the Institutional Ethics Committee of JSS Medical College, JSS Academy of Higher Education and Research.

Exclusion criteria-pregnant women who had any past or present history of thyroid dysfunction/disease, family history of thyroid disease, previous head or neck irradiation, usage of drugs such as levothyroxine, methimazole, iodide, lithium, amiodarone and corticosteroids, patients diagnosed with autoimmune and connective tissue diseases. Detailed history and clinical examination were recorded in a clinical proforma.

Venous blood of about 5 mL was drawn under aseptic conditions and subjected for analysis of haemoglobin (Hb), Thyroid stimulating hormone (TSH), Triiodotyrosine (T3), Tetraiodotyrosine (T4), Anti-TPO antibody and Serum Ferritin level. Levels of T3, T4, TSH and AntiTPO Antibody were measured by chemiluminescence method. Serum Ferritin was estimated by Chemiluminescence Immunoassay in Roche COBAS 6,000 integrated analyser. According to American Thyroid Association and National Guidelines[8], a TSH value >2.5 mIU/L but less than or equal to 10 mIU/L with normal T4 was considered to be subclinical hypothyroidism, but, TSH value >10 mIU/L irrespective of T4 concentration, and TSH value >2.5 with low T4 concentration were considered to be overt hypothyroidism. Individuals with a serum ferritin concentration <20 µg/L were considered as iron deficient. Based on the level of serum ferritin and thyroid status (euthyroid or hypothyroid), the pregnant women were divided into 4 groups viz., iron deficient hypothyroid, iron deficient euthyroid, iron sufficient hypothyroid and iron sufficient euthyroid pregnant women.

Quantitative analysis of thyroid peroxidase enzyme: Estimation of TPO activity was carried-out by luminometric method as detailed by Barae Jomaa *et al*[9]. In brief, serum containing 0.1 to 0.2 mg/mL total protein was incubated with 1.0 M glycine-NaOH (pH9.0) and 1.0 mM EDTA for 30 min with gentle shaking at 37 ° C after which the reaction was initiated by the addition of 20 µL of luminol mix containing 1 M glycine-NaOH (pH 9.0), 1 mM EDTA and 400 µM luminol. Following a 4.0 sec delay, 5.0 µL of 80 mM H_2O_2 was added, and the luminescence was measured as relative luminescence units (RLUs) integrated over 10.0 sec using a PerkinElmer Enspire Multimode Plate Reader (luminol kit-Thermoscientific company, USA).

Statistical analysis

Data collected was entered in Microsoft Excel and analysed using GraphPad Prism version 8. Descriptive statistical measures like percentage, mean and standard deviation were calculated. Paired T-test and Two-way ANOVA (confidence interval 95) for subgroups (comparing TPO enzyme activity with thyroid levels and ferritin level > and <50 ng/mL) was used to calculate the statistical significance at 'p' value of <0.05.

Results

Out of 500 subjects in the study, 9 were excluded because of pre-analytical error, hence data of the remaining 491 pregnant women was analysed. Most of them, 403 (82%) were in age group 21 - 30 years, the majority - 363 (74.75%) belonged to the urban category, 481 (98%) were literate, 228 (46%) were primigravida and 263 (53%) were multigravida. 227 (46%) had normal BMI, 107 (21%) were overweight, 65 (12%) were obese and 92 (18%) were underweight.

The mean TSH was 2.37 ± 3.17 mIU/L, mean T3 was 1.45 ± 0.72 ng/dL and mean T4 was 9.29 ± 2.53 µg/dL among 491 pregnant women.

Among the 491 subjects, 335 had normal thyroid levels (euthyroid) while 156 subjects were considered hypothyroid based on the T3, T4 and TSH levels.

Further, 128 (29.22%) had iron deficiency, 82 (18.72%) had iron deficiency anaemia and rest had normal Hb and ferritin.

1. **Serum Ferritin levels in iron-sufficient and iron-deficient euthyroid as well as hypothyroid patients**

   Serum ferritin level of 491 pregnant women was

analysed. Serum ferritin level in euthyroid women ranged from 0.86 µg/L to 282 µg/L. Ferritin levels less than 20 µg/L were considered as iron deficient. Among the 491 subjects, 335 had normal thyroid levels (euthyroid) while 156 subjects were considered hypothyroid based on the T3, T4 and TSH levels. Among the 335 euthyroid subjects, 185 subjects had normal ferritin levels ranging from 20.8 µg/L to 282 µg/L, while 150 subjects had lower levels of ferritin which ranged from 0.86 µg/mL to 20 µg/L. Among the hypothyroid subjects (n = 156) 96 subjects had normal ferritin levels ranging from 20 µg/L to 240 µg/L while 60 subjects had lower levels of ferritin ranging from 1.69 µg/L to 19.97 µg/L. The results showed that 55% of euthyroid subjects and 61% of hypothyroid subjects had normal levels of serum ferritin, while 44.7% of euthyroid subjects and 38% of hypothyroid subjects had lower levels of serum ferritin.

2. TPO enzyme activity in normal and hypothyroid subjects with correlation to ferritin levels.

TPO enzyme activity was measured in iron sufficient euthyroid and hypothyroid as well as iron deficient euthyroid and hypothyroid women. Quantitative analysis of TPO enzyme was done in different groups. Median TPO enzyme value in iron deficiency hypothyroid group was 225, iron deficiency euthyroid group was 295, normal iron hypothyroid group was 185 and normal iron euthyroid group was 220.

Comparative analysis of TPO enzyme activity among iron sufficient and iron deficient euthyroid women showed elevated TPO activity in iron deficient euthyroid women. However, among the iron sufficient and iron deficient hypothyroid women, the TPO activity was similar. However, for further confirmation, the TPO enzyme activity was compared among euthyroid and hypothyroid

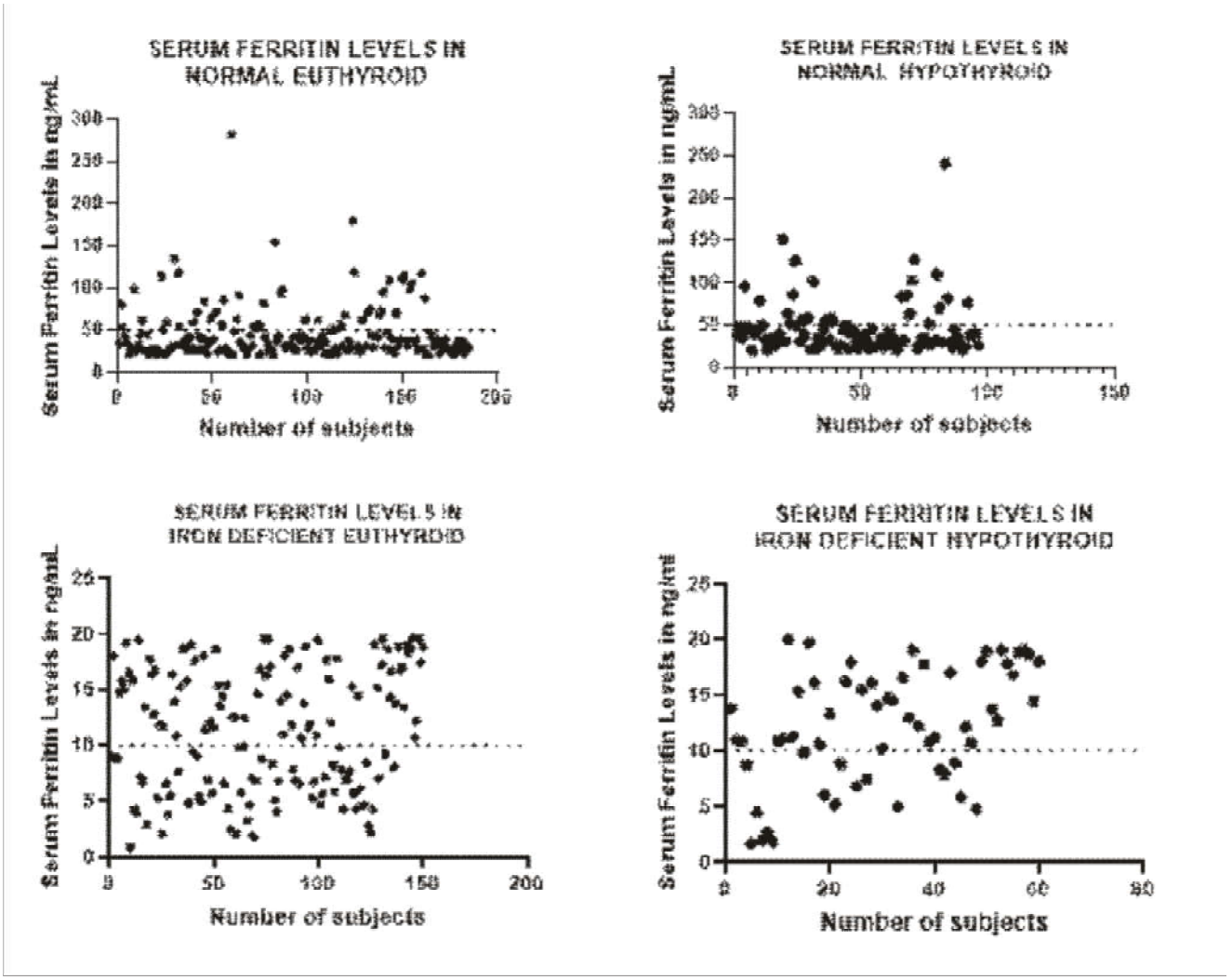

Fig. 1: *Serum ferritin levels in normal iron euthyroid and hypothyroid women as well as iron deficient euthyroid and hypothyroid women.*

women with normal levels of ferritin, which did not show significant differences. Similarly, comparison between Iron deficient euthyroid and hypothyroid women also did not show any significant changes in the TPO enzyme activity as illustrated in Fig. 3.

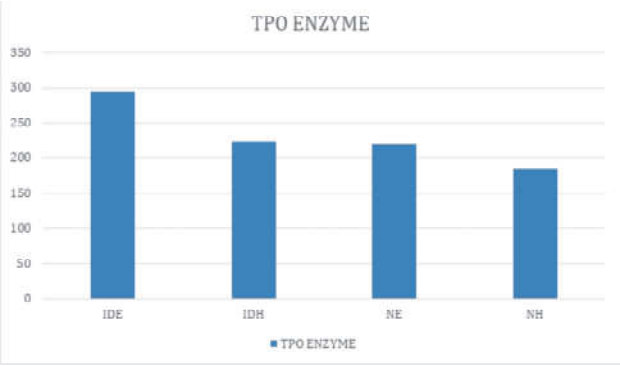

Fig. 2: *TPO Enzyme levels in normal iron euthyroid and hypothyroid women as well as iron deficient euthyroid and hypothyroid women.*

Discussion

Numerous studies have established a direct correlation between iron levels and thyroid gland function[11,12]. Iron deficiency has been shown to directly impact circulating levels of thyroid hormones T3 and T4[11,12]. A recent study published by Pooja *et al* studied the relationship between iron deficiency and thyroid hormone levels in pregnant women. The correlation and regression analysis revealed a significant negative association of TSH and a positive association of FT4 with ferritin, iron, and Hb[13]. However, the data of the current study showed varied levels of serum ferritin in euthyroid and hypothyroid subjects with no significant correlation.

Additional evidence from other studies hypothesize that iron deficiency affects thyroid hormone levels by reducing the

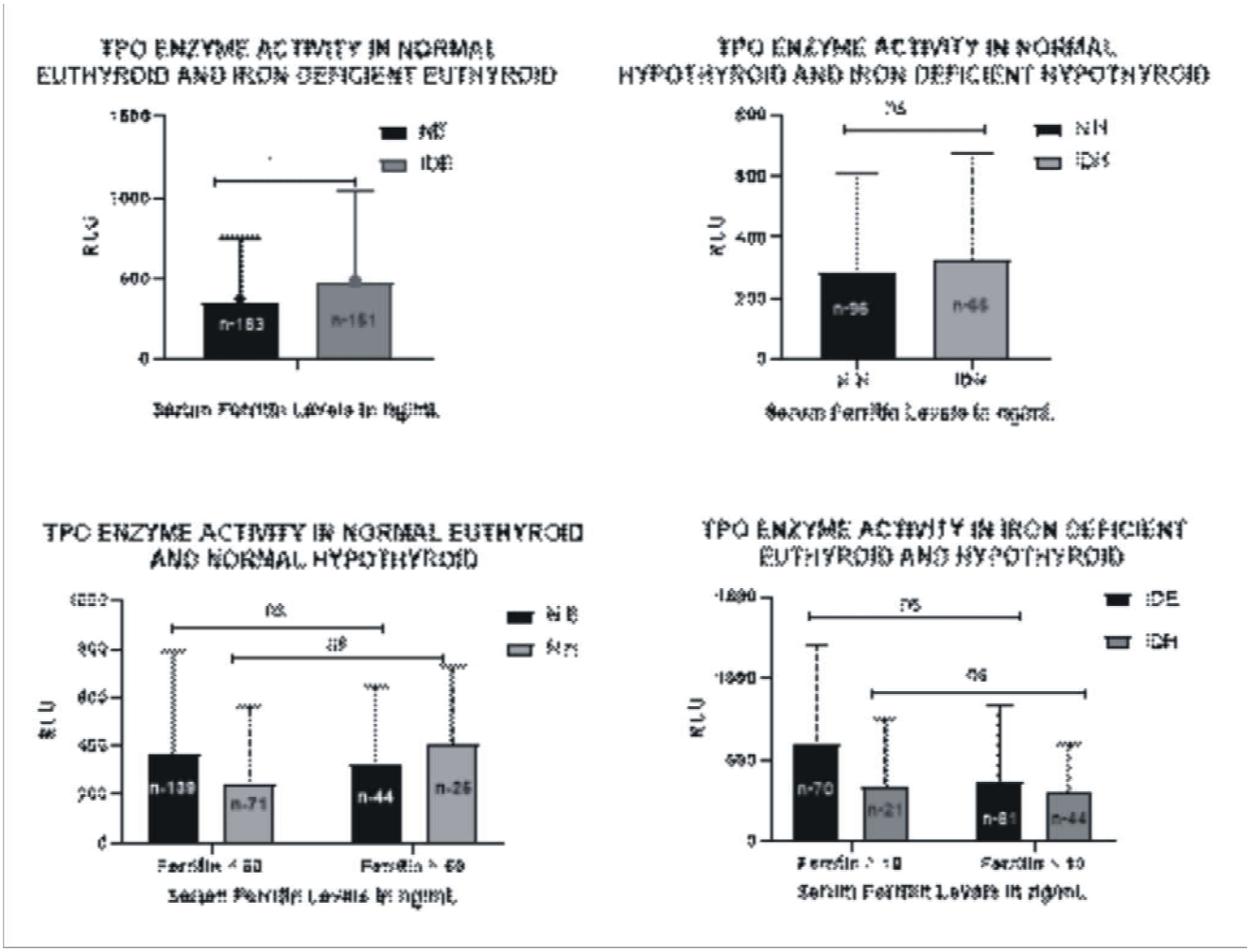

Fig. 3: *Comparative assessment of the activity of TPO in iron sufficient euthyroid and hypothyroid women, as well as iron deficient euthyroid and hypothyroid women. Paired T-test (graph 1 and 2) and Two-way ANOVA (graph 3 and 4 independent variables – ferritin levels and thyroid status is compared with dependent variable TPO enzyme activity) was used to calculate the statistical significance at 'p' value of <0.05.*

activity of the TPO enzyme[14,15,16]. Human thyroid peroxidase (TPO), encoded by the TPO gene located on chromosome 2p25, is subject to regulation by various factors[3]. The TPO mRNA, comprising 17 exons, predominates in human thyrocytes and encodes the 933-amino acid TPO-1 protein. Additionally, alternative splicing generates multiple truncated versions of TPO. TPO-1, a dimeric glycoprotein with a covalently linked heme, features a large N-terminal extracellular region (ectodomain) projecting into the follicular lumen, alongside a short transmembrane domain and intracellular C-terminal region[3].

In the present study, evaluation of TPO enzyme activity among iron sufficient and iron deficient euthyroid and hypothyroid showed significance only among iron sufficient and iron deficient euthyroid. However, no significant correlation was observed among iron sufficient and iron deficient hypothyroid subjects. A study done by Zimmerman *et al*, showed iron deficiency reduces TPO enzyme activity in rats[4]. We couldn't find human studies to compare our study. Potential reasons for these discrepant observations are (a) low sample size; (b) differences in the TPO activity estimation methods, viz., Chemiluminescence versus Fluorimetric methods, c) most women had Subclinical Hypothyroidism rather than Overt Hypothyroidism and (d) sample collection, processing and storage as the exposure of collected samples to light is known to influence the serum ferritin level. Further studies considering these potential influencing factors are needed to conclusively establish a relation between serum ferritin and thyroid status of the individuals.

Conclusion

Contrary to expectations, TPO enzyme activity was not reduced in iron-deficient hypothyroid subjects. Further studies are needed to elucidate the intricate relationship between TPO enzyme activity, serum ferritin levels, and thyroid status in pregnant women, as well as their impact on the overall growth and development of children.

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