ORIGINAL ARTICLE

Study of iron in hypothyroidism and Grave's hyperthyroidism as compared to euthyroidism in Northern Andhra Pradesh

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Abstract

Background: Iron metabolism has an intricate relationship with thyroid metabolism. The deficiency of one parameter affects the other. Iron is an important component of the thyroid peroxidase enzyme and 5'deiodinase, which are integral parts of thyroid metabolism. *Aim and Objectives:* The study evaluates the status of serum iron, ferritin, and Total Iron Binding Capacity (TIBC) in hypothyroidism and Grave's hyperthyroidism with euthyroid subjects as control. *Material and Methods:* This was a hospital-based cross-sectional study that included 100 cases of hypothyroidism and 60 cases of Grave's hyperthyroidism compared with 100 euthyroid subjects aged 15-60 years. After detailed case history and examination, the blood samples were collected and analysed the same day for free T3, free T4, Thyroid Stimulating Hormone (TSH), serum iron, ferritin, and TIBC levels. *Results:* Compared to euthyroid subjects, patients with clinical hypothyroidism had statistically significant ($p<0.05$) lower levels of iron and ferritin levels with higher levels of TIBC, while patients with Grave's hyperthyroidism had statistically significant ($p<0.05$) higher levels of ferritin and iron levels with lower levels of TIBC. *Conclusion:* Our study found a strong relationship between the iron profile and the thyroid profile. The deficiency of one parameter will affect the other.

Keywords: Iron, Ferritin, Total Iron Binding Capacity, Hypothyroidism, Grave's Hyperthyroidism

Introduction

Thyroid disease is widespread in the world. India being no exception, have a significant burden of thyroid diseases. An estimated 42 million patients with thyroid disorders are found in India [1]. The prevalence of hypothyroidism in India is 10.95%compared with only 2% in the UK and 4·6% in the USA [2]. The prevalence of hyper-thyroidism was found to be 1.6%. Thyroid disease prevalence increases with age and is more common in women [2]. The prevalence of sub-clinical hypothyroidism is higher than clinical hypothyroidism, which goes undiagnosed most of the time.

Thyroid hormones are Thyroxine (T4) also called Tetraiodothyronine and Triiodothyronine (T3) secreted by the thyroid gland. They are essential for the body's normal growth, tissue differentiation, and metabolism. Thyroid hormones stimulate the basal metabolic rate, increase glycogenolysis, gluconeogenesis, lipolysis, and enhance the synthesis of proteins [2-3].

They regulate hematopoiesis in the bone marrow and are involved in haemoglobin production in adults and fetal haemoglobin maturation [4]. Disorders of thyroid hormones are associated with abnormalities in red blood cells. Hyperthyroidism

increases erythropoiesis by the proliferation of erythroid progenitor cells. They increase erythropoietin gene expression, thereby increasing erythropoietin secretion. They also stimulate erythroid burst formation and enhance the growth in erythroid colonies like BFU-E and CFU-E [4]. Hyperthyroidism causes hyperplasia of marrow erythroid cells whereas hypothyroidism causes hypoplasia [5-6]. Hyperthyroidism also causes endothelial damage with vascular dysfunction and generalised damage [7].

Iron is essential for the normal functions of the human body. It is vital for the human body's cellular growth and differentiation, enzymatic reactions, oxygen binding, immune function, transport and storage, and cognitive functions such as mental growth. So, deficiency of iron in both physiological and pathological ways affects different functions of the body [8].

Iron is very intricately related to thyroid metabolism [9]. Many human and animal studies show that iron deficiency impairs thyroid metabolism [10]. Thyroid peroxidase is a crucial enzyme for thyroid hormone production. Iron is an essential co-factor needed for the enzyme thyroperoxidase to function effectively [11]. Iron deficiency has been shown to affect the blood levels of T3 and T4. Iron deficiency also lowers the levels of 5' deiodinase, the enzyme which is involved in the peripheral conversion of T4 to T3 [10]. Hence iron deficiency may be the critical factor responsible for hypothyroidism.

Hyperthyroidism increases ferritin production thereby increasing iron storage. Also, over utilisation of iron for various thyroid functions results in iron deficiency, and thereby iron deficiency anemia [12].

Many scientific researches were done in India to understand the association between serum iron levels and thyroid metabolism. The purpose of our study was to better understand this association by comparing the levels of iron with its associated parameters like ferritin and TIBC among hypothyroid/hyperthyroid patients and euthyroid patients.

Material and Methods

Study design

This was a hospital-based cross-sectional study conducted at Maharajah's Institute of Medical Sciences (MIMS), Nellimarla, Vizianagaram, Andhra Pradesh. The study included 100 newly diagnosed cases of hypothyroidism, and 60 newly diagnosed cases of Grave's hyperthyroidism in comparison with 100 euthyroid subjects with age 15-60 years who attended the Department of Medicine and Department of Ophthalmology of Maharajah Institute of Medical Sciences, Nellimarla during 1.5 years from 01.01.2021 to 30.06.2022. The study protocol was approved by the Institutional Ethics Committee of Maharajah's Institute of Medical Sciences, Nellimarla. Informed consent was obtained from all the study participants.

Inclusion criteria

All the cases and controls belonged to the age group of 15-60 years. Newly diagnosed and untreated cases of hypothyroidism and Grave's hyperthyroidism who attended the Outpatient Department (OPD) of Medicine and Ophthalmology, MIMS, Nellimarla were selected as cases. Healthy age and sex-matched 100 euthyroid subjects were enrolled as controls.

Exclusion criteria

Exclusion criteria included pregnant, menopause, or lactating women, other acute or chronic illness, liver or renal disorders, intake of oral contraceptive pills, thyroid malignancies, subjects on iron supplements, and subjects on any medication that could alter thyroid status or iron status in blood.

Methodology

Clinical history was taken from all the subjects who participated in the study. General examination of these patients, including weight, height, heart rate, and blood pressure measurement was done and recorded in a structured protocol format. Grave's hyperthyroid subjects were examined in detail for exophthalmos in the Department of Ophthalmology and details were recorded. After obtaining written informed consent, 5 mL of the blood sample was collected from all subjects under aseptic conditions. After adequate clotting, the serum tube was centrifuged. The serum which got separated in the test tube was aliquoted. It was used for testing thyroid profile (Free T3, Free T4, TSH) and serum levels of iron, TIBC. The aliquoted sample was stored at - 20°C.

The following instruments and methods were used for analysing the parameters.

- Free T3 - Electrochemiluminescence immunoassay (ECLIA),
- Free T4 - Electrochemiluminescence immunoassay (ECLIA)
- TSH - Electrochemiluminescence immunoassay (ECLIA)
- Serum iron: Spectrophotometric method using ferrozine reagent – ERBA EM200 analyser

Statistical methods

Data were analysed using IBM SPSS Statistics Version 21.0. Qualitative variables were expressed as frequency and percentages. Quantitative variables were expressed in Mean and Standard Deviations (SD). Analysis of Variance (ANOVA) with *post hoc* analysis was used for the comparisons between three groups and multiple comparisons. Karl-Pearson correlation coefficient was used to explore the relationship between study variables. For all statistical analyses, $p<0.05$ was considered statistically significant.

Results

Various parameters were analysed in detail. Table 1 shows that age and gender were not significantly different between hypothyroid, hyperthyroid, and euthyroid study groups. Mean±SD of FT3, FT4, and TSH in all three groups were studied. FT3 and FT4 were significantly lower in hypothyroidism ($p<0.05$) and significantly higher in grave's hyperthyroidism ($p=0.00$) in comparison with euthyroid subjects. While TSH was found to be significantly higher in hypothyroidism ($p=0.00$) and significantly lower in Grave's hyperthyroidism ($p <0.05$) in comparison with euthyroid subjects. Table 2 describes the analysis and comparison of Mean±SD of serum iron, ferritin, and TIBC in the three groups and shows a significant association ($p <0.05$). Table 3 shows the *post hoc* analysis of all the variables. Our previous findings were confirmed by ANOVA with *post hoc* analysis. All the parameters were highly statistically significant with p value of 0.00. Tables 4 and 5 show Pearson Correlation and p value within various study groups. It compared variables inside the group. The hypothyroidism group showed a significant relationship between iron with TSH ($p=0.005$) and ferritin ($p=0.000$). The relationship between

ferritin and TSH (p=0.017) was also significant. On the other hand, in the hyperthyroidism group, Pearson Correlation and p value did not show any significance. Figures 1 and 2 show scatterplots of serum iron and ferritin measurements in hypothyroid subjects against TSH.

Table 1: Distribution of subjects based on age and gender

Study group	N	Mean	Gender		SD	95% CI for mean	
			F	M		Lower bound	Upper bound
Euthyroid	100	34.12	72	28	9.060	**Euthyroid**	100
Hypothyroid	100	35.76	78	22	9.151	**Hypothyroid**	100
Hyperthyroid	60	32.87	46	14	6.361	**Hyperthyroid**	60
Total	260	34.46	196	64	8.596	**Total**	260

N: Number, SD: Standard Deviation; F: Female, M: Male

Table 2: Mean, SD and p-value of serum iron, ferritin, and TIBC in various groups

Parameters		Number	Mean ± SD	Std. Error	p
Serum iron	**Euthyroid**	100	91.575 ± 17.468	1.74684	0.000
	Hypothyroid	100	21.652 ± 6.739	0.67391	
	Hyperthyroid	60	181.193 ± 17.417	2.24861	
Ferritin	**Euthyroid**	100	93.759 ± 19.053	1.90534	0.000
	Hypothyroid	100	6.342 ± 3.444	0.34445	
	Hyperthyroid	60	324.100 ± 48.131	6.21371	
TIBC	**Euthyroid**	100	295.128 ± 22.996	2.29965	0.000
	Hypothyroid	100	392.700 ± 17.743	1.77433	
	Hyperthyroid	60	148.820 ± 20.267	2.61654	
	Total	260	298.892 ± 95.060	5.89542	

N: Number, SD: Standard Deviation; F: Female, M: Male

Table 3: Post hoc tests for Serum Iron, Ferritin, and TIBC

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Significance
Serum iron	**Euthyroid**	Hypothyroid	69.92350*	2.02330	0.000
		Hyperthyroid	-89.61763*	2.33630	0.000
	Hypothyroid	Euthyroid	-69.92350*	2.02330	0.000
		Hyperthyroid	-159.54113*	2.33630	0.000
	Hyperthyroid	Euthyroid	89.61763*	2.33630	0.000
		Hypothyroid	159.54113*	2.33630	0.000
Ferritin	**Euthyroid**	Hypothyroid	87.41780*	3.67762	0.000
		Hyperthyroid	-230.34020*	4.24655	0.000
	Hypothyroid	Euthyroid	-87.41780*	3.67762	0.000
		Hyperthyroid	-317.75800*	4.24655	0.000
	Hyperthyroid	Euthyroid	230.34020*	4.24655	0.000
		Hypothyroid	317.75800*	4.24655	0.000
TIBC	**Euthyroid**	Hypothyroid	-97.57140*	2.89584	0.000
		Hyperthyroid	146.30860*	3.34382	0.000
	Hypothyroid	Euthyroid	97.57140*	2.89584	0.000
		Hyperthyroid	243.88000*	3.34382	0.000
	Hyperthyroid	Euthyroid	-146.30860*	3.34382	0.000
		Hypothyroid	-243.88000*	3.34382	0.000

Std: Standard, Sig: Significance, TIBC: Total Iron Binding Capacity; P<0.05 is considered significant

Table 4: Correlations of serum iron within hypothyroid subjects

Hypothyroid		Serum iron	FT3	FT4	TSH	Ferritin	TIBC
Serum iron	Pearson correlation	1	0.246*	0.217*	-0.277**	0.472**	0.216*
	p-value		0.014	0.030	0.005	0.000	0.031
	N	100	100	100	100	100	100

FT3 – Free T3, FT4- Free T4, TSH – Thyroid Stimulating Hormone, TIBC: Total Iron Binding Capacity; N: Number, P<0.05 is considered as significant

Table 5: Correlations of ferritin within hypothyroid subjects

Hypothyroid		TSH
Ferritin	Pearson Correlation	-.239*
	p-value	.017
	N	100

TSH – Thyroid Stimulating Hormone, N: Number; P<0.05 is considered significant.

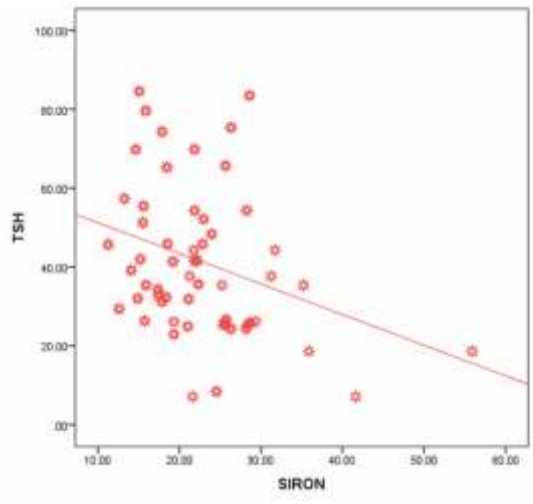

Figure 1: Serum Iron scatterplot of individual subject's measurements in hypothyroidism

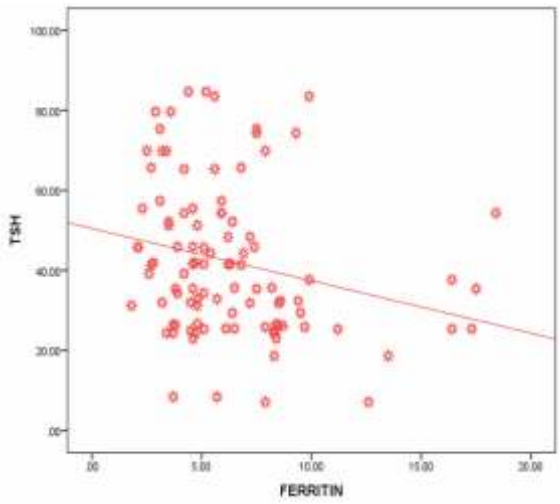

Figure 2: Ferritin scatterplot of individual subject's measurements in hypothyroidism

Discussion

Worldwide, hypothyroidism affects 1% of the general population and about 5% of individuals over the age of 60 years [13]. Hypothyroidism is highly prevalent in India. India had 42 million people with thyroid disorders, as per the national workshop held in Chennai on June 5, 2014 and it is the commonest thyroid disorder affecting one in ten adults in India. Its prevalence in India is 11 % compared with the U.K (2%) and USA (4.6%) as per Bagcchi *et al*. [14].

Age and gender of subjects were matched within the three groups of our study and also with other major studies done in India. In our study, serum iron and ferritin levels were significantly lower in hypothyroidism (p=0.00) and significantly higher in Grave's hyperthyroidism (p=0.00) in comparison with euthyroid subjects. While TIBC was found to be significantly higher in hypothyroidism (p=0.00) and significantly lower in Grave's hyperthyroidism ($p < 0.05$) in comparison with our control subjects. Ferritin and TIBC levels in both clinical hypothyroidism and clinical hyperthyroidism were found to be statistically significant ($p<0.0001$). Our study observed that there is a massive difference in serum iron status, ferritin, and TIBC levels in patients with clinical hypothyroidism and clinical hyperthyroidism compared to the euthyroid group.

Our findings are in agreement with many other studies done in India and the world. Studies by Luo *et al.* (2021) [15] found pregnant women with iron deficiency had significantly increased serum TSH levels and decreased FT4 levels. A study by Moreno-Reyes *et al.* (2021) [16] also showed iron levels determine serum free T4 and T4 levels. Correcting iron deficiency helped in maintaining optimum thyroid function. In a study by Shukla *et al.* (2018) [17], 100 subjects with hypothyroidism were examined compared to patients without hypothyroidism. The study concluded that there was a strong relation between decreased serum iron levels and hypothyroidism. In another study conducted by Banday *et al.* (2018) [18], 70 patients with overt hypothyroidism participated in the research and found that the prevalence of iron deficiency was 34.2%. They concluded in the study that the prevalence of iron deficiency was seen in relatively higher frequency in overt/clinical hypothyroidism, which mandates the measurement of iron profile in overt hypothyroid patients. The study done by Suhail *et al.* (2020) [19] also showed a significant decrease in serum iron and ferritin levels and an increase in TIBC levels in hypothyroidism. Dahiya *et al.* (2016) [8] also showed similar findings.

On the other hand, our findings on hyperthyroidism correlated with studies conducted by Suhail *et al*. (2020) [19]. This study showed a significant increase in serum iron and a decrease in TIBC levels in hyperthyroidism patients compared to euthyroid subjects. A study by Hussein *et al*. (2013) [20] showed a significant increase in serum iron and ferritin levels and a decrease in TIBC levels. This was also seen in studies by Kubota *et al*. [21] and Macaron *et al*. [22]. On the contrary study by Choudhury *et al*. [12] concluded that iron and ferritin levels were decreased and TIBC levels were increased in hyperthyroid subjects with iron deficiency anemia.

The mutual relationship between thyroid hormones and iron can be understood by the fact that thyroperoxidase is the main hormone for the synthesis of thyroid hormones, and it is a heme-

containing enzyme. Hence iron deficiency causes hypothyroidism [23].

Lack of stimulation of the development of the erythroid cell by thyroid hormones in hypothyroidism decreases the erythropoietin levels. So, oxygen distribution to these tissues decreases, which further affects iron metabolism. This is one more cause of hypothyroidism resulting in iron deficiency anemia [24]. Thyroxine supplementation helps in improving erythropoiesis. Thus, erythropoietin levels increase. Increased erythropoiesis results in increased production of RBC, which requires still more iron in already iron-deficient patients. This further worsens the iron deficiency state [8]. Iron deficiency significantly affects the efficiency of the 5' deiodinase enzyme [23,25]. This enzyme causes the peripheral conversion of T4 to T3. Hereby overall peripheral formation of the T3 hormone decreases. Hence the relationship between thyroid hormones and iron is complex. The deficiency of one will eventually cause a deficiency in others, and a vicious cycle sets in. This vicious cycle eventually causes a lack of both the hormone and iron in the body.

In the case of hyperthyroidism, the link between T3 and the regulation of ferritin expression suggest that a positive correlation exists between the levels of T4/T3 and ferritin in the serum [22,26]. Some evidence suggests that ferritin plays a role not only in iron storage but also in iron transport, probably because of its carrying capacity of 4500 iron atoms [27]. So, a marked increase in ferritin can lead to an elevation of serum iron. The elevation of ferritin levels in hyperthyroidism is due to the stimulatory effect of thyroid hormone and thyroid stimulating hormone on ferritin synthesis and release.

Conclusion

In the present study, we concluded that serum iron and ferritin levels decreased in hypothyroidism and increased in Grave's hyperthyroidism when compared to euthyroidism. On the contrary, TIBC levels increased in hypothyroidism and decreased in Grave's hyperthyroidism in comparison to euthyroid subjects. Therefore, patients with hypothyroidism and hyperthyroidism should be routinely screened for the iron profile. This will ensure a more effective thyroid treatment.

Limitations

Our study included both male and female study subjects, and included a significantly higher number of females compared to males. This made it difficult to establish the significance of serum iron, ferritin, and TIBC levels among the male population. The sample size was limited to 60 subjects with Grave's hyperthyroidism. This made it difficult to establish a firm association with hyperthyroidism. This study was limited to rural areas around northern Andhra Pradesh. Widespread research should be conducted in all parts of India including both urban and rural populations to understand the association better.

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