

Correlation of Iron Deficiency Status with Thyroid Profile among Subclinical and Overt Hypothyroidism Patients Attending a Tertiary Care Hospital in Puducherry, India: A Cross-sectional Study

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Abstract

Introduction: Hypothyroidism and iron deficiency are both highly prevalent disorders that share certain similar clinical presentations, implying the possibility for their coexistence. Hence, the objectives of this study were to evaluate serum iron indices and thyroid function tests in subclinical and overt hypothyroidism compared to healthy controls and to investigate the relationship between iron indices and thyroid function in subclinical and overt hypothyroidism. **Materials and Methods:** This hospital-based cross-sectional study included 154 participants, aged between 25–60 years, grouped as 46 subclinical hypothyroid patients, 58 overt hypothyroid patients, and 50 healthy controls. Their serum thyroid function and iron indices, namely ferritin, iron, total iron-binding capacity (TIBC), and transferrin saturation (TSAT), were estimated and compared between the groups. **Results:** The mean age of participants was 37.76 ± 7.13 years. Serum ferritin, iron, and TSAT were significantly lower, while TIBC was higher, in both the subclinical and overt hypothyroid groups compared to healthy subjects. In subclinical and overt hypothyroidism, thyroid-stimulating hormone showed a significant negative correlation with ferritin, iron, and TSAT, but positively correlated with TIBC. Free thyroxine correlated positively with ferritin, iron, and TSAT in both the hypothyroid groups but correlated negatively with TIBC in overt hypothyroidism only. Free triiodothyronine showed a positive correlation with ferritin in overt hypothyroidism. **Conclusion:** The study results suggest that iron deficiency correlates with thyroid function in both subclinical and overt hypothyroidism, demonstrated by decline in ferritin, iron, and TSAT with an increase in TIBC. It implies the likely coexistence of iron deficiency with subclinical and overt hypothyroidism and emphasizes the necessity for early assessment of iron status in patients with thyroid hypofunction to ensure appropriate management.

Keywords: Ferritin, hypothyroidism, iron, iron deficiency, overt hypothyroidism, subclinical hypothyroidism, total iron-binding capacity, transferrin saturation

INTRODUCTION

Hypothyroidism is a common endocrine disorder, affecting diverse populations worldwide, with a prevalence of approximately 5%, including iodine-replete regions. Thyroid autoimmunity and iodine deficiency constitute the major causes of primary hypothyroidism.^[1,2] Subclinical hypothyroidism is a mild form of primary hypothyroidism with 2%–6% risk for progressing to overt hypothyroidism.^[3] Iron deficiency is another global health issue that can present with clinical

manifestations similar to hypothyroidism, like fatigue, lethargy, muscle weakness, intolerance to cold, and depression.^[4,5]

An iron deficiency state is determined mainly by decreased iron stores and poor iron transportation manifesting as low

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circulatory levels of ferritin and transferrin saturation (TSAT), besides decreased iron and increased total iron-binding capacity (TIBC).^[6] Iron deficiency decreases the formation of hemoglobin, resulting in anemia with microcytic hypochromic red cells and associated clinical manifestations.^[7] In hypothyroidism, an underlying iron deficiency can manifest without anemia having overlapping clinical symptoms or as persisting symptoms despite treatment.^[8,9] These situations mandate the need to rule out an underlying iron deficiency irrespective of anemia in hypothyroidism to provide appropriate management.^[10]

Although an association between hypothyroidism and iron deficiency with low ferritin has been reported previously, the relationship between thyroid function and the iron deficient status demonstrated by alteration of other serum iron indices is limited, and their alteration in subclinical hypothyroidism is not well established.^[4] Thus, we formulated this study with the objectives to assess the serum iron indices and thyroid profile in subclinical and overt hypothyroidism compared to healthy controls and to investigate the relationship between iron indices and thyroid profile in subclinical and overt hypothyroidism.

MATERIALS AND METHODS

Study design and study setting

This hospital-based study with a cross-sectional study design was conducted at a tertiary care hospital in Puducherry, India, from April 2022 to March 2023. The study participants were recruited from patients referred to the central laboratory of the hospital for estimation of their thyroid profile and routine blood investigations.

Sample size and sampling technique

One hundred and fifty-four participants were enrolled in this study. The sample size for this study was calculated with the statistical formula, $n = (Z\alpha/2 + Z\beta)^2 \times 2 \times \sigma^2/d^2$ using a confidence interval of 95%, a power of 80%, and a 1:1 ratio between the groups to estimate the difference between two means and standard deviations of thyroid-stimulating hormone (TSH) values (mIU/mL) of 3.72 ± 1.96 and 8.21 ± 3.94 obtained from the available literature.^[11] The study participants were recruited by convenience sampling and further grouped based on their baseline thyroid function status:

- Group I – Healthy controls ($n = 50$): Apparently healthy individuals with euthyroid status described as serum thyroid profile in the normal reference range with TSH: 0.4–5.5 mIU/L, free thyroxine (FT4): 0.8–2.7 ng/dL, and free triiodothyronine (FT3): 2.4–4.2 pg/mL
- Group II – Subclinical hypothyroidism ($n = 46$): Individuals with increased serum TSH (values between 5.5 mIU/L and 10 mIU/L), with FT4 and FT3 in the normal range
- Group III – Overt hypothyroidism ($n = 58$): Newly diagnosed primary hypothyroidism having increased serum TSH (>10 mIU/L), with low FT4 and/or low FT3.

Inclusion criteria

Adult individuals diagnosed with subclinical and overt hypothyroidism, aged between 25–60 years, were recruited as study participants. Apparently, healthy individuals without any previously known disease or treatment history and having a normal thyroid status were recruited as healthy controls.

Exclusion criteria

Patients previously diagnosed with hypothyroidism, anemia or iron deficiency, history of treatment with drugs or supplements affecting thyroid or iron status, recent blood loss, autoimmune conditions, and presence of comorbid conditions such as diabetes mellitus, hypertension, renal dysfunction, liver disease, and coronary heart disease were excluded from the study. Participants found to have possible confounding factors of iron deficiency such as reduced dietary intake, menstrual irregularities, pregnancy, altered gastrointestinal health, and any underlying diseases, were screened during recruitment by detailed history and baseline laboratory data and were also excluded from participation.

Data collection procedure

Participants were selected by convenience sampling technique and their demographic measures with clinical history were documented. Their baseline serum thyroid function status including serum TSH, FT4, and FT3 was assessed by chemiluminescence immunoassay (Siemens Advia Centaur CP). Peripheral venous blood samples were collected and serum was stored at -20°C for estimation of iron indices. Evaluation of serum iron indices included serum iron in $\mu\text{g/dL}$ and TIBC in $\mu\text{g/dL}$ estimated in the chemistry autoanalyzer (DiaChem-300) and serum ferritin in ng/mL estimated by chemiluminescence immunoassay (Siemens AdviaCentaur CP). TSAT expressed in percentage was computed by the formula: Serum iron/TIBC $\times 100$.

Ethical declaration

Approval to conduct the study was obtained from the Institute Ethics Committee for Human Studies (Cert No. 01/SVMCH/IEC-Cert/Mar22), and the Helsinki Declaration 2008 ethical guidelines were also followed. All study participants had given their written informed consent to be enrolled in the study.

Statistical analysis

We analyzed the collected data using statistical software, IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, New York, United States). The continuous variables such as age, baseline laboratory parameters, and iron indices were described in mean \pm standard deviation, while categorical data were described as frequency and percentage. The continuous variables were compared between the three groups using one-way analysis of variance (ANOVA) statistical test with Bonferroni *post hoc* analysis. Comparative analysis of the frequency distribution of categorical data between the three groups was done using the Chi-square test. The correlation between thyroid profile and iron indices was evaluated by Pearson's correlation analysis. Statistically significant results were denoted by a *P* value below 0.05.

RESULTS

Our study included 154 participants categorized into three groups, namely 50 healthy euthyroid subjects, 46 subclinical hypothyroidism patients, and 58 overt hypothyroidism patients. The age distribution of participants among the three groups was comparable with a mean age of 37.76 ± 7.13 years and overall majority of participants in the age range of 31–40 years (44.8%).

Table 1 shows the comparison of demographic and baseline laboratory measures of the healthy control, subclinical hypothyroid, and overt hypothyroid groups. A significant majority of the study participants in all the three groups were females (87%). The mean values of serum TSH were significantly higher, while FT3, FT4, and hemoglobin were lower in the subclinical and overt hypothyroid subjects than euthyroid controls.

Table 2 displays the comparison of the serum iron indices between the three groups. The mean values of serum ferritin, iron, and TSAT were lower, while TIBC was higher, in the subclinical and overt hypothyroid subjects compared to euthyroid controls.

Table 3 shows the correlation of thyroid profile to serum iron indices in the three groups. In both the subclinical and overt hypothyroidism groups, TSH had a significant negative correlation with ferritin, iron, and TSAT and a positive correlation with TIBC. FT4 showed a significant positive correlation with ferritin, iron, and TSAT in both the subclinical and overt hypothyroid groups but negatively correlated with TIBC in the overt hypothyroidism group only. FT3 had no significant correlation with iron indices in the subclinical hypothyroid group but positively correlated with ferritin in overt hypothyroidism. No significant correlation was found between the thyroid profile and serum iron indices in the euthyroid group.

Figure 1 displays the correlation between TSH and markers of iron status. TSH negatively correlated with serum

ferritin ($P < 0.001$), iron ($P < 0.001$), and TSAT ($P < 0.001$) but positively correlated with TIBC ($P < 0.001$).

Figure 2 displays the correlation between thyroid hormone-free T4 and markers of iron status. Thyroid hormone-free T4 positively correlated with serum ferritin ($P < 0.001$), iron ($P < 0.001$), and TSAT ($P < 0.001$) but negatively correlated with TIBC ($P < 0.001$).

DISCUSSION

Primary hypothyroidism is a common endocrine disorder characterized by increased TSH and decreased thyroid hormone levels, manifesting with a variety of clinical features including fatigue, lethargy, depression, and cold intolerance. These symptoms are synonymous to the clinical features of iron deficiency irrespective of the presence of anemia. The possibility for the coexistence of hypothyroidism and iron deficiency may be attributed to the inter-dependent physiological relationship of thyroid hormones and circulatory iron, whereby thyroid hormones are involved in regulating the absorption and utilization of iron in the body, while iron promotes the formation of the biologically active thyroid hormone T3 from T4 by the enzyme thyroid peroxidase. Considering this mutual role, both conditions share clinical symptoms and may exacerbate one another, while underlying iron deficiency in hypothyroidism tends to get underdiagnosed.^[8]

Ferritin is a well-known marker of body iron stores while the percentage TSAT represents the efficiency of iron transport and supply to tissues. Iron deficiency state is defined by the presence of serum ferritin levels lower than 15 ng/mL and TSAT less than 16%.^[6] Serum iron and TIBC are also considered markers of iron status, but isolated assessment of these parameters may not be beneficial in the diagnosis of iron deficiency. The present study focused on investigating the serum markers of iron status in patients with subclinical and overt hypothyroidism compared to healthy controls and their relation to thyroid function in hypothyroidism. Iron

Table 1: Demographic and baseline parameters of the participants

	Euthyroid controls (n=50)	Subclinical hypothyroid (n=46)	Overt hypothyroid (n=58)	P
Age (years)	35.90±7.63	36.67±6.76	40.22±6.35	0.003**
Gender (male/female)	8/42	5/41	7/51	<0.001**
TSH (mIU/L)	2.94±1.07	8.21±1.09	26.05±10.09	<0.001**
FT4 (ng/dL)	1.21±0.25	1.64±0.47	0.53±0.24	<0.001**
FT3 (pg/mL)	3.03±0.63	3.56±0.49	1.74±0.45	<0.001**
Hemoglobin (g/dL)	12.67±0.89	11.89±1.43	11.42±1.41	<0.001**
RDW-CV (%)	15.68±2.27	15.53±2.22	16.16±2.75	0.39
MCHC (g/dL)	34.47±1.06	34.22±1.14	33.99±1.07	0.072
MCH (pg)	29.31±2.66	28.17±2.6	27.88±3.27	0.031*
MCV (fL)	83.53±5.81	82.39±6.21	82.97±8.96	0.748
Hematocrit (%)	36.79±2.84	35.67±4.32	34.57±4.13	0.012*

* $P < 0.05$ is statistically significant, ** $P < 0.001$ indicates high statistical significance. TSH: Thyroid-stimulating hormone, RDW-CV: Red blood cell distribution width coefficient of variation, MCH: Mean corpuscular hemoglobin, MCHC: MCH concentration, MCV: Mean corpuscular volume, FT4: Free thyroxine, FT3: Free triiodothyronine

Table 2: Comparison of serum iron indices between the three groups

	Euthyroid controls (n=50)	Subclinical hypothyroid (n=46)	Overt hypothyroid (n=58)	P
Ferritin (ng/mL)	54.48±16.23	38.51±18.09	28.31±16.89	<0.001**
Iron (µg/dL)	89.28±12.60	68.68±18.41	53.04±22.09	<0.001**
TIBC (µg/dL)	282.83±45.15	317.73±58.66	348.47±51.53	<0.001**
TSAT (%)	32.29±6.52	22.91±8.42	16.12±7.96	<0.001**

P<0.05 is statistically significant, **P<0.001 indicates high statistical significance. TIBC: Total iron-binding capacity, TSAT: Transferrin saturation

Table 3: Correlation of thyroid profile with serum iron indices among the healthy euthyroid control, subclinical hypothyroid, and overt hypothyroid groups

Variable	Ferritin	Iron	TIBC	TSAT
Euthyroid controls				
TSH				
r	-0.071	0.007	0.140	-0.081
P	0.624	0.962	0.332	0.576
FT4				
r	-0.161	-0.171	0.067	-0.154
P	0.263	0.234	0.644	0.284
FT3				
r	0.103	-0.141	-0.241	0.047
P	0.475	0.329	0.092	0.744
Subclinical hypothyroidism				
TSH				
r	-0.362	-0.319	0.342	-0.359
P	0.014*	0.031*	0.020*	0.014*
FT4				
r	0.299	0.310	-0.224	0.320
P	0.043*	0.036*	0.135	0.030*
FT3				
r	0.280	0.172	-0.032	0.141
P	0.060	0.254	0.831	0.354
Overt hypothyroidism				
TSH				
r	-0.424	-0.478	0.390	-0.495
P	0.001*	<0.001**	0.002**	<0.001**
FT4				
r	0.295	0.307	-0.283	0.328
P	0.024*	0.019*	0.032*	0.012*
FT3				
r	0.260	0.241	-0.029	0.220
P	0.049*	0.069	0.829	0.096

*P<0.05 is statistically significant, **P<0.001 indicates high statistical significance. Pearson's correlation coefficient - r. TSH: Thyroid-stimulating hormone, TIBC: Total iron-binding capacity, TSAT: Transferrin saturation, FT4: Free thyroxine, FT3: Free triiodothyronine

deficiency status manifests with laboratory findings of decrease in serum iron, ferritin, and TSAT and increase in TIBC, irrespective of the presence or absence of anemia defined by reduced hemoglobin.^[6,12] Previous studies have demonstrated iron deficiency in hypothyroidism emphasizing on overt hypothyroidism while limited research exists on subclinical hypothyroidism.^[13,14] On the other hand, a study by Erdogan *et al.* did not demonstrate a statistically significant difference in ferritin and iron among subclinical hypothyroidism, overt

hypothyroidism, and healthy controls.^[15] Our study findings support the presence of iron deficiency in both subclinical and overt hypothyroidism as observed by the statistically significant decline in ferritin, iron, and TSAT, with a significant increase in TIBC levels, in both the subclinical and overt hypothyroid groups relative to the healthy subjects.

On assessing the relationship between iron indices and thyroid function, our study findings demonstrated a significant negative correlation of serum TSH to ferritin, iron, and TSAT, with positive correlation to TIBC in both the subclinical and overt hypothyroid groups. Conversely, FT4 positively correlated with ferritin, iron, and TSAT in both the subclinical and overt hypothyroid groups but negatively correlated with TIBC only in overt hypothyroidism. In the subclinical hypothyroid group, FT3 did not show any significant correlations with iron indices in the subclinical hypothyroid group, while in overt hypothyroidism, FT3 positively correlated with ferritin. These findings were concurrent with previous studies which reported ferritin and iron deficiency to be associated with increased TSH and decreased FT4 in primary hypothyroidism.^[11,16] Contrarily, a few previous studies do not show an association between thyroid profile, anemia, and iron indices, which may be due to regional or population differences.^[17,18] A study conducted in Bangladesh by Akhter *et al.* also reported no significant correlation between thyroid profile and ferritin.^[19] Anemia is the most easily identified outcome of iron deficiency; nevertheless, studies have made it evident that iron deficiency can have detrimental effects even when anemia is absent.^[8,20] The findings of our study demonstrate a significant correlation of iron deficiency with subclinical and overt hypothyroidism, thus indicating that iron deficiency can exist as an underlying comorbidity in hypothyroidism.

The significant correlation of increasing TSH levels with a decline in ferritin, iron, and TSAT and an increase in TIBC, as well as the correlation of decreasing FT4 levels with an increase in ferritin, iron, and TSAT and a decline in TIBC observed in this study, implies the coexistence and association of iron deficiency status with thyroid hypofunction. The literature review further suggests several possible mechanisms underlying the relationship between hypothyroidism and iron deficiency including hypochlorhydria and reduced ferritin gene expression.^[21,22] In addition, iron deficiency decreases both the activity of the heme-dependent thyroid peroxidase which affects thyroid hormone synthesis, as well as decreased peripheral deiodination needed for the formation of active T3.^[23] These factors suggest a detrimental interrelationship resulting

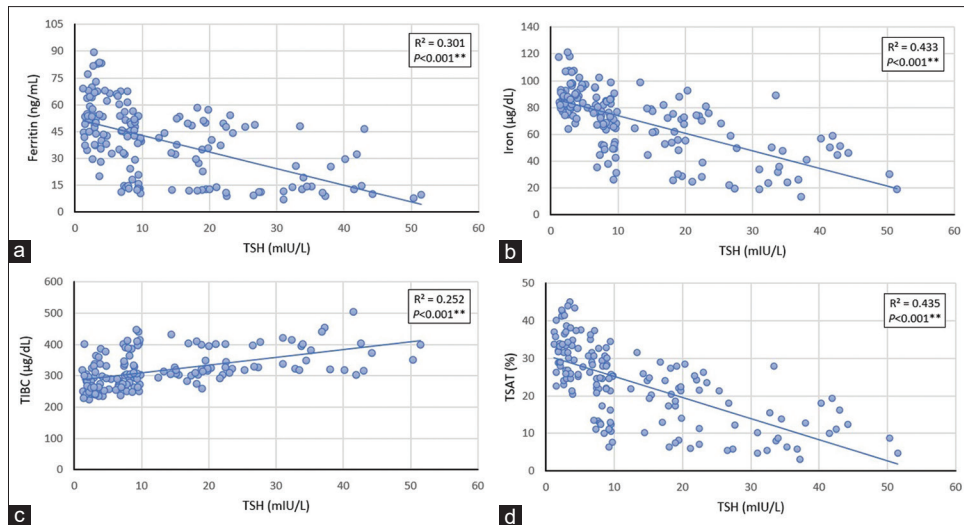


Figure 1: (a-d) Correlation of thyroid-stimulating hormone with serum markers of iron status. $P < 0.05$ is statistically significant. $**P < 0.001$ indicates high statistical significance. TSH: Thyroid-stimulating hormone

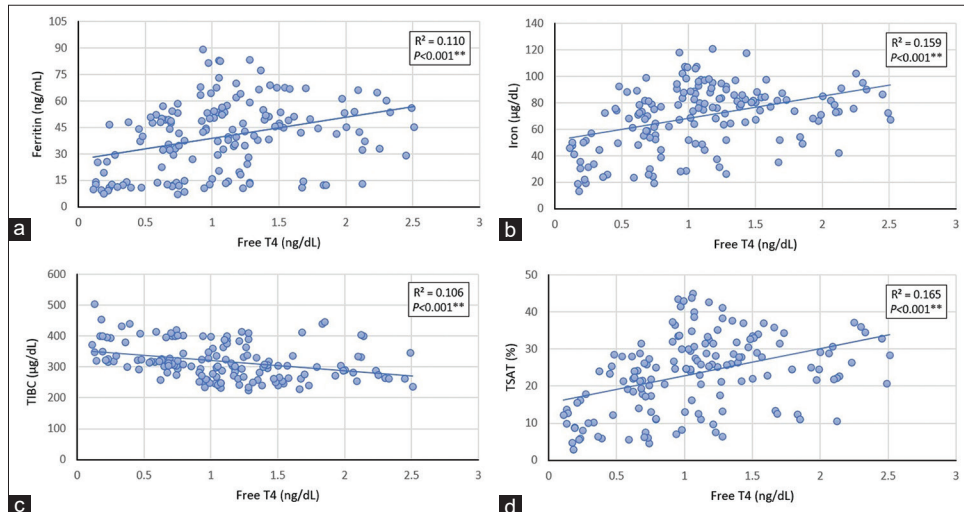


Figure 2: (a-d) Correlation of the thyroid hormone-free thyroxine with serum markers of iron status. $P < 0.05$ is statistically significant. $**P < 0.001$ indicates high statistical significance. T4: Thyroxine, TIBC: Total iron-binding capacity, TSAT: Transferrin saturation

in the predisposition for co-occurrence of hypothyroidism and iron deficiency.

Attaining an euthyroid status or resolving iron deficiency in hypothyroidism can be challenging if only one of either aspect is addressed since both conditions have the tendency to coexist and trigger the onset of each other. Including the assessment of serum ferritin, iron, TIBC, and TSAT, as part of the routine laboratory investigation profile of thyroid dysfunction may offer valuable insights for early diagnosis and better management of underlying iron deficiency in hypothyroidism.

Some limitations of our study include its cross-sectional design since causal mechanisms and the associated factors contributing to iron deficiency in hypothyroidism could not be inferred. Assessment of antithyroid peroxidase antibodies also was not done, which would have been beneficial to study the impact of autoimmune thyroid dysfunction with regard

to iron deficiency. Further research on the effect of thyroid hypofunction on iron homeostasis across various types of anemia could also contribute to unveil better therapeutic approaches for the diagnosis and management of persisting symptoms in hypothyroidism.

CONCLUSION

We conclude that an underlying iron deficiency state tends to coexist with subclinical and overt primary hypothyroidism, characterized by decreased serum ferritin, iron, TSAT, and increased TIBC. Furthermore, increased TSH levels in subclinical hypothyroidism and overt hypothyroidism correlate with a decrease in serum ferritin, iron, and TSAT and increased TIBC, which demonstrates its correlation with iron deficiency status. This possible co-dependent occurrence of hypothyroidism and iron deficiency emphasizes the necessity

for simultaneous assessment of iron indices in patients with hypothyroidism for better management.

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Conflicts of interest

There are no conflicts of interest.

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