Serum Copper, Iron, and Total Iron Binding Capacity in Hypothyroidism: A Case Control Study

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Background: Thyroid hormone metabolism is linked to iron metabolism. Thyroperoxidase, a key enzyme in the biosynthesis of thyroid hormones, is iron-dependent. Thus, iron deficiency might be the primary cause of hypothyroidism. Copper is another trace element that has been linked to thyroid status. Copper regulates excessive thyroxine (T4) absorption and reduces cell damage during thyroid hormone synthesis. The present study clarified the possible correlations between iron and copper levels as well as total iron binding capacity (TIBC) and triiodothyronine (T3), T4, as well as thyroid-stimulating hormone (TSH) levels in healthy and hypothyroid subjects.

Materials and methods: Thirty-five healthy subjects and 35 hypothyroid subjects were included in this study. Serum T3, T4, and TSH levels were measured using the enzyme linked fluorescence assay. Serum iron levels and TIBC were estimated using ferrozine/magnesium carbonate method, while serum copper levels were estimated using colorimetric method.

Results: Copper levels were not significantly different between healthy and hypothyroid subjects. Iron and T4 levels were significantly lower in hypothyroid subjects compared with those in healthy subjects, while TIBC and TSH levels were significantly higher. There was no significant correlation between copper levels and T3, T4, and TSH levels.

Conclusion: There were inverse correlations between TIBC and T4 as well as iron levels, and there was no significant correlation between copper levels and all thyroid function parameters. Routine examination of iron levels and thyroid function is highly recommended for early diagnosis and therapy of hypothyroidism.

Keywords: total iron binding capacity, iron, copper, hypothyroidism

Introduction

Hypothyroidism is a clinical disease characterised by low thyroid function. It is characterised by decrease in blood triiodothyronine (T3) and thyroxine (T4) levels, which is followed by an increase in serum thyroid-stimulating hormone (TSH) concentration. Thyroid hormones are required for normal body growth and are one of the key...
hormonal components in controlling the primary metabolic rate of target organs such as the liver, heart, kidney, and brain.1

Primary gland failure or lack of thyroid stimulation by the hypothalamus or pituitary gland can lead to hypothyroidism. Primary gland failure may be caused by infiltrative illness, iodine deficiency, Hashimoto disease, or congenital defects.2 The most prevalent cause of hypothyroidism in the United States is thyroid autoimmune disease. Central causes of hypothyroidism are often linked to different signs of pituitary or hypothalamic dysfunction and are identified by low TSH levels in proportion to insufficient thyroid hormone. Hypothyroidism, if left untreated, can lead to dyslipidemia, hypertension, neuromuscular dysfunction, cognitive impairment, and infertility.2

The symptoms of hypothyroidism might differ depending on age and gender. Lethargy and failure to thrive are more common in infants and children. Women with hypothyroidism may have menstrual irregularities and fertility problems. In the elderly, cognitive deterioration may be the only sign.2

Goiter, peripheral edema, dry skin, thin or brittle hair, and delayed relaxation phase of deep tendon reflexes are examination findings linked with hypothyroidism. Bradycardia, flattened T waves, and low voltage are all common electrocardiography results. Severe hypothyroidism can cause pericardial effusion, pleural effusion, megacolon, hemodynamic instability, and coma.2 Moreover, hypothyroidism can cause normocytic anemia, hypoxia, hypercapnia, hypotension, increased creatine kinase, hyperlipidemia, and hyperprolactinemia.3

Anemia is frequent in adults with hypothyroidism. Anemia is usually mild to moderate, with hemoglobin concentrations below 8-9 g/dL.4 In hypothyroidism, anemia is generally normochromic and normocytic, but it can rarely be moderately macrocytic.5 This variation has been linked to concurrent iron and folic acid deficits, which are mostly due to decreased absorption.4,6 T4 insufficiency causes decreased haemoglobin synthesis in hypothyroidism.6

Thyroid hormone is involved in haemoglobin synthesis in adults and haemoglobin maturation in foetus, and hypothyroidism causes anaemia by reducing oxygenation and disrupting the hematopoietic process.7 Iron deficiency, on the other hand, inhibits T4 production by decreasing the activity of haem-dependent thyroid peroxidase.8 Many studies suggest that iron deficiency impairs thyroid hormone metabolism in both animals and humans.9,10,11 Iron deficiency has also been shown to decrease plasma T3 and T4 concentrations while increasing in vitro hepatic reverse T3 (rT3) deiodination, implying that iron deficiency affects thyroid hormone metabolism via a deactivating pathway. T4 is transformed to T3, however a large part is converted to rT3, a physiologically inactive metabolite. It is yet unknown how anaemia impacts deiodinase activity.11

Trace elements have been shown to regulate hormones at several levels of action, including hormone release, activity, and binding to target tissue.12 Copper, one of the most abundant minerals in the human body, is essential for thyroid metabolism, particularly hormone production and absorption. Copper increases T4 synthesis and inhibits T4 overabsorption in blood cells via managing calcium levels in the body. Apart from that, copper is necessary to produce phospholipids, which are essential for TSH stimulation.13

The present study clarified the possible correlations between the levels of iron, copper, T3, T4, TSH, as well as total iron binding capacity (TIBC) in healthy and hypothyroid subjects.

Materials and methods

Study Design and Subject Selection

The current case-control study was carried out at the Integral Institute of Medical Sciences and Research Department of Biochemistry in Lucknow, India. A total of 70 subjects, including 35 newly diagnosed hypothyroidism cases and 35 age- and gender-matched healthy controls were chosen for the study after imposing certain inclusion criteria and exclusion criteria for cases and controls. Subjects included for hypothyroidism cases group were subjects aged 20-60 years old diagnosed with hypothyroidism as per American Thyroid Association Criteria14, while healthy individuals aged 20-60 years were included as subjects for the control group. Subjects with a history of iron malabsorption, iron deficiency anemia, gastrointestinal and respiratory blood losses, and pregnant and lactating women were excluded from the hypothyroidism cases. Meanwhile, subjects with a history of any acute or chronic illness were excluded from the control group.

A data collection form was used to collect clinical history from each subject. The Institutional Ethics Committee, Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, granted permission for human participant enrolment and blood sample collection (No.: IEC/IIMS&R/2022/10).
Sample Collection and Serum Separation
Five mL of venous blood were drawn from each subject under aseptic conditions. The blood sample was allowed to clot at room temperature before being centrifuged for 5 minutes at 3000 rpm to separate the serum, which was then used to assess the levels of T3, T4, TSH, copper, iron, and TIBC.

Estimation of Thyroid profile, Iron, Total Iron Binding Capacity, and copper
T3, T4, and TSH levels were measured using the enzyme-linked fluorescence assay (ELFA) technique with a Biomerieux miniidas automated immunoassay analyzer (BioMérieux, Marcy-l’Étoile, France) with reference range of T3 = 0.92-2.33 nmol/L; T4 = 60-120 nmol/L; and TSH = 0.25-5.0 mIU/L. Iron and TIBC were estimated by the ferrozine/magnesium carbonate method using commercially available kit (Tulip Diagnostic (P) Ltd, Verna, India) with ERBA CHEM 7 semi autoanalyzer machine (ERBA Mannheim, Mannheim, Germany). Copper levels were estimated by the colorimetric method using commercially available kit (Tulip Diagnostic (P) Ltd) with ERBA CHEM 7 semi autoanalyzer machine (ERBA Mannheim).

Statistical Analysis
Microsoft Excel 2021 version (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 2023 version (GraphPad Software, Boston, MA, USA) were used for statistical analysis. All data were expressed as mean±standard deviation. An unpaired t-test was used to compare study parameters between case and control groups. To determine the link between variables, Pearson’s correlation coefficient was used. A p-value of <0.05 was deemed statistically significant.

Results
Table 1 shows the biochemical and anthropometric parameter values between the two study groups. There was no significant difference in copper and T3 levels between the case and control groups. However, the T4 and iron levels were significantly lower in the case group compared with those in the control group. Whereas the BMI, TIBC and TSH levels were significantly higher in the case group compared to control group.

There was no significant correlation between copper levels and T3, T4, and TSH levels. The only significant correlations were between TIBC and T4 levels, as well as TIBC and iron levels (Table 2). Figure 1 shows the negative correlations between TIBC and T4 levels, while Figure 2 shows the negative correlations between TIBC and iron levels (Figure 2).

Discussion
In this study, T4 and iron levels were shown lower while TSH and TIBC levels were higher in hypothyroid patients compared to healthy controls. These findings are consistent with previous research that found iron insufficiency to be

Table 1. Characteristics of subjects.

<table>
<thead>
<tr>
<th>Anthropometric &amp; Biochemical Parameters</th>
<th>Cases (n=35)</th>
<th>Controls (n=35)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean±SD</td>
<td>36.23±8.21</td>
<td>30.51±7.20</td>
<td>0.003*</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (45.71)</td>
<td>16 (45.71)</td>
<td>0.499</td>
</tr>
<tr>
<td>Female</td>
<td>19 (54.29)</td>
<td>19 (54.29)</td>
<td>0.499</td>
</tr>
<tr>
<td>BMI (kg/m²), mean±SD</td>
<td>41.23±5.17</td>
<td>38.84±3.70</td>
<td>0.023*</td>
</tr>
<tr>
<td>T3 (nmol/L), mean±SD</td>
<td>1.55±0.49</td>
<td>1.63±1.34</td>
<td>0.741</td>
</tr>
<tr>
<td>T4 (nmol/L), mean±SD</td>
<td>89.53±21.31</td>
<td>109.00±24.00</td>
<td>0.000*</td>
</tr>
<tr>
<td>TSH (mIU/mL), mean±SD</td>
<td>11.40±5.02</td>
<td>1.92±1.19</td>
<td>0.000*</td>
</tr>
<tr>
<td>Copper (µg/dL), mean±SD</td>
<td>51.60±20.44</td>
<td>59.62±21.40</td>
<td>0.114</td>
</tr>
<tr>
<td>Iron (µg/dL), mean±SD</td>
<td>96.63±44.56</td>
<td>127.54±48.37</td>
<td>0.007*</td>
</tr>
<tr>
<td>TIBC (µg/dL), mean±SD</td>
<td>156.68±53.02</td>
<td>133.49±35.02</td>
<td>0.034*</td>
</tr>
</tbody>
</table>

*p<0.05 is considered significant.
Table 2. Correlation between variables in hypothyroidism cases.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age</th>
<th>BMI</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
<th>Iron</th>
<th>Copper</th>
<th>TIBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.000</td>
<td>0.276</td>
<td>0.171</td>
<td>0.623</td>
<td>0.718</td>
<td>1.000</td>
<td>0.878</td>
<td>0.358</td>
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<tr>
<td>BMI</td>
<td>1.000</td>
<td>0.599</td>
<td>0.165</td>
<td>0.845</td>
<td>0.453</td>
<td>0.617</td>
<td>0.644</td>
<td></td>
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<tr>
<td>T3</td>
<td>1.000</td>
<td>0.172</td>
<td>0.365</td>
<td>0.253</td>
<td>0.922</td>
<td>0.509</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>1.000</td>
<td>0.305</td>
<td>0.341</td>
<td>0.561</td>
<td>0.017*</td>
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<tr>
<td>TSH</td>
<td>1.000</td>
<td>0.104</td>
<td>0.379</td>
<td>0.057</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>1.000</td>
<td>0.566</td>
<td>0.042*</td>
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<td></td>
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<td></td>
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<tr>
<td>Copper</td>
<td>1.000</td>
<td>0.939</td>
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<tr>
<td>TIBC</td>
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<td></td>
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<td>1.000</td>
</tr>
</tbody>
</table>

*Correlation is significant if \( p<0.05 \) level (2-tailed).

associated with decreased thyroid hormone levels. Many animal and human studies have shown that a lack of dietary iron reduces thyroxine and triiodothyronine circulation levels, as well as the conversion of T4 to T3. It is still unclear how iron deficiency affects deiodinase activity.

Iron is required for the activity of thyroperoxidase, which produces thyroid hormone. It was previously noted that iron is required for the process that transports thyroid hormone into cells, and a shortage of it can result in thyroid hormone pooling, resulting in a clinically hypothyroid appearance even when T3 levels remain normal, resulting in a thyroxine resistance-like condition. This increases the demand for iron and may result in iron deficient symptoms.

According to another research, individuals with hypothyroidism showed decreased erythroid cell numbers and proliferative activity in the bone marrow as a result of low plasma erythropoietin levels and inadequate oxygenation of the tissues. As a result, there was a substantial negative association between TSH and Hb, which resulted in iron shortage, which may be both a cause and an effect of hypothyroidism. In accordance with the previous study another study reported that poor gut absorption due to low digestive acid/enzyme levels or by linked autoimmune illnesses such as celiac disease may be the reason for iron deficiency in hypothyroidism. Following, a review study indicated that thyroid hormones activate erythropoietin gene

![Figure 1. The correlation between serum TIBC and T4 levels.](image1)

![Figure 2. The correlation between serum TIBC and iron levels.](image2)
expression, leading to an increase in erythropoietin secretion and reduced thyroid peroxidase enzyme activity an iron dependent crucial enzyme involved in thyroid hormone production.\textsuperscript{21} Menorrhagia and microcytic anaemia can result from hypothyroidism due to iron malabsorption.\textsuperscript{2}

Thus, iron is an important factor that should be taken in account while treating hypothyroid individuals and also measuring the iron profile in hypothyroid individuals may be beneficial, as iron shortage may be the underlying reason. In relation to another trace element copper, we did not find any significant correlation between copper and thyroid profile in hypothyroidism cases. The study’s findings were consistent with those of a previously conducted study.\textsuperscript{22} Anemia and a low level of body iron are frequent symptoms of hypothyroidism. Thus, determining the iron profile may give a new insight in early detection of anemia in hypothyroidism patients.

**Conclusion**

Our study reported that there were inverse correlations between TIBC and T4 as well as iron levels, and there was no significant correlation between copper levels and all thyroid function parameters. Since hypothyroidism is frequently connected with anaemia and a low iron storage in the body and there is no such major clinical manifestation of hypothyroidism in the early stages of anaemia, it is highly recommended to routinely examine iron levels and thyroid function for early diagnosis and therapy. However, more studies in a larger sample size are warranted to confirm the current findings.

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**Authors Contribution**

PTM, GKY, DT, RA and DPV were involved in the conception and planning of the research. GKY and DPV performed the data acquisition. PTM, DT and RA analysed the experimental data. PTM, GKY, and RA prepared the manuscript as well as designed the tables and figures. All authors were involved in result interpretation and critical revision of the manuscript.

**References**


