



Original Article

# Interpretation of Biochemical Tests for Iron Metabolism in Hyperthyroidism

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## Abstract:

**Objective:** Several studies suggest that thyroid hormones may affect erythropoiesis. However the mechanism by which thyroid hormones alter the ferritin concentration is not well known. Therefore, the present case-control study was designed to determine the changes due to hyperthyroidism in serum ferritin, iron and transferrin levels and to investigate the inter-relationship between these parameters.

**Material:** This study was conducted on 50 newly diagnosed hyperthyroid patients and the results were compared with 50 age and sex matched healthy controls. Serum ferritin was assessed by two site sandwich immunoassay using direct chemiluminometric technology. TIBC and serum iron were estimated by colorimetric method.

**Results:** Serum ferritin ( $314.43 \pm 68.7$  ng/mL) and iron concentration ( $159.88 \pm 36.28$   $\mu$ g/dL) were found to be increased in hyperthyroid patients as compared to healthy controls ( $255.23 \pm 45.5$  ng/mL and  $110.52 \pm 20.52$   $\mu$ g/dL respectively). There was a significant difference between hyperthyroid patients and healthy controls in serum levels of ferritin and iron ( $p < 0.05$  for both). Serum ferritin and iron were correlated significantly positive with thyroid parameters while a significant negative correlation was found with transferrin.

**Conclusion:** Our data suggest that alterations in thyroid status in a given individual produce significant changes in serum ferritin, iron and transferrin levels. Increased ferritin levels seem to be protective against increased oxidative stress seen in hyperthyroidism but these also increase atherosclerotic risk. However, a large scale study is recommended to establish the fact.

**Keywords:** Hyperthyroidism; total triiodothyronine; total thyroxine; thyroid stimulating hormone; ferritin

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## Introduction

Thyroid hormone, 3, 3', 5-triiodo-L-thyronine (T3) has an important role to play in eukaryotic cell development, differentiation and metabolism. Hyperthyroidism occurs when there is increased secretion of hormones over a short or long period of time. The symptoms of hyperthyroidism include increased excitability, intolerance to heat, increased perspiration, mild to extreme weight loss, varying degrees of diarrhea, muscle weakness, anxiety or psychic disorders, extreme fatigue, lack of sleep and tremors of the hands [1].

Several minerals and trace elements like iodine, iron, selenium and zinc are essential for normal thyroid hormone metabolism. Iron is an essential nutrient and a potential toxin. It is physiologically essential for growth and survival, playing important roles in many biological processes, such as electron and oxygen transport, and DNA replication. However, free iron can be toxic to cells due to the ability of iron to catalyze free radical formation that can destroy lipid membranes and other cellular constituents. Intracellular iron homeostasis is mainly controlled by cytoplasmic iron response proteins (IRP1 and IRP2), which are known as iron-sensing proteins [2].

Plasma ferritin is a measure of iron stores and the best single test to confirm iron deficiency. It is also a marker of some neoplastic diseases. It has been reported in literature that thyroid hormones may affect erythropoiesis. Transferrin is a plasma glycoprotein for iron delivery which is expressed in all mammals and is synthesized in the liver. Serum ferritin levels have been observed to be altered in patients with hyperthyroidism. Some studies have reported increased ferritin levels while others observed these levels to be decreased. Also, the mechanism by which thyroid hormones alter the ferritin concentration is not well known [3-6]. Therefore, the present case-control study was designed to determine the changes due to hyperthyroidism in serum ferritin, iron and transferrin levels and to investigate the interrelationship between these parameters.

## Material and methods

This study was conducted on 50 newly diagnosed hyperthyroid patients and 50 healthy controls with thyroid profile in the normal range. None of the patients had anemia, liver diseases, malignancy, or inflammatory illness during the study period. All individuals suffering from chronic diseases, such as diabetes mellitus, diseases of the liver, kidney, cardiac and other endocrine and immunological disorders were excluded from both patient groups and healthy controls with the help of suitable investigations.

After obtaining informed consent from the subjects venous blood was collected from median anti cubital vein aseptically. Serum was separated and stored at -20 °C until analysis. Serum total triiodothyronine (TT3) and total thyroxine (TT4) levels were estimated by radioimmunoassay and TSH (thyroid stimulating hormone) levels were estimated by immunoradiometric assay (IRMA) to group them as normal subjects and hyperthyroid patients. Serum-free triiodothyronine (FT3) and free thyroxine (FT4) were assayed by a chemiluminescent assay method using Advia Centaur CP analyzer with original kits obtained from Siemens healthcare diagnostics Ltd. (Bayswater Victoria, Australia). Serum ferritin levels were analysed on chemiluminescence using Siemens kits. The ADVIA Centaur CP Ferritin assay is a two site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two anti ferritin antibodies. The first antibody, in the lite reagent is a polyclonal goat anti ferritin antibody labeled with acridinium ester. The second antibody, in the solid phase is a monoclonal mouse antiferritin antibody, which is covalently coupled to paramagnetic particles [7]. Total iron binding capacity (TIBC) was analysed by colorimetric method with precipitation using kits of Giese diagnostics srl (Colle Presentio, Rome,

Italy). The iron carrier protein transferrin is saturated with the addition of trivalent iron ions. The unbound iron is removed by precipitation with basic carbonate of magnesium and supernatant tied to the iron dose is the total iron binding capacity [8]. Serum iron was estimated by colorimetric method using kits of Giese diagnostics srl (Colle Presentio, Rome, Italy). Transferrin bound iron is released at an acid pH in serum. Fe (III) and Fe (II) react with chromazurol B(2,6-dichloro-4-hydroxy-3,3-dimethylfuchstone-5,5-dicarboxylic acid) and the cetyltrimethylammonium bromide (CTMA), forming a ternary blue colour complex. The intensity of colour is proportional to sample iron concentration [9]. Free transferrin levels were then calculated using the formulae (Transferrin = TIBC-serum iron) [7]. Erythrocytic parameter analyses were done by hemocytometry.

Normal ranges of different parameters used in the study are as following: TSH (0.3-5.0  $\mu$ IU/mL), TT<sub>3</sub> (70-200 ng/dL), TT<sub>4</sub> (5.5-13.5  $\mu$ g/dL), FT<sub>3</sub> (2.3-4.2 pg/mL), FT<sub>4</sub> (0.89-1.76 ng/dL), ferritin (22-322 ng/mL in males and 10-291 ng/mL in females), TIBC (250-420 mg/dL), iron (60-150  $\mu$ g/dL in males and 40-145  $\mu$ g/dL in females) and transferrin (150-340 mg/dL).

All statistical analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS) version 20 for windows. Values shown in the text, tables and figures are mean  $\pm$ SD. Student t test was applied for comparison of means of study groups. p value < 0.05 was considered significant. Correlations between groups were analyzed using Pearson correlation coefficient (r) formula.

## Results

The mean age of the patients in hyperthyroid group was  $48.45 \pm 12.35$  (20-65) years, while in control group was  $45.14 \pm 10.8$  (20-63) years. Out of 50 patients, 7 were males and 43 were females in hyperthyroid group while there were 5 males and 45 females in control group. The biochemical parameters are shown in table 1. BMI (body mass index) was found to be slightly lower in hyperthyroid patients ( $27.34 \pm 0.78$  kg/m<sup>2</sup>) as compared to controls ( $31.25 \pm 0.84$  kg/m<sup>2</sup>;  $p < 0.001$ ). Our results revealed that TT<sub>3</sub>, TT<sub>4</sub>, FT<sub>3</sub> and FT<sub>4</sub> levels were significantly elevated while TSH levels were significantly decreased in hyperthyroid patients. Serum ferritin ( $314.43 \pm 68.7$  ng/mL) and iron concentration ( $159.88 \pm 36.28$   $\mu$ g/dL) was found to be increased in hyperthyroid patients as compared to healthy controls ( $255.23 \pm 45.5$  ng/mL and  $110.52 \pm 20.52$   $\mu$ g/dL respectively). There was a significant difference between hyperthyroid patients and healthy controls in serum levels of ferritin and iron ( $p < 0.05$  for both). Ferritin levels were correlated significantly positive with FT<sub>3</sub> ( $r = 0.200$ ,  $p = 0.032$ ; Fig. 1). A positive correlation was found with iron ( $r = 0.021$ ,  $p = 0.922$ ) and negative with transferrin levels ( $r = -0.069$ ,  $p = 0.742$ ) but the correlation was not significant statistically. Serum iron was correlated negatively with TSH ( $r = -0.440$ ,  $p = 0.028$ ; Fig. 2) and serum transferrin levels ( $r = -0.853$ ,  $p = 0.000$ ) and the correlation was statistically significant. Transferrin levels were correlated positively and statistically significantly with TSH ( $r = 0.448$ ,  $p = 0.025$ ; Fig. 3).

## Discussion

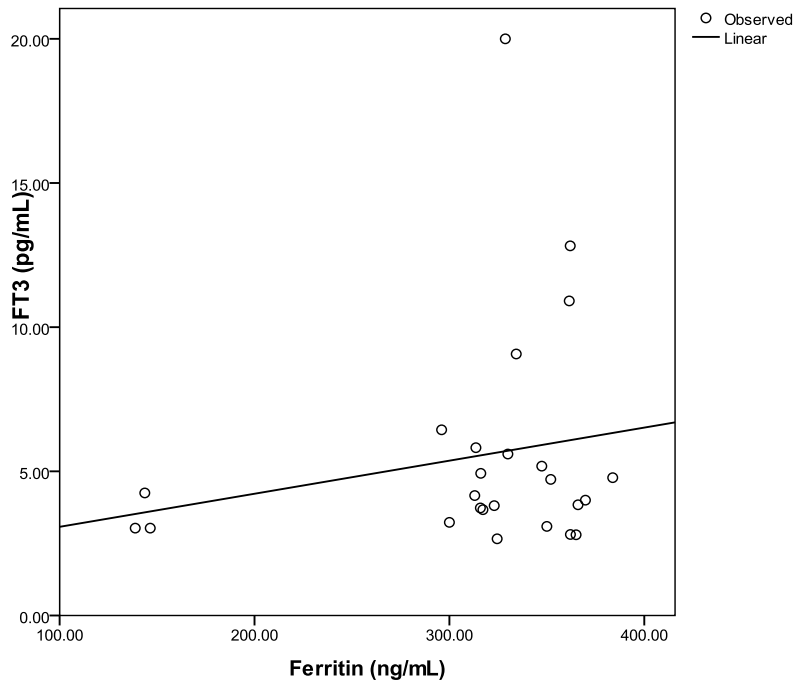
The results of present study showed an increased level of serum ferritin and iron while decreased levels of transferrin in hyperthyroid patients. Increase in serum ferritin levels in patients with hyperthyroidism may be due to the direct action of thyroid hormones on its synthesis and release [4]. However, the molecular mechanisms involved in the hepatic regulation of ferritin expression by T<sub>3</sub> remain to be determined. These results are in accordance with other studies which also showed an increased level of serum ferritin in hyperthyroid patients [5, 6]. Serum ferritin has been widely accepted as an acute-phase reactant (any substance in blood which increases in response to an acute condition such as infection, injury, inflammation etc.) and is nonspecifically elevated in a wide

variety of inflammatory states including infection, malignancy, and autoimmune diseases [10, 11]. This could be one of the reasons for increased ferritin levels.

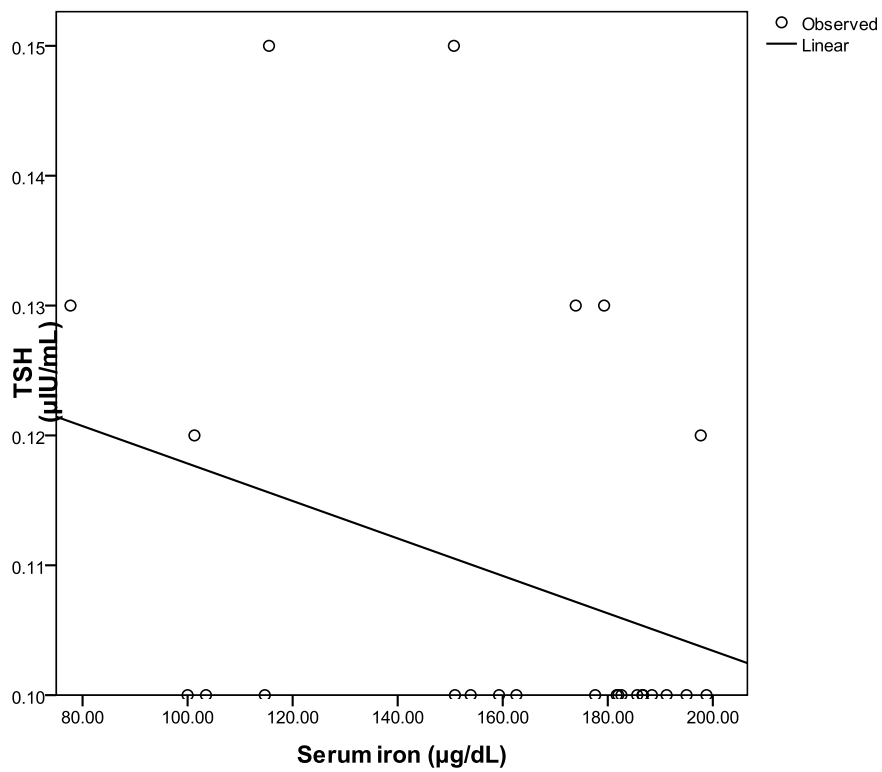
**Table 1** Biochemical parameters of hyperthyroid patients and healthy controls

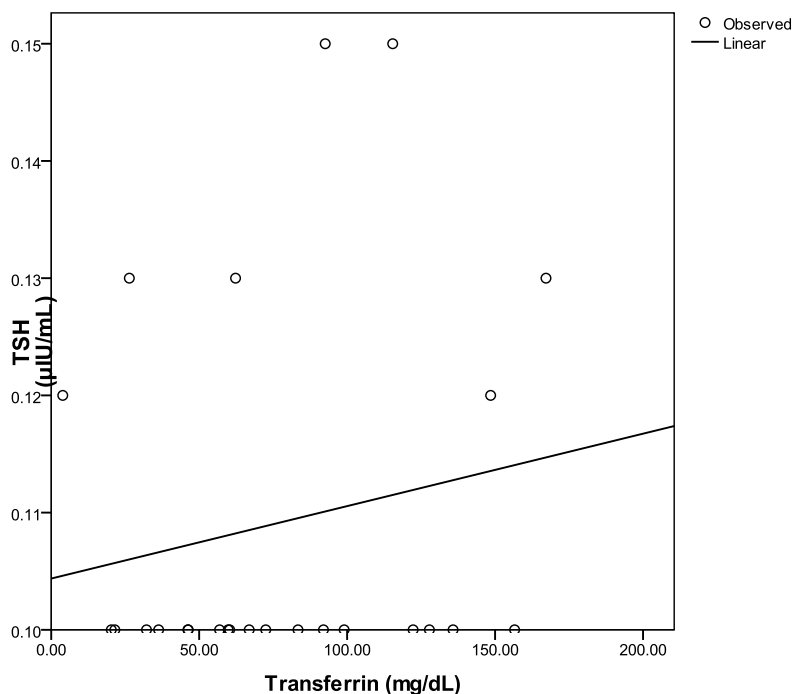
Parameter	Healthy controls	Hyperthyroid patients	p value
Number	50	50	-
Age	45.14 ± 10.8	48.45 ± 12.35	0.5
BMI (kg/m <sup>2</sup> )	23.25 ± 0.84	21.34 ± 0.78	0.03*
Hb (g/dL)	14.05 ± 6.66	12.73 ± 0.35	0.02*
Hematocrit (ratio%)	42.94 ± 0.83	39.21 ± 1.50	0.03*
TT3 (ng/dL)	131.08 ± 22.52	249.24 ± 63.80	0.000**
TT4 (µg/dL)	7.98 ± 2.32	13.15 ± 5.67	0.009*
TSH (µIU/mL)	1.67 ± 1.77	0.10 ± 0.01	0.000**
FT3 (pg/mL)	3.67 ± 1.11	5.53±3.93	0.001*
FT4 (ng/dL)	1.64 ± 0.98	2.17±1.78	0.001*
Ferritin (ng/mL)	255.23 ± 45.5	314.43 ± 68.7	0.004*
TIBC (mg/dL)	350.55 ± 36.6	237.98 ± 24.33	0.02*
Serum iron (µg/dL)	110.52 ± 20.52	159.88 ± 36.28	0.005*
Transferrin (mg/dL)	240.03 ± 16.1	78.09 ± 46.18	0.02*

\*Significant; \*\*Highly significant; all values are in mean ± SD.



**Figure 1** Scatter diagram showing correlation of serum ferritin with FT3 in cases ( $r = 0.200$ ,  $p = 0.032$ ).



**Figure 2** Figure showing correlation of TSH with serum iron in cases ( $r = -0.440$ ,  $p = 0.028$ ).**Figure 3** Scatter diagram showing correlation of TSH with serum transferrin in cases ( $r = 0.448$ ,  $p = 0.025$ ).

In relation to thyroid hormone and ferritin, many controversial reports are available in literature. It has been observed in one study that thyroidectomy induced hypothyroidism was associated with increased rat hepatic ferritin content, which may be because of post-transcriptional changes in the ferritin synthetic rate [1, 12, 13]. Since, there is controversy regarding effect of thyroid hormones on ferritin levels, post-surgical state being responsible for these findings cannot be ruled out in this case. Also, administration of T3 to hypothyroid individuals produced a significant increase in the serum ferritin level supporting our findings [3]. More recently, hyperthyroid rats with elevated T3 and T4 levels were found to have 38% increased liver ferritin protein synthesis rate over controls [1].

We previously reported that total iron content was increased in patients of hyperthyroidism; although the origin of increased iron content could not be established. 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 compared with transferrin's carrying capacity of only 2 per transferrin. Thus, increase in iron can be due to an elevated level of serum ferritin [3].

Thyropoxidase (TPO) is an iron-containing enzyme that initiates the first two steps in thyroid hormone synthesis [14]. In thyrotoxicosis, activity of this enzyme is increased, so more iron will be required for its proper action which may lead to increased ferritin. As it is well known that when iron levels are low, ferritin synthesis is decreased; similarly, when iron levels are high, ferritin synthesis increases [15]. It has long been known that iron is involved in the translational regulation of ferritin and it has been shown in a study that the initiation of synthesis of ferritin can be reversibly inhibited by removing iron from the culture medium with the iron chelator deferoxamine [16]. Thyroid hormone stimulates erythropoiesis. This is attributed to direct hormonal effect on the erythroid

marrow and to increased erythropoietin secretion leading to increased iron turnover [17] Findings of the present study may be consequent to increased iron turnover.

Ferritin occurs in two forms, H- and L-ferritin. H-ferritin is known to reduce iron-induced oxidative stress by capturing and decreasing intracellular free iron. So, increased ferritin in hyperthyroidism could play a role in protection from oxidative damage. Previous studies have shown the presence of oxidative stress in hyperthyroid patients [18]. Ferritin H chain is transcriptionally regulated by oxidative stress [19, 20]. The increase in ferritin synthesis is mediated by heme oxygenase (HO), an enzyme induced by oxidative stress that liberates iron from heme. This may lead to increased iron. The released iron stimulates ferritin synthesis through the IRE-IRP machinery and the newly synthesized ferritin reduces the amount of iron available for reactive oxygen species (ROS) production and cellular damage. So, increased ferritin synthesis may be closely associated with protection against iron-induced oxidative stress [2].

Transferrin is synthesized in the liver. It is a plasma glycoprotein which is expressed in all mammals and it helps in iron transport. The low concentration of transferrin appears to reflect the increased iron stores. In addition, transferrin is a negative acute phase protein (protein whose concentration decreases in response to acute phase reaction like in inflammation) which leads to its decreased concentration in hyperthyroidism. Hyperthyroidism is an oxidative stress like condition causing damage to transferrin by neutrophil-derived superoxide which leads to protein fragmentation and increased ferrous iron release [11] leading to increase in iron and decrease in transferrin levels as depicted from present study also.

Serum ferritin levels are assumed to be an atherosclerotic risk factor. Salonen et al. demonstrated that a ferritin concentration  $\geq 200$   $\mu\text{g/L}$  was associated with a 2.2fold increase in the risk of acute myocardial infarction in men [21]. Iron catalyzes the formation of reactive oxygen species through the Fenton and Haber-Weiss reactions. Free radicals cause lipid peroxidation, leading to the modification of LDL at the molecular level, facilitating its deposition and leading to the formation of atherosclerotic plaque [22, 23]. Thus, increased ferritin levels enhance oxidation of LDL increasing cardiovascular abnormalities. Transferrin is one of the most important antioxidant. Apotransferrin, at physiological concentrations, is known to inhibit lipid peroxidation in the liposome model by iron-binding [11]. But decreased level of transferrin in study population is also pointing towards increased cardiovascular risk factor. So, increased ferritin and iron along with decreased transferrin levels in hyperthyroidism increase the overall cardiovascular risk. Further investigations are required to confirm this observation.

## Conclusion

Our data suggests that alterations in thyroid status in a given individual produce changes in serum ferritin, iron and transferrin levels. Increased ferritin levels seem to be protective against increased oxidative stress seen in hyperthyroidism but, at the same time, changes in other parameters may point towards atherosclerotic risk also.

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