

## ORIGINAL RESEARCH

## IRON PROFILE, VITAMIN B12 STATUS AND SERUM LACTATE DEHYDROGENASE IN HYPOTHYROIDISM AND THEIR CORRELATION WITH THYROID FUNCTION: A CROSS-SECTIONAL STUDY

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### ABSTRACT

**Background:** The systemic effects of hypothyroidism extend beyond the thyroid axis to iron handling, vitamin B12 status and markers of cellular and muscle stress such as lactate dehydrogenase (LDH). Most published work has examined these parameters separately rather than together. **Objective:** To evaluate iron status, LDH activity and vitamin B12 levels in patients with hypothyroidism and to examine their association with thyroid function. **Methods:** A single-centre, laboratory-based, cross-sectional observational study was conducted over 18 months in the Department of Biochemistry of a tertiary teaching hospital in Rewa, Madhya Pradesh. One hundred and fifty-five patients with hypothyroidism aged 18–60 years were enrolled by convenience sampling from the outpatient and inpatient departments. Free T3, free T4 and thyroid-stimulating hormone (TSH) were measured by chemiluminescent immunoassay, and serum iron, ferritin, total iron-binding capacity (TIBC), LDH and vitamin B12 by standard automated methods. Normality was assessed by the Shapiro–Wilk test and associations by Pearson correlation, with significance set at  $p < 0.05$ . **Results:** Mean age was  $45.33 \pm 8.96$  years (67 men, 88 women). Mean free T3 was  $2.21 \pm 0.18$  pg/mL, free T4  $0.61 \pm 0.07$  ng/dL and TSH  $13.27 \pm 2.38$   $\mu$ IU/mL. Serum iron ( $47.93 \pm 4.33$   $\mu$ g/dL) and ferritin ( $24.14 \pm 3.59$  ng/mL) were low with elevated TIBC ( $399.58 \pm 16.04$   $\mu$ g/dL); LDH was raised ( $276.05 \pm 14.96$  U/L), vitamin B12 was low ( $168.94 \pm 15.65$  pg/mL) and haemoglobin indicated mild anaemia ( $11.21 \pm 0.55$  g/dL). TSH correlated negatively with iron ( $r = -0.76$ ), ferritin ( $r = -0.84$ ), vitamin B12 ( $r = -0.66$ ) and haemoglobin ( $r = -0.43$ ), and positively with TIBC ( $r = 0.74$ ) and LDH ( $r = 0.57$ ; all  $p < 0.001$ ); free T3 and free T4 showed the mirror-image pattern. Neither white cell count nor red cell count correlated with thyroid function, and platelet count showed only a weak association with free T4. **Conclusion:** In this cohort hypothyroidism was accompanied by a consistent pattern of iron depletion, vitamin B12 depletion, raised LDH and mild anaemia, each correlating with the degree of thyroid dysfunction. The findings support assessment of iron and vitamin B12 status, alongside LDH, in the clinical evaluation of hypothyroid patients, although a cross-sectional design without a euthyroid comparison group cannot establish causal direction.

**Keywords:** Hypothyroidism; Iron profile; Ferritin; Vitamin B12; Lactate dehydrogenase; Anaemia

### INTRODUCTION

Hypothyroidism is a common endocrine disorder characterised by insufficient thyroid hormone secretion which, untreated, can produce serious and occasionally life-threatening systemic complications [1]. An epidemiological survey across eight Indian cities found a prevalence of approximately one in ten adults, making the condition a substantial public health concern rather than a narrow endocrine problem [2]. Because its clinical features fatigue, cold intolerance, weight gain, dry skin are non-specific and overlap with many other conditions, diagnosis rests principally on biochemistry: an elevated TSH with reduced free T4 in overt disease, and an elevated TSH with normal free T4 in the subclinical form.

Thyroid hormones and iron metabolism are linked in both directions. Iron is an essential cofactor for thyroid peroxidase (TPO), the enzyme that catalyses iodination and coupling in thyroid hormone synthesis, and experimental iron deficiency

reduces TPO activity directly [3]. The relationship also runs the other way: thyroid hormones stimulate erythropoietin production and support erythroid activity in the bone marrow, so hypothyroidism may itself impair iron utilisation and storage, creating a plausible bidirectional and potentially self-reinforcing loop between the two systems [4].

Vitamin B12 status has attracted separate but related interest. Deficiency is common in autoimmune thyroid disease, plausibly through shared autoimmune mechanisms such as antiparietal cell antibodies and atrophic gastritis, and a systematic review and meta-analysis estimated the pooled prevalence of B12 deficiency among hypothyroid patients at approximately 27% [5].

Skeletal muscle involvement is a further and less frequently measured facet of the disorder. Reduced membrane stability, diminished enzyme clearance and subtle myofibre stress are reflected biochemically in raised creatine kinase, aspartate aminotransferase and LDH, and LDH in particular has been reported to track disease severity and to normalise after thyroid hormone replacement [6].

Most published work has examined iron status, vitamin B12 and LDH largely in isolation, and data evaluating all three concurrently against thyroid function in an Indian population remain limited. This study was therefore designed to assess iron profile, LDH and vitamin B12 together in patients with hypothyroidism and to correlate each against TSH, free T3 and free T4. The current is undertaken To evaluate alterations in iron status, lactate dehydrogenase activity and vitamin B12 levels in patients with hypothyroidism and to examine their association with thyroid dysfunction.

## **MATERIALS AND METHOD**

### **Study design and setting**

This was a single-centre, laboratory-based, cross-sectional observational study carried out in the Department of Biochemistry, Shyam Shah Medical College, Rewa, Madhya Pradesh, with participants recruited from the outpatient and inpatient departments of the affiliated Sanjay Gandhi Memorial Hospital. Data collection extended over 18 months following receipt of ethical clearance from May 2024 to October 2025.

### **Ethical considerations**

The study protocol, participant information sheet and consent documents were reviewed and approved by the Institutional Ethics Committee (SSMC/IEC/2024/13751) and the study was conducted in accordance with the Declaration of Helsinki and the ICMR National Ethical Guidelines for Biomedical and Health Research Involving Human Participants [7]. Written informed consent was obtained by the investigator after explanation of the purpose and procedures of the study, and confidentiality was maintained through coded identification numbers and secure electronic data storage.

### **Participants**

Patients aged 18 to 60 years with hypothyroidism confirmed by the attending physician on the basis of clinical presentation and laboratory findings were eligible. Patients were excluded if they had comorbid conditions known to affect the parameters under study, including diabetes mellitus (taking antidiabetic medications), hypertension (taking antihypertensive medications), chronic cardiac, renal or hepatic disease, or chronic infection; conditions associated with blood loss such as peptic ulcer, haemorrhoids or other bleeding disorders; bone marrow suppression; blood transfusion within the preceding three months; pregnancy or the puerperium (within six weeks of delivery); or unwillingness to consent.

### **Sample size**

Sample size was calculated with the formula  $n = Z^2pq/d^2$ , using the reported prevalence of hypothyroidism in India ( $p = 0.11$ ,  $q = 0.89$ ) [2], a 95% confidence level ( $Z = 1.96$ ) and an absolute precision of 5% ( $d$ ), giving  $n = (1.96)^2 \times 0.11 \times 0.89 / (0.05)^2 = 150.4$ . This was rounded up and 155 consecutive consenting eligible patients were enrolled by non-random convenience sampling during the study period.

Clinical and anthropometric measurements

Blood pressure was measured with a calibrated mercury sphygmomanometer using standard clinical technique. Weight and height were measured with a calibrated scale and stadiometer respectively, and body mass index was calculated as weight in kilograms divided by height in metres squared.

Sample collection and laboratory analysis

Venous blood (approximately 5–10 mL) was drawn by venepuncture under aseptic conditions and distributed into EDTA tubes for the complete blood count and plain tubes for biochemical analyses. Serum was separated by centrifugation at 3,000 rpm for 10 minutes with precautions against haemolysis, and samples were either processed promptly, stored at 2–8 °C, or frozen at –20 °C if analysis was delayed. Avoidance of haemolysis is of particular importance for LDH, since erythrocytes contain LDH at concentrations far exceeding those of serum and even slight haemolysis produces spuriously elevated results [8].

Free T3 and free T4 were measured by paramagnetic-particle chemiluminescent immunoassay on the Beckman Coulter Access Immunoassay System, using a competitive-binding format for free T3 and a two-step format for free T4, each calibrated against multi-point calibration curves and run alongside routine internal quality control. TSH was measured on the same platform by the corresponding chemiluminescent assay. Serum iron and TIBC were measured by standard automated colorimetric methods, ferritin by immunoturbidimetric assay, LDH by a standard kinetic enzymatic assay and vitamin B12 by chemiluminescent immunoassay, all on calibrated analysers with routine internal quality control.

Statistical analysis

Data were entered, cleaned and verified before analysis. Continuous variables are presented as mean ± standard deviation and categorical variables as frequencies. Normality was assessed with the Shapiro–Wilk test; as the variables were approximately normally distributed, parametric methods were used throughout. Associations between thyroid hormones and biochemical or haematological parameters were assessed with the Pearson correlation coefficient, with a two-sided p value below 0.05 considered significant. Analyses were performed in SPSS version 20 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

RESULTS

One hundred and fifty-five patients with hypothyroidism meeting the eligibility criteria were enrolled and analysed.

General and biochemical characteristics

Mean age was 45.33 ± 8.96 years, with 67 men and 88 women (female-to-male ratio 1.3:1). Mean systolic and diastolic blood pressures were 138.13 ± 6.29 and 87.49 ± 5.35 mmHg. The thyroid profile confirmed hypothyroidism, with reduced free T3 (2.21 ± 0.18 pg/mL) and free T4 (0.61 ± 0.07 ng/dL) alongside elevated TSH (13.27 ± 2.38 µIU/mL). Iron indices showed low serum iron (47.93 ± 4.33 µg/dL) and ferritin (24.14 ± 3.59 ng/mL) with elevated TIBC (399.58 ± 16.04 µg/dL). LDH was raised (276.05 ± 14.96 U/L) and vitamin B12 was low (168.94 ± 15.65 pg/mL). Mean haemoglobin was 11.21 ± 0.55 g/dL, a value below the World Health Organization thresholds for anaemia in both men and non-pregnant women, while white cell, red cell and platelet counts lay within conventional reference limits (Table 1).

Table 1. General, biochemical and haematological characteristics of the study population (n = 155)

| Variable                        | Mean ± Standard Deviation |
|---------------------------------|---------------------------|
| Demographic and clinical        |                           |
| Age (years)                     | 45.33 ± 8.96              |
| Sex (male / female), n          | 67 / 88                   |
| Systolic blood pressure (mmHg)  | 138.13 ± 6.29             |
| Diastolic blood pressure (mmHg) | 87.49 ± 5.35              |

| Variable                             | Mean ± Standard Deviation |
|--------------------------------------|---------------------------|
| Body Mass Index                      | 26.5 ± 2.47               |
| Thyroid profile                      |                           |
| Free T3 (pg/mL)                      | 2.21 ± 0.18               |
| Free T4 (ng/dL)                      | 0.61 ± 0.07               |
| TSH (μIU/mL)                         | 13.27 ± 2.38              |
| Iron indices and other biochemistry  |                           |
| Serum iron (μg/dL)                   | 47.93 ± 4.33              |
| Ferritin (ng/mL)                     | 24.14 ± 3.59              |
| TIBC (μg/dL)                         | 399.58 ± 16.04            |
| LDH (U/L)                            | 276.05 ± 14.96            |
| Vitamin B12 (pg/mL)                  | 168.94 ± 15.65            |
| Haematological indices               |                           |
| White cell count (cells/μL)          | 7,558.43 ± 939.05         |
| Red cell count (10 <sup>6</sup> /μL) | 4.32 ± 0.38               |
| Haemoglobin (g/dL)                   | 11.21 ± 0.55              |
| Platelet count (/μL)                 | 255,697.92 ± 33,166.72    |

SD, standard deviation; TSH, thyroid-stimulating hormone; TIBC, total iron-binding capacity; LDH, lactate dehydrogenase.

## Correlation of thyroid parameters with iron indices, LDH and vitamin B12

TSH correlated negatively with serum iron ( $r = -0.76$ ), ferritin ( $r = -0.84$ ) and vitamin B12 ( $r = -0.66$ ), and positively with TIBC ( $r = 0.74$ ) and LDH ( $r = 0.57$ ), all highly significant ( $p < 0.001$ ) (Table 2). Free T3 and free T4 showed the mirror-image pattern (Tables 3 and 4): both correlated positively with iron ( $r = 0.64$  and  $r = 0.53$ ), ferritin ( $r = 0.70$  and  $r = 0.67$ ) and vitamin B12 ( $r = 0.55$  and  $r = 0.52$ ), and negatively with TIBC ( $r = -0.57$  and  $r = -0.55$ ) and LDH ( $r = -0.42$  and  $r = -0.35$ ). All correlations in Tables 2 to 4 were significant at  $p < 0.001$ .

**Table 2. Correlation of TSH with iron indices, LDH and vitamin B12 (n = 155)**

| Variable            | r (with TSH) | p value |
|---------------------|--------------|---------|
| Serum iron (μg/dL)  | -0.76        | < 0.001 |
| Ferritin (ng/mL)    | -0.84        | < 0.001 |
| TIBC (μg/dL)        | 0.74         | < 0.001 |
| LDH (U/L)           | 0.57         | < 0.001 |
| Vitamin B12 (pg/mL) | -0.66        | < 0.001 |

**Table 3. Correlation of free T3 with iron indices, LDH and vitamin B12 (n = 155)**

| Variable            | r (with free T3) | p value |
|---------------------|------------------|---------|
| Serum iron (μg/dL)  | 0.64             | < 0.001 |
| Ferritin (ng/mL)    | 0.70             | < 0.001 |
| TIBC (μg/dL)        | -0.57            | < 0.001 |
| LDH (U/L)           | -0.42            | < 0.001 |
| Vitamin B12 (pg/mL) | 0.55             | < 0.001 |

**Table 4. Correlation of free T4 with iron indices, LDH and vitamin B12 (n = 155)**

| Variable           | r (with free T4) | p value |
|--------------------|------------------|---------|
| Serum iron (μg/dL) | 0.53             | < 0.001 |

| Variable            | r (with free T4) | p value |
|---------------------|------------------|---------|
| Ferritin (ng/mL)    | 0.67             | < 0.001 |
| TIBC (µg/dL)        | -0.55            | < 0.001 |
| LDH (U/L)           | -0.35            | < 0.001 |
| Vitamin B12 (pg/mL) | 0.52             | < 0.001 |

## Correlation of thyroid parameters with haematological indices

Haemoglobin correlated significantly with all three thyroid parameters: positively with free T3 ( $r = 0.31$ ,  $p < 0.001$ ) and free T4 ( $r = 0.36$ ,  $p < 0.001$ ), and negatively with TSH ( $r = -0.43$ ,  $p < 0.001$ ). Platelet count showed a weak positive correlation with free T4 only ( $r = 0.16$ ,  $p = 0.043$ ). Neither white cell count nor red cell count correlated significantly with any thyroid parameter (Table 5).

**Table 5. Correlation of thyroid hormones with haematological parameters (n = 155)**

| Haematological parameter             | Free T3 (r / p) | Free T4 (r / p) | TSH (r / p)     |
|--------------------------------------|-----------------|-----------------|-----------------|
| White cell count (cells/µL)          | -0.05 / 0.577   | -0.04 / 0.616   | 0.02 / 0.796    |
| Red cell count (10 <sup>6</sup> /µL) | 0.03 / 0.716    | 0.15 / 0.068    | -0.07 / 0.420   |
| Haemoglobin (g/dL)                   | 0.31 / < 0.001  | 0.36 / < 0.001  | -0.43 / < 0.001 |
| Platelet count (/µL)                 | 0.03 / 0.718    | 0.16 / 0.043    | -0.11 / 0.176   |

Significant associations are those with  $p < 0.05$ .

## DISCUSSION

This cohort of 155 patients with hypothyroidism had a mean age of  $45.33 \pm 8.96$  years and a female predominance (88 of 155), consistent with the recognised pattern of rising prevalence through middle age and a higher burden in women, attributable to autoimmune predisposition, hormonal influences and genetic susceptibility [2,9,10]. Mean systolic and diastolic pressures lay towards the upper end of the normal range, in keeping with the established relationship between thyroid hormone deficiency and increased systemic vascular resistance [11,12].

The most consistent finding was a clear pattern of iron depletion: low serum iron and ferritin with elevated TIBC, the classic biochemical signature of iron deficiency, accompanied by strong correlations with thyroid function in the expected directions. This mirrors the findings of Kumar and colleagues, who reported markedly lower serum iron and ferritin with elevated TIBC in hypothyroid patients together with positive correlations between ferritin and T3 and T4 and a negative correlation with TSH [13], and those of Akhter and colleagues, who linked low ferritin to altered thyroid status through modulation of TPO activity [14]. Chatterjee and colleagues similarly described a significant relationship between iron metabolism and the thyroid hormone profile in hypothyroidism [15]. Mechanistically this is consistent with the role of iron as an essential TPO cofactor, demonstrated experimentally by Hess and colleagues in iron-deficient rats [3], while the relationship plausibly operates in both directions, since thyroxine replacement increases erythropoietin drive and with it iron demand, potentially unmasking or worsening iron deficiency [4].

Serum LDH was elevated and correlated positively with TSH and negatively with free T3 and free T4, consistent with the report of Kadam and colleagues, who found raised LDH alongside altered iron and ferritin levels in hypothyroid patients at a tertiary centre [16]. Thyroid hormone deficiency affects hepatic clearance, mitochondrial oxidative capacity and skeletal muscle membrane stability, and subclinical myopathy has been proposed as a contributor to the LDH elevation observed in hypothyroidism. The correlation pattern seen here is compatible with LDH acting as an adjunct marker of disease severity rather than an incidental finding, although LDH is an entirely non-specific enzyme and its elevation cannot be attributed to thyroid status alone in the absence of concurrent creatine kinase measurement or exclusion of haemolysis in individual samples.

Vitamin B12 was low and correlated negatively with TSH and positively with free T3 and free T4, in line with the findings of Mallick and colleagues [17], Abdulateef and colleagues [18] and Hoon and colleagues [19], and directionally consistent with the systematic review of Benites-Zapata and colleagues [5]. It should be noted, however, that the mean vitamin B12 concentration in the present cohort ( $168.94 \pm 15.65$  pg/mL) implies that the great majority of participants fell below the conventional deficiency threshold of 200 pg/mL, a proportion far exceeding the pooled prevalence of approximately 27% reported in that meta-analysis. This discrepancy may reflect the largely vegetarian dietary pattern of the population served by this hospital, differences in assay calibration and cut-off, or referral characteristics of the cohort, and it deserves explicit comment rather than being presented simply as agreement with the published literature. The mechanistic link is in any case unsettled: autoimmune thyroid disease frequently coexists with atrophic gastritis and pernicious anaemia, impairing B12 absorption, but deficiency also occurs independently of autoimmune mechanisms and may reflect diet alone, and the relative contribution of each pathway cannot be resolved from a cross-sectional design.

Mean haemoglobin was  $11.21 \pm 0.55$  g/dL and correlated positively with free T3 and free T4 and negatively with TSH, consistent with the role of thyroid hormones in stimulating erythropoietin secretion and erythroid progenitor proliferation [20] and with previous reports of reduced haemoglobin in hypothyroidism [21,22]. This value represents mild anaemia by World Health Organization criteria in both sexes rather than a haemoglobin at the lower end of the normal range, and essentially the whole cohort would be classified as anaemic on this basis. Neither white cell count nor red cell count correlated with thyroid function, and the association with platelet count was weak, suggesting that the haematological effect in this cohort was concentrated in the red cell line plausibly reflecting the combined influence of reduced erythropoietin drive, iron deficiency and vitamin B12 depletion rather than generalised marrow suppression. The dissociation between a clearly reduced haemoglobin and an essentially normal red cell count is itself of interest and would be clarified by red cell indices, which were not reported.

Taken together, these findings position hypothyroidism as more than a purely endocrine disturbance: the consistent correlations between thyroid function and iron status, LDH, vitamin B12 and haemoglobin point to a condition with measurable metabolic and haematological consequences that track the degree of thyroid dysfunction.

## Limitations

The study was conducted at a single centre with non-random convenience sampling, so the cohort may not represent hypothyroid patients generally. There was no euthyroid comparison group, which means that the observed deficiencies cannot be attributed to hypothyroidism rather than to the background prevalence of iron and vitamin B12 deficiency in this population a serious constraint in a region where both are common in the general community. Thyroid autoantibodies were not measured, so autoimmune from non-autoimmune hypothyroidism could not be distinguished, and this bears directly on the interpretation of the vitamin B12 findings. Treatment status was not recorded, so newly diagnosed and previously treated patients could not be separated. Dietary intake, which is a major determinant of both iron and vitamin B12 status, was not assessed. Finally, correlation coefficients quantify association only; the strength of the correlations reported here should not be read as evidence of causation.

## CONCLUSION

In this cohort of 155 patients with hypothyroidism, thyroid dysfunction was accompanied by a consistent pattern of iron deficiency, reduced vitamin B12, elevated LDH and mild anaemia, each correlating significantly with TSH, free T3 and free T4, while white cell and platelet counts were largely unaffected. These findings support the assessment of iron and vitamin B12 status, together with LDH, as part of the clinical evaluation of patients with hypothyroidism. Given the absence of a euthyroid comparison group, they should be regarded as hypothesis-generating, and prospective studies with matched controls and post-treatment follow-up are needed to establish whether these associations are causal and whether they are reversible with thyroid hormone replacement.

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## Conflicts of Interest

The authors declare no conflict of interest.

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