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Original Research Article

Serum Iron, Vitamin B12, and Folic Acid Levels in Hypothyroid Patients Compared with Euthyroid Controls: A Hospital-Based Cross-Sectional Comparative Study from a Tertiary Care Centre in Jaipur

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Conflict of interest: Nil

Abstract:

Background: Hypothyroidism is a common endocrinopathy with haematopoietic effects, and concurrent deficiencies of iron, vitamin B12, and folic acid contribute to its clinical burden. Integrated assessment in north Indian cohorts remains limited.

Aim: To compare serum iron, vitamin B12, folic acid, and thyroid hormones between hypothyroid patients and euthyroid controls at a tertiary care centre in Jaipur.

Materials and Methods: Hospital-based cross-sectional comparative study at the Department of Biochemistry, SMS Medical College, Jaipur. Sixty subjects — 30 newly diagnosed primary hypothyroid patients (TSH >4.5 μIU/mL) as cases and 30 age- and gender-matched euthyroid controls — were enrolled. Serum TSH, fT3, fT4, iron, B12, and folic acid were measured by CLIA and colorimetric methods. Student's t-test, Chi-square, and Pearson's correlation were applied; $p<0.05$ was significant.

Results: Cases and controls were age, sex, and diet matched. BMI was higher in cases (27.1±2.4 vs 22.6±2.1 kg/m²; $p<0.001$). Cases had higher TSH (19.91±7.72 vs 2.40±0.91 μIU/mL) and lower fT3 (2.13±0.47 vs 3.15±0.43 pg/mL) and fT4 (0.70±0.15 vs 1.30±0.22 ng/dL); all $p<0.001$. Cases also had significantly lower mean iron (65.3 vs 109.5 μg/dL), B12 (196.2 vs 512.8 pg/mL), and folic acid (4.42 vs 10.22 ng/mL); all $p<0.001$. Deficiency prevalences (cases vs controls): iron 43.3% vs 10% (OR=6.76); B12 60% vs 6.7% (OR=21.00); folate 30% vs 6.7% (OR=6.00). TSH correlated negatively with iron ($r=-0.412$, $p=0.024$); fT4 positively with iron ($r=+0.478$, $p=0.008$) and B12 ($r=+0.385$, $p=0.036$).

Conclusion: Hypothyroid patients have significantly lower iron, B12, and folic acid than euthyroid controls. Routine screening with targeted supplementation should accompany the diagnosis of hypothyroidism.

Keywords: Hypothyroidism, Serum Iron, Vitamin B12, Folic Acid, TSH, Micronutrient Deficiency, Thyrogastric Syndrome, India.

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Introduction

Hypothyroidism is one of the most prevalent endocrine disorders worldwide, characterised biochemically by reduced free triiodothyronine (fT3) and free thyroxine (fT4) accompanied by elevated thyroid-stimulating hormone (TSH).[1] Primary hypothyroidism accounts for approximately 95% of all cases and affects multiple organ systems given the fundamental role of thyroid hormones in cellular metabolism. In India, the prevalence of hypothyroidism among adults is reported at approximately 11% — notably higher than the 4–5% prevalence observed in Western populations. [2,3] A multi-centric study across eight major Indian cities reported a prevalence of 15.86% in women versus 5.02% in men, confirming the marked female and age-related susceptibility. [2]

Thyroid hormones exert critical regulatory effects on haematopoiesis through enhancement of erythroid progenitor proliferation, stimulation of erythropoietin production, and direct genomic regulation of erythropoietic gene expression. [4,5] Consequently, hypothyroidism induces a hypoplastic state across myeloid lineages and is associated with anaemia in 20–60% of affected patients — earning anaemia the designation of the "haematological mask" of hypothyroidism. [6,7] The anaemia spectrum encompasses normocytic normochromic, hypochromic microcytic, and

macrocytic variants, reflecting the heterogeneous nutritional and inflammatory contributors. [8]

Iron is an indispensable component of thyroid peroxidase (TPO), the enzyme catalysing iodination of tyrosine residues during thyroid hormone synthesis.[9,10] Iron deficiency therefore impairs thyroid hormone synthesis, while hypothyroidism in turn reduces iron absorption through hypochlorhydria and altered transferrin synthesis — establishing a bidirectional relationship documented in recent systematic reviews and analytical studies.[11,17] Vitamin B12 deficiency is highly prevalent in hypothyroidism, with reports ranging from 39.6% to 68% across recent series — driven largely by the autoimmune "thyrogastric syndrome" linking autoimmune thyroid disease with autoimmune atrophic gastritis, parietal-cell destruction, and consequent loss of intrinsic factor.[18-21] A systematic review and meta-analysis of 64 studies confirmed significantly lower B12 levels in hypothyroid patients (pooled mean difference −60.67 pg/mL; p=0.01) and elevated anti-parietal-cell antibody positivity.[20]

Folic acid deficiency in hypothyroidism is less consistently studied than iron and B12 but has been documented in several Indian cross-sectional series, with concurrent deficiencies of all three micronutrients increasingly recognised as a relevant clinical entity. [23-25] Despite this body of evidence, integrated assessment of iron, B12, and folic acid alongside thyroid hormone status in north Indian cohorts remains limited, and data from Rajasthan are sparse.[13]

Against this background, the present hospital-based cross-sectional comparative study was undertaken to evaluate serum iron, vitamin B12, and folic acid levels in patients with newly diagnosed hypothyroidism in comparison with age- and gender-matched euthyroid controls at SMS Medical College and Attached Hospital, Jaipur, and to characterise the correlations between thyroid hormone status and these micronutrients. The findings aim to inform whether routine micronutrient screening should accompany the diagnosis of hypothyroidism in this population.

Materials and Methods

Study Design and Setting: This was a hospital-based cross-sectional comparative study conducted at the Department of Biochemistry, in collaboration with the Department of Medicine, Sawai Man Singh (SMS) Medical College and Attached Hospital, Jaipur, Rajasthan, India — a tertiary care teaching institution catering to patients from Jaipur and surrounding districts of Rajasthan. The study was carried out over a one-year period following approval from the Institutional Ethics Committee and was conducted in accordance with the principles of the Declaration of Helsinki and ICMR ethical guidelines. Written informed consent was obtained from every participant before enrolment.

Study Population and Sample Size: The study population comprised two groups: 30 patients newly diagnosed with primary hypothyroidism (TSH >4.5 μIU/mL with low or low-normal fT3 and fT4) attending the Medicine outpatient and inpatient services as cases; and 30 age- and gender-matched euthyroid individuals (TSH 0.4–4.5 μIU/mL with normal fT3 and fT4) attending for routine health check-up or unrelated minor complaints as controls. Sample size was estimated using the formula for comparison of two means: $n = 2 \cdot (Z\alpha + Z\beta)^2 \cdot \sigma^2 / (\mu_1 - \mu_2)^2$, with $Z\alpha = 1.96$, $Z\beta = 0.84$ (80% power), and a pooled standard deviation and effect size derived from previous studies; a minimum of 30 per group (total 60) was calculated to detect clinically significant differences in serum iron, vitamin B12, and folic acid between groups.

Inclusion and Exclusion Criteria: For cases (hypothyroid group): adults ≥18 years of either sex; primary hypothyroidism (overt or subclinical) confirmed by elevated TSH (>4.5 μIU/mL); newly diagnosed and not yet on thyroid replacement therapy; written informed consent. For controls (euthyroid group): age- and gender-matched healthy adults with TSH in 0.4–4.5 μIU/mL and normal fT3 and fT4. Common exclusions: secondary or tertiary hypothyroidism; current levothyroxine therapy; vitamin B12, folic acid, or iron supplementation within the preceding three months; known chronic kidney disease, hepatic cirrhosis, malabsorption syndromes, or inflammatory bowel disease; chronic blood loss, recent transfusion, or haemoglobinopathy; pregnancy or lactation; malignancy; medications interfering with thyroid function (amiodarone, lithium, interferon-α) or nutrient absorption (proton pump inhibitors, metformin, anticonvulsants); acute infection.

Clinical Assessment: Each enrolled participant underwent a structured clinical assessment by the principal investigator. Recorded variables included age, sex, dietary habits (vegetarian / non-vegetarian), measured anthropometry (height, weight, body mass index computed as weight/height² in kg/m²), and a targeted history of constitutional, gastrointestinal, and haematological symptoms. Patients meeting eligibility criteria were enrolled consecutively until the planned 30 cases and 30 controls were achieved.

Laboratory Investigations: Venous blood samples (5 mL) were collected from all participants under aseptic precautions, with fasting samples preferred for lipid and metabolic studies. Serum was separated within two hours and analysed in the Central Laboratory of SMS Medical College. Thyroid profile (TSH, fT3, fT4), serum vitamin B12, and

serum folic acid were measured by chemiluminescence immunoassay (CLIA) on automated immunoassay analysers; serum iron was measured by colorimetric methods on an automated biochemistry analyser. Reference ranges were: TSH 0.4–4.5 µIU/mL; fT3 2.3–4.2 pg/mL; fT4 0.8–1.8 ng/dL; serum iron 60–170 µg/dL; vitamin B12 200–900 pg/mL; folic acid 3–17 ng/mL. Deficiency cut-offs were: iron <60 µg/dL; vitamin B12 <200 pg/mL; folate <3 ng/mL.

Statistical Analysis: Data were entered in Microsoft Excel and analysed using SPSS version 26.0. Continuous variables are expressed as mean ± SD and were compared between groups using Student's independent t-test after confirming normality with the Shapiro-Wilk test. Categorical variables are expressed as frequencies and percentages and were compared using the Chi-square test, with odds ratios reported where applicable. Correlations between thyroid hormones (TSH, fT4) and micronutrient levels (iron, B12, folic acid) were assessed using Pearson's correlation coefficient. A two-tailed p-value <0.05 was considered statistically significant.

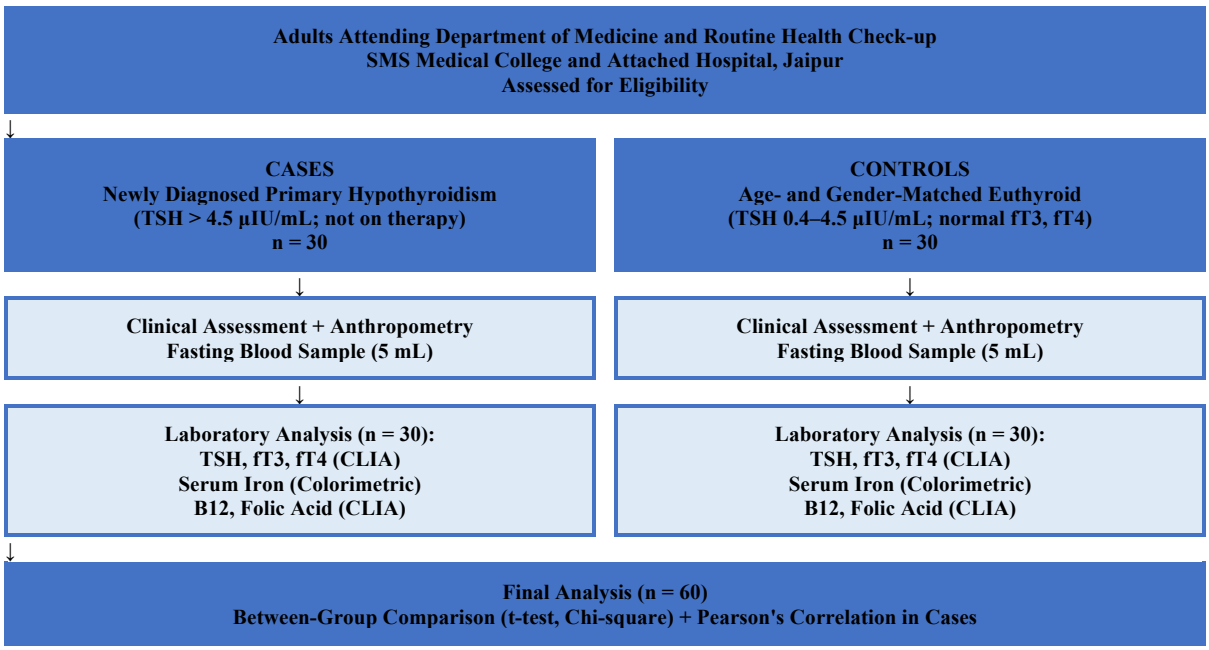

Figure 1: Study Participant Flow Diagram (Comparative Two-Arm Design)

Results

A total of 60 subjects were enrolled — 30 newly diagnosed primary hypothyroid patients (cases) and 30 age- and gender-matched euthyroid individuals (controls), with full data captured for every enrolled participant (Figure 1).

Table 1: Demographic, Anthropometric, and Dietary Profile of Cases and Controls

Variable	Category	Cases (n=30)	Controls (n=30)
Age Distribution — Mean age (cases) 40.0 ± 9.5 y; controls 40.8 ± 8.6 y (p = 0.728)	18–30 years	6 (20.0%)	5 (16.7%)
	31–40 years	9 (30.0%)	10 (33.3%)
	41–50 years	10 (33.3%)	11 (36.7%)
	51–60 years	5 (16.7%)	4 (13.3%)
Gender Distribution (χ^2 = 0.131, p = 0.717)	Female	26 (86.7%)	25 (83.3%)
	Male	4 (13.3%)	5 (16.7%)
Anthropometry			
Height (cm), mean ± SD	—	159.2 ± 6.8	160.5 ± 7.2 (p=0.475)
Weight (kg), mean ± SD	—	68.4 ± 8.9	58.2 ± 7.5 (p<0.001)
BMI (kg/m²), mean ± SD	—	**27.1 ± 2.4**	**22.6 ± 2.1 (p<0.001)**
Dietary Habits (χ^2 = 0.659, p = 0.417)	Vegetarian	21 (70.0%)	18 (60.0%)
	Non-vegetarian	9 (30.0%)	12 (40.0%)

Cases and controls were well matched for age (mean 40.0 ± 9.5 vs 40.8 ± 8.6 years; p = 0.728), gender (86.7% vs 83.3% female; p = 0.717), and dietary habits (70.0% vs 60.0% vegetarian; p = 0.417). The mean BMI was significantly higher in cases (27.1 ± 2.4 kg/m²) than controls (22.6 ± 2.1 kg/m²; p < 0.001), placing cases in the overweight category — consistent with the well-recognised metabolic

effects of thyroid hormone deficiency. Demographic profiles are summarised in Figure 2.

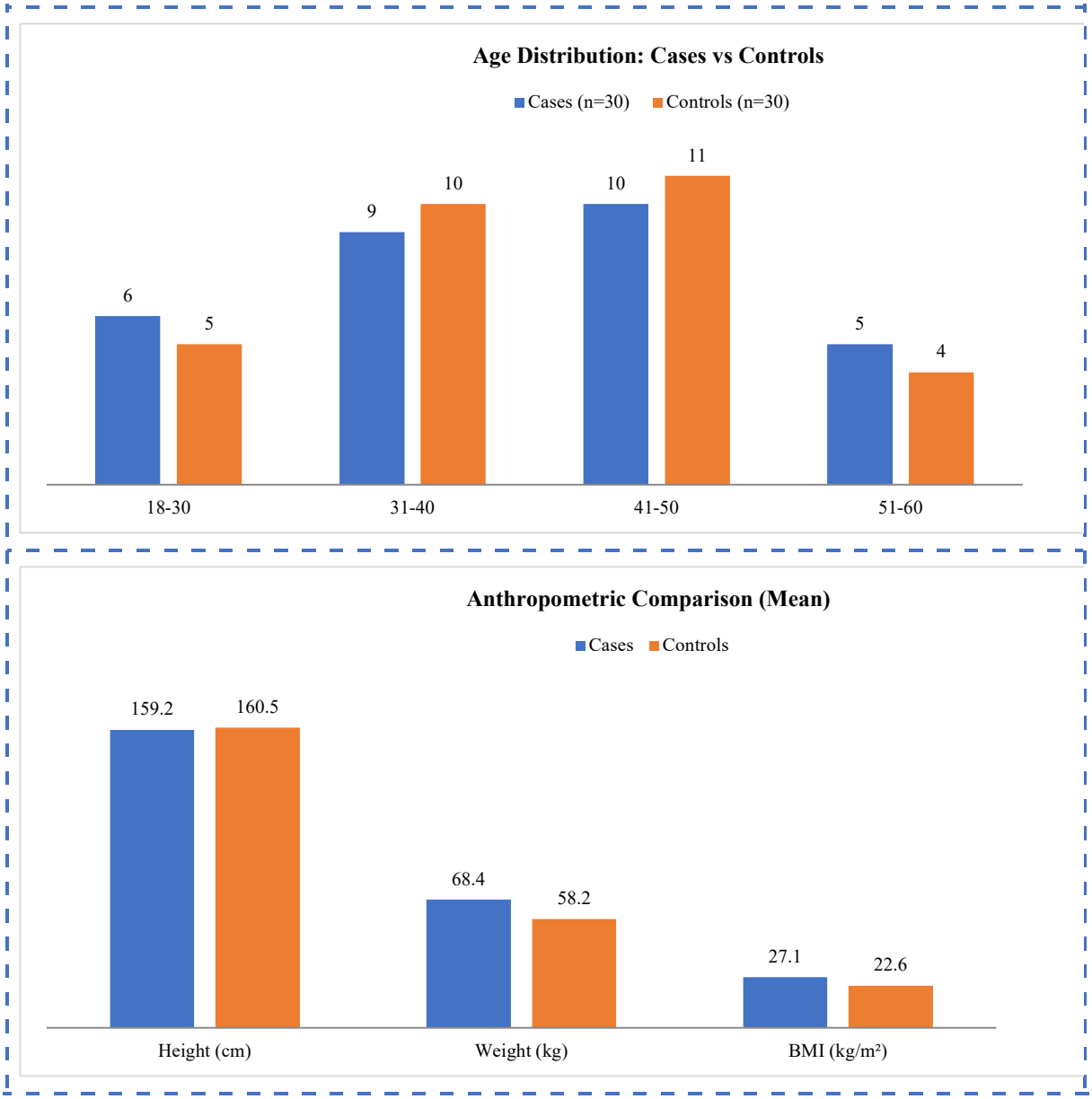

Figure 2: Demographic and Anthropometric Profile of Cases versus Controls

Table 2: Comparison of Thyroid Hormone Profile Between Cases and Controls

Parameter	Cases (n=30) Mean ± SD	Controls (n=30) Mean ± SD	Reference Range	p-value (t-test)
TSH (µIU/mL)	**19.91 ± 7.72**	**2.40 ± 0.91**	0.4–4.5	**<0.001**
fT3 (pg/mL)	**2.13 ± 0.47**	**3.15 ± 0.43**	2.3–4.2	**<0.001**
fT4 (ng/dL)	**0.70 ± 0.15**	**1.30 ± 0.22**	0.8–1.8	**<0.001**
Cases demonstrate the expected hypothyroid biochemical pattern — markedly elevated TSH and reduced fT3 and fT4 — confirming overt primary hypothyroidism; controls have all three values within reference range.				

The thyroid hormone profile clearly distinguished cases from controls. Mean serum TSH was 19.91 ± 7.72 µIU/mL in cases versus 2.40 ± 0.91 µIU/mL in controls ($p < 0.001$) — approximately an 8-fold higher mean. Mean fT3 (2.13 ± 0.47 vs 3.15 ± 0.43 pg/mL) and mean fT4 (0.70 ± 0.15 vs 1.30 ± 0.22 ng/dL) were both significantly lower in cases ($p < 0.001$ for both), with mean values in cases falling below the lower reference limit and confirming overt primary hypothyroidism in the case group.

Table 3: Comparison of Serum Micronutrient Levels and Prevalence of Deficiencies Between Cases and Controls

Parameter	Cases (n=30)	Controls (n=30)	Reference	p-value	OR
Mean Micronutrient Levels (Mean ± SD)					
Serum Iron (µg/dL)	**65.3 ± 19.2**	**109.5 ± 31.8**	60–170	**<0.001**	—
Vitamin B12 (pg/mL)	**196.2 ± 77.6**	**512.8 ± 134.1**	200–900	**<0.001**	—
Folic Acid (ng/mL)	**4.42 ± 1.84**	**10.22 ± 3.45**	3–17	**<0.001**	—
Prevalence of Deficiencies, n (%) — Chi-Square with Odds Ratio					
Iron deficiency (<60 µg/dL)	**13 (43.3)**	3 (10.0)	<60	**0.003**	**6.76**
B12 deficiency (<200 pg/mL)	**18 (60.0)**	2 (6.7)	<200	**<0.001**	**21.00**
Folate deficiency (<3 ng/mL)	**9 (30.0)**	2 (6.7)	<3	**0.020**	**6.00**
Independent t-test for continuous variables; Chi-square for categorical | OR = Odds Ratio | Cases have ~21-fold odds of B12 deficiency, ~7-fold odds of iron deficiency, and ~6-fold odds of folate deficiency compared with controls					

All three micronutrients were significantly lower in cases than controls. Mean serum iron fell from 109.5 ± 31.8 µg/dL in controls to 65.3 ± 19.2 µg/dL in cases ($p < 0.001$); mean vitamin B12 fell from 512.8 ± 134.1 pg/mL to 196.2 ± 77.6 pg/mL ($p < 0.001$) — with the case-group mean lying below the lower reference limit of 200 pg/mL; mean folic acid fell from 10.22 ± 3.45 to 4.42 ± 1.84 ng/mL ($p < 0.001$). Correspondingly, deficiency prevalence was strikingly higher in cases — iron 43.3% vs 10.0% (OR = 6.76, $p = 0.003$); B12 60.0% vs 6.7% (OR = 21.00, $p < 0.001$); folate 30.0% vs 6.7% (OR = 6.00, $p = 0.020$). Among hypothyroid cases, micronutrient deficiencies tended to be more frequent in females (iron 46.2% vs 25.0% in males; B12 61.5% vs 50.0%; folate 30.8% vs 25.0%), though these gender differences did not reach statistical significance owing to the small male subset (n = 4).

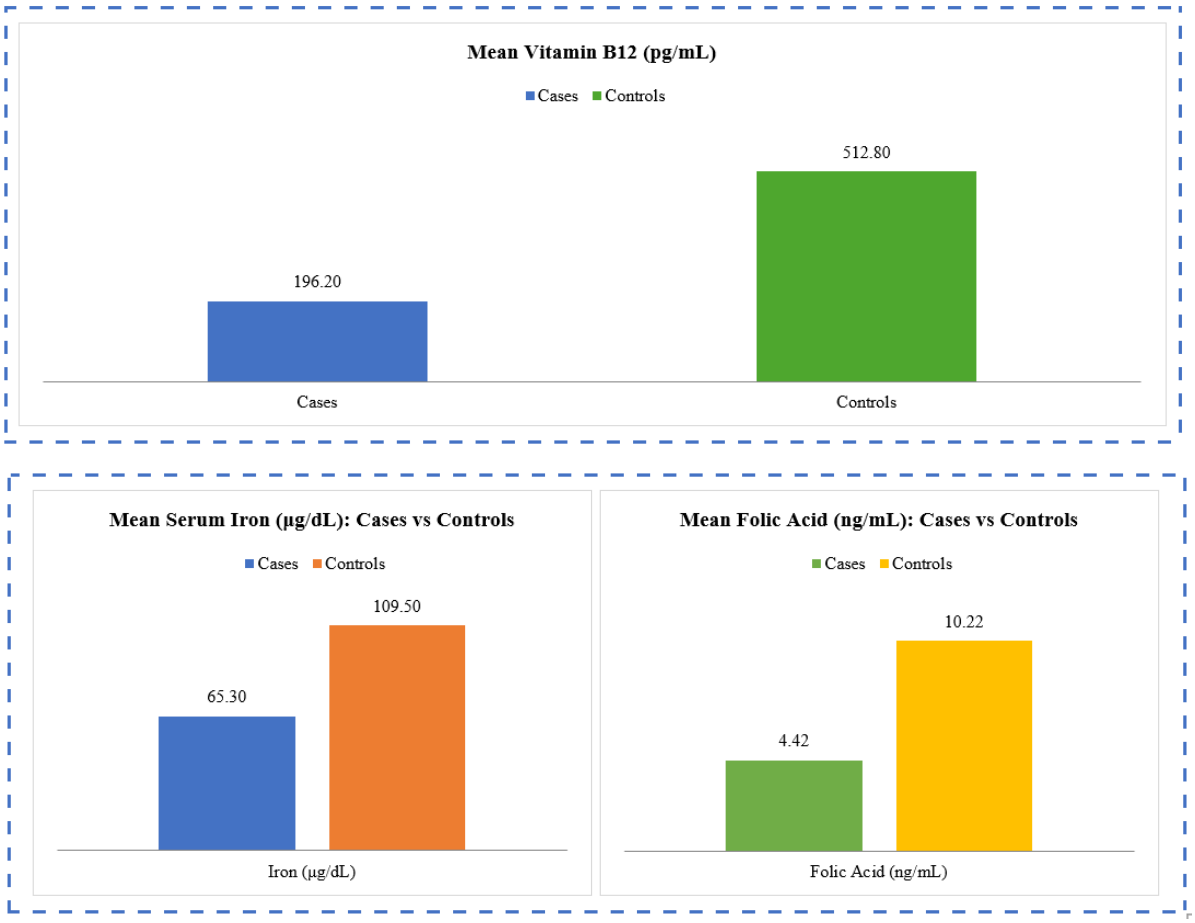

Figure 3: Mean Serum Micronutrient Levels in Cases versus Controls

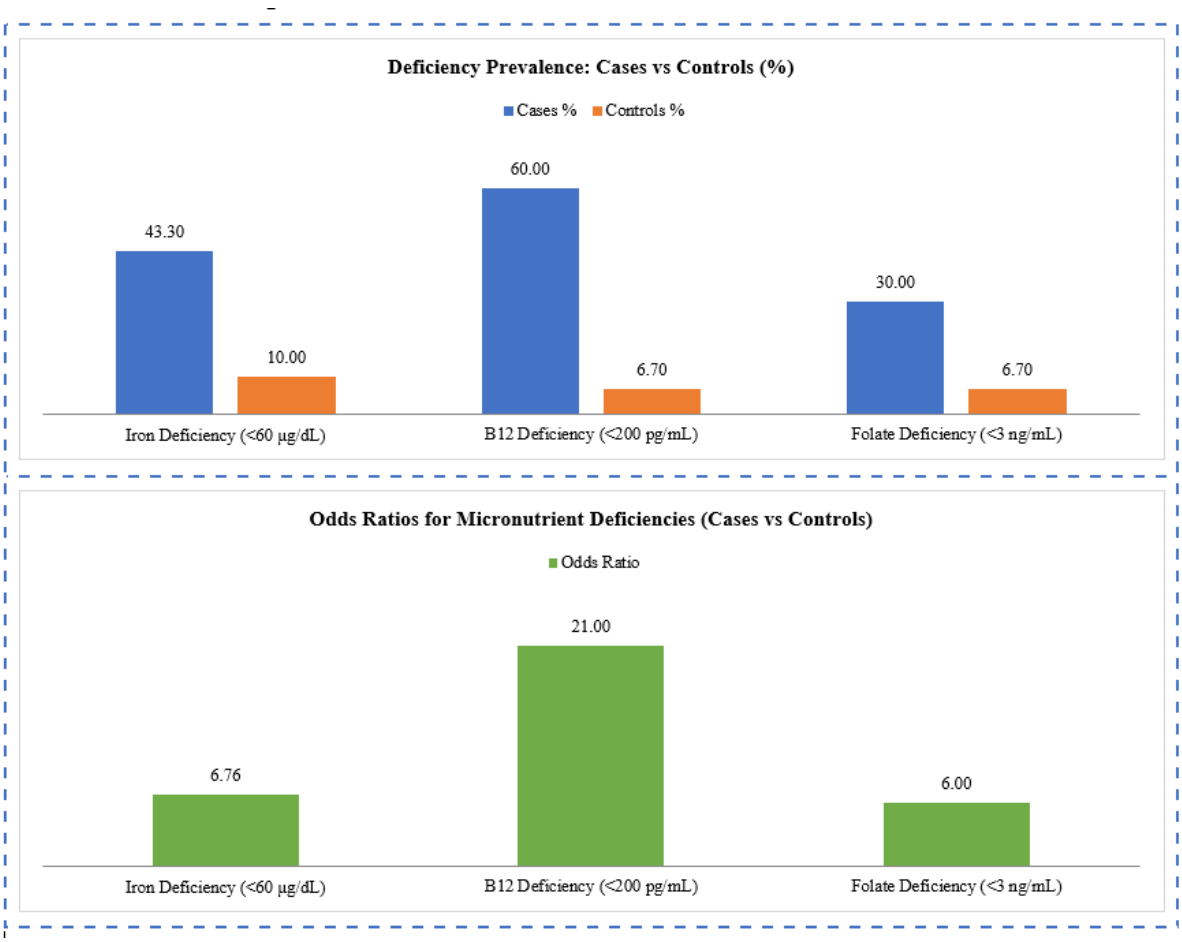

Figure 4: Prevalence of Micronutrient Deficiencies and Odds Ratios

Table 4: Pearson's Correlation of Thyroid Hormones with Serum Micronutrient Levels in Hypothyroid Cases (n = 30)

Parameters Correlated	Pearson r	p-value	Strength and Significance
TSH vs Serum Iron	**−0.412**	**0.024**	**Moderate, Significant**
TSH vs Vitamin B12	−0.356	0.053	Weak, NS
TSH vs Folic Acid	−0.289	0.121	Weak, NS
fT4 vs Serum Iron	**+0.478**	**0.008**	**Moderate, Significant**
fT4 vs Vitamin B12	**+0.385**	**0.036**	**Weak, Significant**
fT4 vs Folic Acid	+0.312	0.094	Weak, NS
Pearson's correlation coefficient (r); two-tailed test | NS = Not Significant ($p \geq 0.05$) | TSH correlates negatively with iron (the only individually significant TSH-micronutrient relationship at this sample size); fT4 correlates positively with both iron and B12, supporting the bidirectional thyroid-micronutrient relationship			

In the hypothyroid case group, TSH demonstrated a moderate significant negative correlation with serum iron ($r = -0.412$, $p = 0.024$) and weak non-significant negative correlations with B12 ($r = -0.356$, $p = 0.053$) and folic acid ($r = -0.289$, $p = 0.121$). Conversely, fT4 demonstrated a moderate significant positive correlation with serum iron ($r = +0.478$, $p = 0.008$), a weak significant positive correlation with B12 ($r = +0.385$, $p = 0.036$), and a non-significant positive correlation with folic acid ($r = +0.312$, $p = 0.094$) — collectively confirming that worsening thyroid hormone status tracks with progressive micronutrient depletion. The correlation pattern is illustrated in Figure 5.

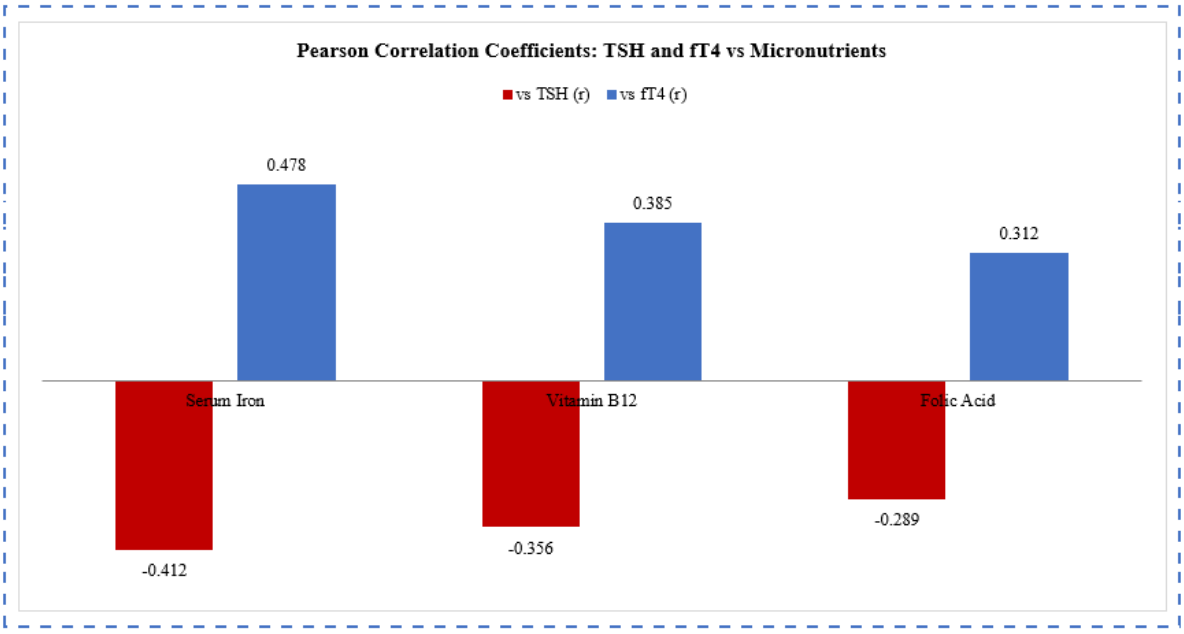

Figure 5: Pearson's Correlation Coefficients of TSH and fT4 with Serum Micronutrient Levels in Cases

Discussion

The present hospital-based cross-sectional comparative study evaluated serum iron, vitamin B12, and folic acid levels alongside thyroid hormone status in 30 newly diagnosed primary hypothyroid patients and 30 age- and gender-matched euthyroid controls at a tertiary care centre in Jaipur. Three principal findings emerged: hypothyroid patients have significantly lower serum iron, vitamin B12, and folic acid than euthyroid controls; the prevalence of micronutrient deficiencies is markedly elevated in hypothyroidism with odds ratios of 6–21; and thyroid hormone status correlates significantly with micronutrient levels, supporting a bidirectional thyroid–micronutrient relationship.

Demographic Profile and BMI: The mean age of hypothyroid cases (40.0 ± 9.5 years) and the 86.7% female predominance are consistent with the established demographic profile of Indian hypothyroid populations [2,3] Gupta et al. (2023) reported a similar mean age of 36.09 ± 12.86 years and 69% female predominance in 100 hypothyroid patients; [18] Aon et al. (2022) reported 82% female predominance in Kuwait; [19] and Aktaş (2020) reported a similar 30–50 year peak age range in autoimmune hypothyroidism. [21] The significantly higher mean BMI in our cases (27.1 vs 22.6 kg/m²; $p < 0.001$) reflects the well-recognised metabolic effects of thyroid hormone deficiency — decreased basal metabolic rate, reduced thermogenesis, and altered lipid metabolism — and matches observations from prior series.

Vitamin B12 Levels and Deficiency in Hypothyroidism: The most striking finding of our study was the markedly reduced vitamin B12 in hypothyroid cases (mean 196.2 vs 512.8 pg/mL; $p < 0.001$), with 60% of cases deficient versus 6.7% of controls — an odds ratio of 21.00. Our prevalence is consistent with the upper end of published rates: Gupta et al. (2023) reported 68% B12 deficiency in 100 hypothyroid patients;[18] Aktaş (2020) reported 46% in autoimmune hypothyroidism;[21] and Aon et al. (2022) reported 33.3% in overt hypothyroidism and 47.9% in subclinical hypothyroidism.[19] The mean B12 level of 196.2 pg/mL in our cases closely mirrors Tripathi et al. (2019) at 210.45 ± 129.30 pg/mL and Sinha et al. (2022) at the same magnitude.[23,24] The systematic review by Benites-Zapata et al. (2023), pooling 64 studies and 28,597 subjects, confirmed significantly lower B12 in hypothyroid patients (pooled mean difference −60.67 pg/mL; $p = 0.01$) and an aggregate B12 deficiency prevalence of 27% in thyroid disorders.[20]

The high B12 deficiency in hypothyroidism is mechanistically explained by the *thyrogastric syndrome* — the autoimmune cluster of thyroid disease, atrophic gastritis, parietal-cell destruction, and consequent intrinsic-factor deficiency leading to B12 malabsorption. Aon et al. found that 7.5% of B12-deficient hypothyroid patients had positive intrinsic-factor antibodies and 13.3% had positive tissue transglutaminase antibodies (suggesting concurrent celiac disease).[19] Sahoo et al. (2025) demonstrated a significant negative correlation between B12 and anti-TPO antibodies ($r = −0.159$, $p = 0.020$) in 214 subjects, supporting the autoimmune mechanism.[22] The high vegetarian proportion of our cohort (70%) likely amplifies this risk.

Iron Status and Iron Deficiency: Serum iron was significantly lower in cases (65.3 vs 109.5 µg/dL; $p < 0.001$), with iron deficiency in 43.3% of cases versus 10% of controls (OR = 6.76). Our findings align with multiple recent Indian and international series: Swapnika et al. (2024) reported 86% iron deficiency anaemia in subclinical hypothyroidism;[14] Verma et al. (2024) reported 70% with serum iron significantly lower than controls ($p = 0.0036$); [15] Sylus (2024) found significantly lower iron, ferritin, and TSAT in both overt and subclinical hypothyroidism ($p < 0.05$); [16] and Saini et al. (2024) reported approximately 15-fold lower ferritin in hypothyroid patients (9.95 vs 149.35 ng/mL; $p \leq 0.001$). [13] The systematic review by Garofalo et al. (2023) pooling 10 studies with 4,395 patients and 23,354 controls provided strong evidence for the relationship between iron deficiency and thyroid dysfunction. [11] Singh et al. (2024) summarised the bidirectional mechanism: iron is an essential cofactor for thyroid peroxidase (catalysing iodination of tyrosine residues), and hypothyroidism in turn impairs iron absorption through hypochlorhydria, altered transferrin synthesis, and impaired tissue iron utilisation.[17]

Folic Acid Levels and Deficiency: The folic acid findings in our cohort (mean 4.42 vs 10.22 ng/mL; $p < 0.001$; 30% vs 6.7% deficient, OR = 6.00) are consistent with Tripathi et al. (2019) who reported folate of 2.51 vs 6.67 ng/mL ($p < 0.0001$) and a significant positive correlation between TSH and folate ($r = 0.187$, $p = 0.013$),[24] Sinha et al. (2022) with comparable magnitudes,[23] and Dutt et al. (2023) who confirmed that folate alongside ferritin and B12 was significantly associated with thyroid hormone status.[25] The relatively lower prevalence of folate (30%) compared with B12 deficiency (60%) in our cohort is consistent with the observation that folate absorption from the upper small intestine is generally better preserved in hypothyroidism than B12 absorption — which is dependent on intrinsic factor from gastric parietal cells often affected in autoimmune atrophic gastritis.

Correlation Between Thyroid Hormones and Micronutrients: Our correlation findings — TSH negatively correlated with iron ($r = −0.412$), and fT4 positively correlated with iron ($r = +0.478$) and B12 ($r = +0.385$) — are concordant with prior series. Verma et al. (2024) reported TSH-iron $r = −0.448$ ($p = 0.0011$);[15] Sylus (2024) reported TSH-iron $r = −0.38$;[16] Saini et al. (2024) reported significant TSH-ferritin negative correlation;[13] and Aktaş (2020) reported anti-TPO–B12 $r = −0.394$ ($p < 0.001$) supporting the autoimmune link.[21] The weaker correlations for B12 and folate in our cohort (compared with iron) probably reflect the modest sample size (n = 30 in cases), and would likely strengthen in a larger series.

Clinical Implications: Three implications follow. First, the high prevalence and the magnitude of micronutrient deficiencies in hypothyroidism — particularly the 21-fold odds of B12 deficiency — justify routine screening for serum iron, vitamin B12, and folic acid at the time of hypothyroidism diagnosis, especially in vegetarian and female patients. Second, given the bidirectional nature of the iron–thyroid relationship, correction of iron deficiency may itself enhance thyroid hormone synthesis and improve response to levothyroxine therapy. Third, in patients with confirmed deficiency, supplementation should be regarded as a complementary therapeutic component of comprehensive hypothyroidism management, not as an optional adjunct.

Strengths and Limitations: Strengths of the present study include the prospective design, the simultaneous measurement of three clinically relevant micronutrients alongside the full thyroid panel, rigorous age- and gender-matching between cases and controls, and the use of gold-standard CLIA methodology for all hormonal and micronutrient assays. Limitations include the modest sample size (n = 60 total), which constrains the power for sub-group analyses (notably gender comparisons within cases); the single-centre design limiting external generalisability; the cross-sectional design which cannot establish temporal or causal relationships; the absence of anti-TPO antibody measurement, which would have allowed direct examination of the autoimmune thyrogastric link to B12 deficiency; and the lack of follow-up data on response to thyroid hormone replacement or micronutrient supplementation. Future larger multicentre prospective studies incorporating anti-TPO and intrinsic-factor antibody testing alongside longitudinal follow-up are warranted.

Conclusion

This hospital-based cross-sectional comparative study demonstrated that newly diagnosed primary hypothyroid patients have significantly lower serum iron, vitamin B12, and folic acid than age- and gender-matched euthyroid controls (all $p < 0.001$), with markedly elevated deficiency prevalences in cases (iron 43.3% vs 10%, OR = 6.76; B12 60% vs 6.7%, OR = 21.00; folate 30% vs 6.7%, OR = 6.00). TSH correlated negatively with serum iron ($r = −0.412$, $p = 0.024$); fT4 correlated positively with iron ($r = +0.478$, $p = 0.008$) and B12 ($r = +0.385$, $p = 0.036$). These findings establish a robust association between hypothyroidism and concurrent iron, B12, and folate deficiency, and support routine screening for these micronutrients alongside thyroid function testing in patients with hypothyroidism, with targeted supplementation as an integral component of comprehensive management.

Declarations

Ethical Approval: The study was approved by the Institutional Ethics Committee of SMS Medical College, Jaipur, prior to commencement and was conducted in accordance with the Declaration of Helsinki and ICMR ethical guidelines.

Informed Consent: Written informed consent was obtained from all participants before enrolment, after explanation of the study procedures in a language understood by them.

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Conflict of Interest: The authors declare no conflict of interest.

Authors' Contributions: Singh P conceptualised the study, recruited participants, performed laboratory and clinical assessments, analysed data, and drafted the manuscript. Gupta R supervised the work, contributed to methodological design, and critically revised the manuscript. All authors have read and approved the final version.

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