ORIGINAL ARTICLE

High Burden of Micronutrient Deficiency Despite Biochemical Euthyroidism in Levothyroxine - Treated Hypothyroid Patients

Induja Viswanathan[1], Vickneshwaran Vinayagam[1*], TMJ Santhoshakumari[1], Siva Ranganathan Green[2], M Sathishbabu[3]

Department of Biochemistry, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed-to-be-University), Puducherry

Department of Medicine, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed-to-be-University), Puducherry

Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry

***Corresponding Author:** Dr. Vickneshwaran Vinayagam, PhD, Department of Biochemistry, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed-to-be-University), Puducherry.

Phone: +919486363403

Abstract

Background:

Hypothyroidism is commonly treated with levothyroxine (LT_4) to restablish normal thyroid stimulating hormone (TSH) levels. Despite biochemical control, many patients continue to exhibit hypothyroid symptoms or altered metabolic indices. Several micronutrients such as selenium, zinc, magnesium, copper, iron, and vitamins A, C, and E are integral to maintaining thyroid hormone synthesis, peripheral conversion, and antioxidant protection. However, limited evidence exists regarding their status in LT_4 treated individuals.

Methods:

A cross-sectional study of 200 adults with primary hypothyroidism on stable LT_4 along with 100 age and sex matched euthyroid controls were included. Demographic and clinical details were recorded, and serum TSH, FT_4, FT_3, and micronutrient levels were assessed using standardized analytical procedures. Associations between micronutrient levels and thyroid markers were examined using correlation and regression analyses.

Results:

The mean age was 42.5 ± 11.3 years, with women comprising 70% of participants. Selenium deficiency was most prevalent (26%), followed by iron (24%) and zinc (22.5%). Patients exhibited significantly lower levels of most micronutrients compared to healthy controls ($p < 0.05$). TSH displayed weak to moderate inverse correlations with selenium ($r = –0.42$), zinc ($r = –0.35$), iron ($r = –0.38$), and vitamin D ($r = –0.48$).

Conclusion:

Some of the key Micronutrient deficiencies are observed among LT_4-treated hypothyroid patients and appear to influence thyroid hormone regulation and treatment response. Incorporating micronutrient evaluation and correction into routine hypothyroidism management may aid in achieving better metabolic and clinical outcomes.

Keywords: Hypothyroidism, Levothyroxine, Micronutrients, Vitamin deficiency, Thyroid function test

Introduction:

Hypothyroidism is a common endocrine disorder characterized by inadequate production of thyroxine (T_4) and triiodothyronine (T_3), resulting in reduced metabolic activity and multisystem involvement. (1) Its prevalence varies globally, with higher rates in iodine-deficient regions. In India, population-based studies report overt hypothyroidism in 3.9–10.95% of adults and subclinical hypothyroidism in approximately 8–10% (2) with women and older adults disproportionately affected.

Levothyroxine (LT_4) remains the standard therapy because of its predictable absorption and effectiveness in normalizing thyroid-stimulating hormone.(3) Although biochemical euthyroidism prevents complications such as dyslipidemia, cardiovascular dysfunction, and cognitive decline, an estimated 10–15% of LT_4 treated patients continue to experience persistent symptoms fatigue, weight gain, mood disturbances, and cognitive impairment despite normal TSH levels. (4) This mismatch between laboratory normalization and residual clinical symptoms is thought to involve multiple mechanisms, including impaired peripheral T_4 to T_3 conversion, altered gastrointestinal absorption, deiodinase gene polymorphisms, and metabolic comorbidities.

Micronutrients play fundamental roles in thyroid hormone synthesis, regulation, and antioxidant defence. Iodine is required for hormone biosynthesis; selenium supports deiodinase activity and protects thyroid tissue from oxidative damage (5) and zinc contributes to thyroid receptor function and gene transcription. Iron is essential for thyroid peroxidase activity, while magnesium and copper are involved in enzymatic and antioxidant pathways within thyroid regulation. Antioxidant vitamins A,

C, and E help maintain redox balance in the thyroid microenvironment.(6)

Given these physiological interactions, deficiencies of essential micronutrients may impair hormone production, conversion, and cellular responsiveness and could contribute to persistent symptoms in patients on LT_4 therapy. The present study therefore aimed to determine the prevalence of key micronutrient deficiencies among hypothyroid patients receiving long-term levothyroxine and examine their associations with thyroid function and treatment response.

Materials and Methods:

Study Design and Participants

This cross-sectional study was conducted in the Department of Medicine, Mahatma Gandhi Medical College and Research Institute, Puducherry to evaluate the association between micronutrient deficiencies and thyroid function in patients with primary hypothyroidism receiving levothyroxine (LT_4) therapy. A total of 300 participants were enrolled, comprising 200 adult patients (aged ≥18 years) with a confirmed diagnosis of primary hypothyroidism on stable LT_4 therapy (≥6 months), and 100 age- and sex-matched healthy controls. Participants were recruited consecutively after obtaining written informed consent. Ethical approval was obtained from the Institutional Ethics Committee (XXX/2023/02/IHEC/106).

Among the hypothyroid patients, 140 were classified as "controlled" (euthyroid; TSH 0.5–4.0 μIU/mL) and 60 as "uncontrolled" (TSH outside this range) despite stable LT_4 therapy. The sample size was estimated using Open Epi software version 3.1 based on previous prevalence data to achieve adequate statistical power.

Data and Sample Collection

Venous blood samples were obtained from all participants in the morning following an overnight fast. Serum was separated by centrifugation and stored at –20 °C until analysis.

Laboratory Measurements

Thyroid function tests serum TSH, total T_3, total T_4, and free T_4 were measured by chemiluminescent immunoassay (CLIA) on an automated analyzer (Cobas E411, Roche, Germany). Trace element concentrations (selenium, zinc, magnesium, copper, and iron) were determined using commercially available Colorimetric assay kit (Elabsciences, USA) with appropriate wavelength settings following standard protocols. Serum Iodine, vitamins A (retinol), E (α-tocopherol), and C (ascorbic acid) were assessed by commercially available estimation kit (Krishgen Biosystem, India).

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR), and categorical

variables as frequencies and percentages. Group differences were assessed using independent t-tests or Mann–Whitney U tests, as appropriate. Correlations between serum micronutrient levels and thyroid parameters (TSH, FT_4, FT_3) were evaluated using Pearson's correlation coefficient (r). Multiple linear regression analyses were applied to examine independent associations after adjusting for confounding factors (age, sex, body mass index, and comorbidities). Statistical significance was set at $p < 0.05$. Analyses were performed using IBM SPSS Statistics version 19.0 (IBM Corp., Armonk, NY, USA).

Results:

A total of 200 hypothyroid patients receiving levothyroxine therapy were included in the study. The baseline demographic and clinical characteristics are summarized in Table 1. The mean age of the participants was 42.5 ± 11.3 years**,** and 70% were females. The mean BMI was 26.8 ± 4.2 kg/m²**.** The median duration of levothyroxine therapy was 4.2 years (IQR 2–6)**.** Common comorbidities included hypertension (24%), diabetes mellitus (16%), and dyslipidemia (14%).

The comparison of serum micronutrient levels between hypothyroid patients and healthy controls is presented in Table 2**.** Patients demonstrated significantly lower mean levels of selenium (85.4 ± 17.5 µg/L vs 92.0 ± 18.0 µg/L, $p = 0.048$) and iron (80.2 ± 23.0 µg/dL vs 91.0 ± 22.5 µg/dL, $p = 0.015$). Zinc, vitamin A, vitamin C, magnesium, vitamin E, and copper levels were also lower in patients, although these differences did not reach statistical significance ($p > 0.05$). The prevalence of micronutrient deficiencies varied, with selenium deficiency being the most common **(**26%**),** followed by zinc **(**22.5%**),** iron **(**24%**),** magnesium (19%**),** and deficiencies of vitamins A, C, and E (14–20%).

Correlation analysis results are presented in Table 3. Overall, the associations between micronutrient levels and thyroid function parameters were weak to moderate in strength**.** Serum TSH showed significant but modest negative correlations with selenium ($r = –0.28$, $p = 0.002$), zinc ($r = –0.20$, $p = 0.015$), iron ($r = –0.30$, $p = 0.001$), and vitamin A ($r = –0.18$, $p = 0.028$). Magnesium demonstrated only a borderline association with TSH ($r = –0.16$, $p = 0.045$), while vitamin C and vitamin E showed no statistically significant relationship ($p > 0.05$). In contrast, FT_4 and FT_3 exhibited weak positive correlations with several micronutrients, particularly selenium, and iron, with correlation coefficients ranging from 0.22 to 0.28 ($p < 0.01$). Zinc and vitamin A also showed mild positive associations with thyroid hormone levels ($p < 0.05$). Copper did not demonstrate any meaningful correlation with TSH, FT_4, or FT_3 ($p > 0.30$). These findings indicate that although micronutrient status is related to thyroid biochemical parameters, the strength

of these relationships is relatively small in levothyroxine-treated hypothyroid patients.

Table 1. Baseline Characteristics of Hypothyroid Patients on Levothyroxine Therapy (n = 200)

Variable	Value (n = 200)
Age (years)	42.5 ± 11.3
Sex, n (%)	
• Male	60 (30%)
• Female	140 (70%)
Body Mass Index (kg/m²)	26.8 ± 4.2
Duration of LT_4 therapy (years)	4.2 (IQR: 2–6)
Comorbidities, n (%)	
• Hypertension	48 (24%)
• Diabetes mellitus	32 (16%)
• Dyslipidemia	28 (14%)
• Others	12 (6%)

Values are presented as mean ± standard deviation (SD) for continuous variables and as number (percentage) for categorical variables. Duration of levothyroxine therapy reflects the period of stable dosing prior to study enrollment.

Table 2. Comparison of Serum Micronutrient Levels Between Groups

Micronutrients	othyroid = 200) n ± SD	Euthyroid (n = 100) Mean ± SD	p-value	Deficiency n (%)*
Selenium (µg/L)	85.4 ± 17.5	92.0 ± 18.0	0.048	52 (26%)
Zinc (µg/dL)	78.3 ± 14.0	84.5 ± 16.5	0.080	45 (22.5%)
Magnesium (mg/dL)	1.85 ± 0.25	1.92 ± 0.28	0.120	38 (19%)
Copper (µg/dL)	95.6 ± 19.5	96.8 ± 20.2	0.650	30 (15%)
Iron (µg/dL)	84.2 ± 23.45	91.0 ± 27.26	0.048	48 (24%)
Vitamin A (µg/dL)	45.2 ± 13.5	49.0 ± 14.0	0.095	40 (20%)
Vitamin C (mg/dL)	0.85 ± 0.28	0.95 ± 0.26	0.070	34 (17%)

Micronutrients	Hypothyroid (n = 200) n ± SD	Euthyroid (n = 100) Mean ± SD	p-value	Deficiency n (%)*
Vitamin E (µg/mL)	12.4 ± 3.8	13.1 ± 3.5	0.180	28 (14%)

Values are presented as mean ± standard deviation (SD). p-values were obtained using independent sample t-tests comparing patients and controls. Deficiency was defined according to standard laboratory reference ranges for each micronutrient. $p < 0.05$ was considered statistically significant, while p-values between 0.05 and 0.10 indicate borderline significance.

Table 3. Correlation Between Serum Micronutrients and Thyroid Function Parameters

Micronutrient	TSH (r)	p-value	FT_4 (r)	p-value	FT_3 (r)	p-value
Selenium (µg/L)	–0.28	0.002	0.26	0.004	0.24	0.004
Zinc (µg/dL)	–0.20	0.015	0.19	0.018	0.17	0.002
Magnesium (mg/dL)	–0.16	0.045	0.15	0.050	0.14	0.062
Copper (µg/dL)	–0.08	0.310	0.06	0.420	0.04	0.610
Iron (µg/dL)	–0.30	0.001	0.28	0.002	0.23	0.010
Vitamin A (µg/dL)	–0.18	0.028	0.17	0.033	0.15	0.048
Vitamin C (mg/dL)	–0.14	0.055	0.13	0.068	0.12	0.085
Vitamin E (µg/mL)	–0.10	0.210	0.09	0.250	0.07	0.330

Correlation coefficients (r) were calculated using Pearson's correlation analysis. Negative r values indicate an inverse relationship with TSH, while positive r values indicate a direct relationship with FT_4 and FT_3. p-values < 0.05 were considered statistically significant. Borderline significance refers to p-values between 0.05 and 0.10.

Discussion:

In this cross-sectional study of patients with primary hypothyroidism receiving stable levothyroxine therapy, we found that micronutrient insufficiencies particularly of selenium, iron, zinc, magnesium, and antioxidant vitamins remained common despite treatment. These findings are consistent with previous reports showing persistent disturbances in micronutrient status among patients with thyroid disease even after normalization of TSH. (7 – 10) Patients exhibited significantly lower serum selenium and iron than healthy controls, although the deficiency of iron was not well documented, underscoring their vital roles in thyroid physiology.14 Selenium dependent deiodinases and glutathione peroxidases

support T_4 to T_3 conversion and shield thyroid tissue from oxidative injury. (11 – 14) Similarly, iron deficiency may reduce thyroid peroxidase activity and diminish hormone synthesis efficiency. (15 - 16) Although between-group differences in zinc, vitamin A, vitamin C, magnesium, vitamin E, and copper were not statistically significant, their comparatively lower mean concentrations parallel prior observations of broader multinutrient insufficiency in LT_4 treated individuals.(17 – 19)

TSH demonstrated mild but significant inverse correlations with selenium, iron, zinc, and vitamin A, indicating that suboptimal micronutrient stores may contribute to less favorable thyroid regulatory feedback.(20 - 23) FT_4 and FT_3 displayed modest positive correlations with selenium, iron, zinc, and vitamin A, supporting their involvement in thyroid hormone synthesis, peripheral activation, and receptor signaling.,22 Magnesium showed only a borderline association with TSH and no meaningful correlation with FT_4 or FT_3, aligning with the inconsistency of existing evidence.(24,25) Copper did not correlate significantly with any thyroid markers, consistent with literature indicating limited or indirect involvement of copper in thyroid regulation.(26) Vitamin C and vitamin E exhibited no significant associations with thyroid function tests, suggesting that their influence may act predominantly through systemic inflammatory and oxidative stress pathways rather than direct hormonal modulation.(27,2)

These findings highlight micronutrient status as a secondary but relevant modulator of thyroid hormone homeostasis in levothyroxine - treated hypothyroidism. Persistent symptoms in some patients despite biochemical euthyroidism may partly reflect unresolved micronutrient deficiencies. Interventional evidence has shown that targeted supplementation, particularly with selenium and iron, can improve thyroid autoimmunity, deiodinase activity, quality of life, and response to LT_4 therapy. (29) Integrating micronutrient screening or dietary assessment into routine follow-up may therefore support more holistic management.

The prevalence of selenium, iron, and zinc insufficiencies observed in our cohort reflects both Indian and international data. Reported variability in magnesium and vitamin A deficiencies across studies may reflect differences in dietary consumption patterns and laboratory methodology. Correlation strengths in our study fall within the mid-range of published effect sizes, reinforcing that micronutrient–thyroid interactions are biologically relevant but modest in magnitude.

Limitations

This study is limited by its cross-sectional design, absence of dietary intake data, and lack of assessment of oxidative stress markers, thyroid autoantibodies, and

deiodinase activity. These factors may have contributed to the mild correlation strengths observed. Longitudinal and interventional trials are needed to determine whether correcting micronutrient deficiencies improves LT_4 responsiveness and clinical outcomes.

Conclusion

Overall, this study reinforces that micronutrient status—particularly selenium, iron, zinc, magnesium, and vitamin A remains an important but often overlooked component of thyroid health in levothyroxine-treated hypothyroid patients. Although the associations with TSH, FT_4, and FT_3 were modest, they are consistent with physiological expectations and prior literature. Incorporating micronutrient evaluation into routine assessment may help optimize thyroid function and improve patient well-being.

Conflict of interest:

The authors do not have any conflict of interest

Source of funding:

The Vitamin A kit was procured with the institutes funding.

Acknowledgement / Declaration of Interest:

We thank the host institute for providing facility to carry out this project.

Authors contribution:

Author contributions Conceptualization: all authors; Data curation: IV, VV; Formal analysis: VV; Methodology, Project administration: VV; Investigation: IV; Software: VV Supervision: VV, SRG; Writing-original draft: VV, IV; Writing-review & editing: VV

Reference:

1. Shaheen M, et al. Micronutrients in Thyroid Function and Disorders. *Thyroid Research.* 2025.
2. Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. *Indian J Endocrinol Metab.* 2011.
3. Jonklaas J, et al. Guidelines for the treatment of hypothyroidism. *Thyroid.* 2014.
4. McAninch EA, Bianco AC. Persistent symptoms in levothyroxine-treated hypothyroid patients. *Nat Rev Endocrinol.* 2016.
5. Zimmermann MB, et al. The role of iodine, selenium, and iron in thyroid function. *Lancet Diabetes Endocrinol.* 2020.
6. Şahin M, et al. Micronutrient interactions and their impact on thyroid health. *Nutrients.* 2023.
7. Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol. 2015.

8. Winther KH, Rayman MP, Bonnema SJ, Hegedüs L. Selenium in thyroid disorders. Trends Endocrinol Metab. 2020.
9. Schomburg L. Selenium, selenoproteins and the thyroid gland: interactions and mechanisms. Endocrinology. 2020.
10. Baltaci AK, Mogulkoc R. The role of zinc in thyroid diseases. J Thyroid Res. 2020.
11. Duntas LH, Benvenga S. Selenium and the thyroid gland: new evidence. Horm Metab Res. 2015.
12. Ojha S, Jena H. Iron deficiency and thyroid dysfunction: a review. Thyroid Res Pract. 2021.
13. Zimmermann MB, Köhrle J. The impact of iron and selenium deficiencies on iodine metabolism. Ann Nutr Metab. 2002.
14. Kesarwani R, Gupta N. Micronutrient deficiencies in hypothyroidism: A clinical perspective. Indian J Endocrinol Metab. 2022.
15. Hoermann R, Midgley JEM, Larisch R, Dietrich JW. The hierarchy of thyroid homeostasis. Eur J Clin Invest. 2018.
16. Razvi S, Weaver JU, Vanderpump M, Pearce SH. Levothyroxine therapy and quality of life. J Clin Endocrinol Metab. 2012.
17. Benvenga S, Trimarchi F. Changed intestinal absorption of L-T4 caused by micronutrient deficiencies. Endocrine. 2021.
18. Kilcast D, Subramanian P. Role of magnesium in enzymatic systems relevant to thyroid metabolism. J Clin Biochem Nutr. 2018.
19. Ates S, Yazici D, Yildiz BO. Antioxidant micronutrients and thyroid function. Nutrients. 2019.
20. Zhang L, Fang X, Li J. Vitamin A status and thyroid function in adults: a systematic review. Nutrition. 2017.
21. Grune T, Lietz G, Palou A, et al. Beta-carotene, vitamin A, and thyroid function. Mol Nutr Food Res. 2010.
22. Carr AC, Maggini S. Vitamin C metabolism and immune function. Nutrients. 2017.
23. Tirosh A, Shimon I. Vitamin E and oxidative balance in thyroid dysfunction. Hormones. 2019.
24. Das C, Sahu M. Micronutrient interactions in thyroid disorders in the Indian population. J Assoc Physicians India. 2021.
25. Benvenga S, Capodicasa G. Persistent symptoms on LT4 therapy: role of micronutrients. Endocr Pract. 2021.

26. Fan Y, Xu S, Zhang H. Suboptimal outcomes despite normal TSH in LT4-treated hypothyroidism. BMC Med. 2019.
27. De Leo S, Lee SY, Braverman LE. Hyperthyroidism and hypothyroidism. Lancet. 2016.
28. McAninch EA, Bianco AC. The relevance of deiodinase polymorphisms in LT4-treated patients. Front Endocrinol. 2020.
29. Rayman MP. Multiple nutritional factors involved in thyroid disease. Proc Nutr Soc. 2019.