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Correlation of Vitamin B12, Folate, and Homocysteine with Thyroid Function in Thyroid Disorders

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Abstract

Background: Thyroid disorders are frequently accompanied by disturbances of one-carbon and micronutrient metabolism. Vitamin B12 is the coenzyme of methionine synthase, which remethylates homocysteine (Hcy) to methionine, and thyroid hormone modulates the activity of the enzymes governing Hcy metabolism. Deficiencies of vitamin B12 and folate, together with hyperhomocysteinemia, may therefore contribute to the metabolic and haematological complications of thyroid dysfunction.

Objective: To evaluate the correlation of serum vitamin B12, folate, and homocysteine with thyroid-stimulating hormone (TSH) in patients with thyroid disorders compared with euthyroid controls.

Materials and Methods: This comparative cross-sectional study enrolled 150 subjects — 100 with thyroid disorders (75 hypothyroid, 25 hyperthyroid) and 50 euthyroid controls — at the Central Biochemistry Laboratory, G.S.L. Medical College -Rajahmundry. Serum Total T3, Total T4, TSH, vitamin B12, vitamin D, homocysteine, and folate were measured by chemiluminescent immunoassay (CLIA). Data were analysed using the unpaired t-test, chi-square test, and Pearson correlation in SPSS v21.0; a p-value < 0.05 was considered significant.

Results: In hypothyroid patients, TSH correlated strongly and positively with homocysteine ($r = 0.908$, $p < 0.001$) and inversely with vitamin B12 ($r = -0.514$, $p < 0.001$) and folate ($r = -0.638$, $p < 0.001$); folate

correlated inversely with homocysteine ($r = -0.562$, $p < 0.001$). These associations were weak and non-significant in the hyperthyroid group and in controls, except for a modest inverse TSH–folate correlation in controls ($r = -0.361$, $p = 0.01$).

Conclusion: Hypothyroidism is associated with a coordinated pattern of rising homocysteine and falling vitamin B12 and folate as TSH increases. Routine assessment of vitamin B12, folate, and homocysteine in hypothyroid patients may aid early identification of at-risk individuals and guide timely supplementation.

Keywords: Hypothyroidism; Homocysteine; Vitamin B12; Folate; Thyroid-stimulating hormone; Thyroid disorders

Introduction

Thyroid disorders (TD) are among the most common endocrine conditions worldwide, and their frequency has increased over the past several decades. Beyond the classical hypothalamic–pituitary–thyroid axis, thyroid function is closely linked to micronutrient status and one-carbon metabolism. Several vitamins — notably A, D, E, and the B-complex — modulate thyroid function, and vitamin deficiencies have repeatedly been reported in patients with TD.

Vitamin B12 (cobalamin) is central to haematopoiesis and serves as a component of enzymes such as methylmalonyl-coenzyme A mutase and, critically, as the coenzyme of methionine synthase (homocysteine methyltransferase), which converts homocysteine to methionine; thyroxine regulates the activity of this enzyme. Although the causes of vitamin B12 deficiency in patients with TD are multifactorial, they relate predominantly to the comorbidity of other autoimmune disorders, impaired absorption, and dietary habits. The prevalence of vitamin B12 deficiency also increases with age. Patients with concurrent vitamin B12 deficiency and hypothyroidism commonly present with fatigue, weakness, poor memory, pruritus, and sensory loss.

Folate is a second vitamin whose intestinal absorption is impaired in hypothyroidism and which may cause macrocytic anaemia. Because humans cannot synthesise folate, it must be obtained from dietary sources such as fresh and frozen green leafy vegetables, citrus fruits and juices, wheat bread, and legumes; folate deficiency is therefore linked chiefly to low intake of these foods. Folate additionally prevents neuronal degeneration in adults, improves cognitive function, and reduces depressive symptoms. Notably, vitamin B12 deficiency may secondarily lower functional folate through the "folate trap," in which available tetrahydrofolate cannot be utilised.

Homocysteine (Hcy) is a naturally occurring sulfur-containing amino acid and an intermediate of the methionine cycle, with important roles in nucleic acid synthesis, DNA methylation, amino acid homeostasis, epigenetic maintenance, and redox processes. Under physiological conditions, about 80% of Hcy is bound to plasma proteins such as albumin through disulfide bonds, most of the remainder is bound to thiols as cystine or related forms, and only a small fraction circulates free. A higher-than-normal blood Hcy concentration is termed hyperhomocysteinemia (HHcy); the Expert Consensus on the Diagnosis and Treatment of Hyperhomocysteinemia sets the diagnostic threshold at 10 μmol/L. Homocysteine can impair deiodinase activity through oxidative stress and inflammatory mechanisms, and a biochemical link between Hcy and glutamine metabolism mediated by stress-related pathways has been described.

Homocysteine is generated by demethylation during the methionine cycle — its only source — and is metabolised mainly through two pathways: remethylation and transsulfuration. In remethylation, which occurs in a variety of tissues, Hcy acquires a methyl group from 5-methyltetrahydrofolate (derived from folic acid) or from betaine to regenerate methionine, catalysed by methionine synthase (MS). In transsulfuration, Hcy is converted to cysteine with the assistance of vitamin B6, catalysed by cystathionine-β-synthase (CBS). The two pathways coexist and complement one another but cannot fully substitute for each other; only when they are balanced is Hcy maintained within the normal range (Figure 1).

Vitamin D is a steroid molecule produced in the skin and acts through a large number of genes involved in bone metabolism and calcium and phosphorus homeostasis. Vitamin D3 insufficiency has been linked to non-skeletal effects including cancer and cardiovascular, metabolic, and autoimmune disease, such as Hashimoto's thyroiditis and Graves' disease, and impaired vitamin D signalling has been reported to promote thyroid tumorigenesis. Because vitamin D receptors are present on thyrotropes that secrete TSH, a link between vitamin D status and thyroid activity is plausible. Vitamin D deficiency in thyroid disease may be attributable to inadequate intake, malabsorption, limited sun exposure, or reduced outdoor activity. Against this background, the present study assessed the association of vitamin B12, folate, and homocysteine — with vitamin D as an additional measured parameter — with thyroid function in patients with thyroid disorders.

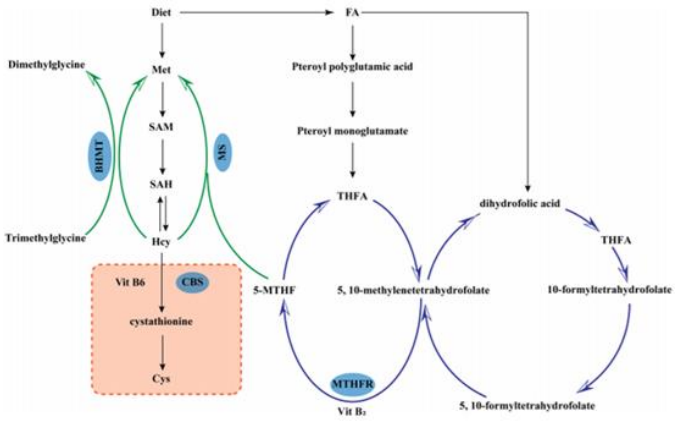

Figure 1: Metabolism of homocysteine. 5-MTHF, 5-methyltetrahydrofolate; BHMT, betaine–homocysteine methyltransferase; CBS, cystathionine-β-synthase; Cys, L-cysteine; FA, folic acid; Hcy, homocysteine; Met, methionine; MS, methionine synthase; SAM, S-adenosylmethionine; SAH, S-adenosyl-L-homocysteine; MTHFR, methylenetetrahydrofolate reductase; THFA, tetrahydrofolic acid.

Objective

To evaluate the correlation of serum vitamin B12, folate, and homocysteine with thyroid function (TSH) in patients with thyroid disorders compared with euthyroid controls.

Materials And Methods

Study design and setting

This comparative cross-sectional study was conducted in the Central Biochemistry Laboratory, G.S.L. Medical College -Rajahmundry. A total of 150 participants were enrolled, comprising 100 patients with thyroid disorders — 75 hypothyroid and 25 hyperthyroid — and 50 age-matched euthyroid controls.

Inclusion criteria

- Adults aged 15–65 years.
- Euthyroid (control) and thyroid-disorder (hypothyroid or hyperthyroid) groups.

Exclusion criteria

- Hypertension or diabetes mellitus.
- Secondary hypothyroidism.

Sample collection

After an overnight fast, 5 mL of venous blood was collected from each subject. Samples were allowed to clot completely at room temperature and were then centrifuged for 10 minutes at approximately 3,500 rpm to separate serum for analysis.

Biochemical analysis

Serum concentrations of Total T3, Total T4, TSH, vitamin B12, vitamin D, homocysteine, and folate were determined by chemiluminescent immunoassay (CLIA).

Statistical analysis

Results are expressed as mean ± standard deviation (SD) and percentages. The chi-square test was used to compare categorical variables between the hypothyroid and control groups, and the unpaired t-test was used to compare study parameters between cases and controls. Pearson correlation coefficients were calculated among the study variables. A p-value < 0.05 was considered statistically significant. All analyses were performed using SPSS version 21.0.

Results

A total of 150 subjects were included in the study: 100 with thyroid abnormalities (75 hypothyroid, 25 hyperthyroid) and 50 euthyroid controls. The Pearson correlations among TSH, homocysteine, folate, and vitamin B12 across the three groups are summarised in Table 1.

In the hypothyroid group, TSH showed a strong positive correlation with homocysteine ($r = 0.908$, $p < 0.001$) and significant inverse correlations with vitamin B12 ($r = −0.514$, $p < 0.001$) and folate ($r = −0.638$, $p < 0.001$). Folate was inversely correlated with homocysteine ($r = −0.562$, $p < 0.001$). By contrast, none of these correlations reached significance in the hyperthyroid group, and only the TSH–folate correlation was significant among controls ($r = −0.361$, $p = 0.01$).

To characterise the strength and precision of the significant hypothyroid correlations, derived inferential statistics were computed from the reported coefficients and sample size (Table 2). The TSH–homocysteine relationship was the strongest, explaining approximately 82% of the shared variance ($r^2 = 0.824$), with a narrow 95% confidence interval (0.858 to 0.941). The inverse TSH–folate, folate–homocysteine, and TSH–vitamin B12 correlations were moderate ($r^2 = 0.41$, 0.32, and 0.26, respectively) and all statistically significant. The corresponding scatter diagrams are shown in Figures 2–5.

Table 1: Pearson correlation coefficients (r) and significance (p) among TSH, homocysteine, folate, and vitamin B12 by thyroid status.

Correlation	Hypothyroid (n=75)	Hyperthyroid (n=25)	Euthyroid controls (n=50)
TSH – Homocysteine	r = 0.908	r = 0.191	r = 0.222
	p < 0.001*	p = 0.360	p = 0.122
TSH – Folate	r = −0.638	r = −0.0002	r = −0.361
	p < 0.001*	p = 0.999	p = 0.010*
Folate – Homocysteine	r = −0.562	r = −0.230	r = 0.018
	p < 0.001*	p = 0.269	p = 0.901

TSH – Vitamin B12	r = −0.514	r = 0.161	r = −0.060
	p < 0.001*	p = 0.442	p = 0.679

* Statistically significant (p < 0.05).

Table 2: Derived inferential statistics for the significant correlations in the hypothyroid group (computed from r and n).

Correlation (Hypothyroid, n=75)	95% CI (Fisher z)	r	r^2	t	df	p
TSH – Homocysteine	0.858 to 0.941	0.908	0.824	18.52	73	<0.001
TSH – Folate	−0.756 to −0.481	−0.638	0.407	−7.08	73	<0.001
Folate – Homocysteine	−0.700 to −0.384	−0.562	0.316	−5.81	73	<0.001
TSH – Vitamin B12	−0.664 to −0.325	−0.514	0.264	−5.12	73	<0.001

CI, confidence interval; df, degrees of freedom. Confidence intervals were derived via Fisher z-transformation; $t = r\sqrt{(n-2)}/\sqrt{(1-r^2)}$

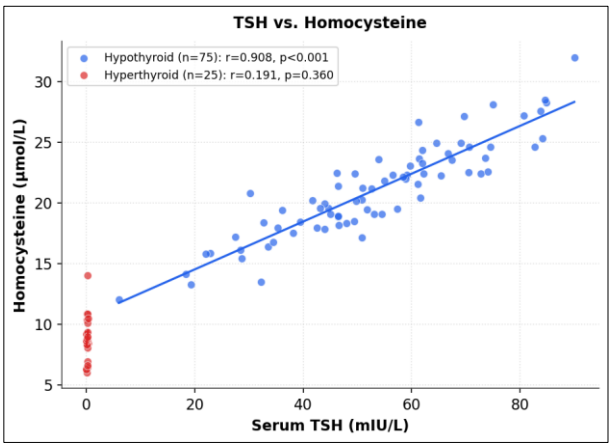

Figure 2: Scatter diagram showing the association between TSH and homocysteine in the hypothyroid and hyperthyroid groups.

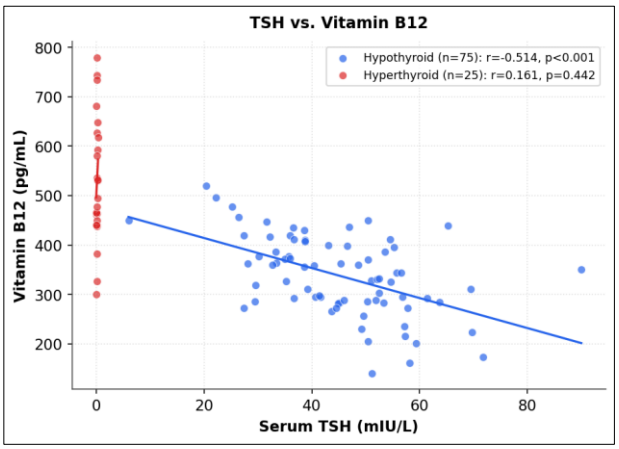

Figure 3: Scatter diagram showing the association between TSH and vitamin B12 in the hypothyroid and hyperthyroid groups.

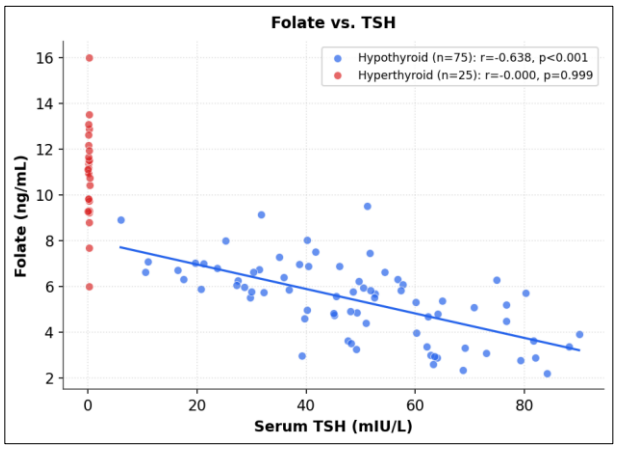

Figure 4: Scatter diagram showing the association between folate and TSH in the hypothyroid and hyperthyroid groups.

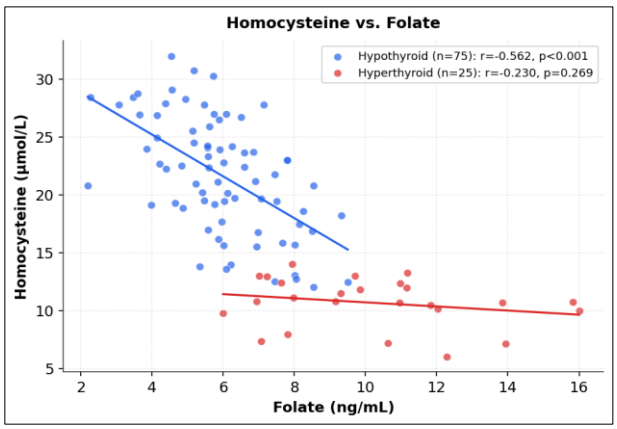

Figure 5: Scatter diagram showing the association between homocysteine and folate in the hypothyroid and hyperthyroid groups.

Discussion

The present study compared vitamin B12, folate, and homocysteine — with vitamin D as an additional measured parameter — between patients with thyroid disorders and euthyroid controls. The central finding is that, in hypothyroid patients, rising TSH is accompanied by increasing homocysteine and falling vitamin B12 and folate, whereas these relationships are absent in hyperthyroid patients and largely absent in controls.

TSH correlated positively with homocysteine ($r = 0.908$, $p < 0.001$) and negatively with vitamin B12 and folate in hypothyroid patients. This pattern is biologically coherent: because vitamin B12 and folate are the cofactor and methyl donor of the remethylation pathway, their depletion slows the conversion of homocysteine to methionine and thereby raises homocysteine. Thyroid hormone deficiency further impairs one-carbon flux and reduces the renal clearance and metabolism of homocysteine, which together explain the elevated homocysteine of hypothyroidism.

Our observations are consistent with previous reports. A study from Turkey reported vitamin B12 deficiency in 25.6% of patients with subclinical hypothyroidism and 18.6% of patients with overt hypothyroidism. Tripathi et al. reported markedly lower folate in hypothyroid patients (2.51 ± 0.99) than in controls (6.67 ± 0.83), and Catargi et al. likewise found lower folic acid in hypothyroid patients and recommended folate supplementation alongside thyroid therapy. Ziaee et al. observed that combined levothyroxine and folic acid was more effective than levothyroxine alone in lowering serum homocysteine. Conversely, in hyperthyroidism, homocysteine tends to fall: Demirbas et al. reported reduced serum homocysteine in untreated hyperthyroid patients, and Nechiporuk et al. showed that experimental hyperthyroidism accelerates transsulfuration, which may explain the lower homocysteine frequently observed in hyperthyroid states — mirroring the non-significant correlations seen in our hyperthyroid group.

The haematopoietic system is among the systems most affected by thyroid dysfunction, and anaemia is its most important manifestation. Alterations in thyroid parameters may therefore predispose to anaemia; conversely, iron-deficiency anaemia may impair thyroid function by lowering plasma T3 and T4, reducing peripheral T4-to-T3 conversion, and raising TSH. Low iron and low ferritin are among the most frequently overlooked contributors to reduced thyroid function and low vitamin B12 status. Taken together, TSH shows a positive correlation with homocysteine and negative correlations with vitamin B12 and folate, underscoring that considerable awareness is still required at the physician level in developing countries such as India for optimal management of thyroid patients.

Conclusion

Thyroid hormones exert metabolic control across the endocrine system, and a bidirectional link exists between thyroid disease and homocysteine that may exacerbate disease progression. In this study, hypothyroid patients had lower vitamin B12 and folate and higher homocysteine than euthyroid individuals, a pattern that may perturb the haematopoietic system and predispose to anaemia. Clinically, thyroid function testing — and, where indicated, thyroid ultrasound — should be performed promptly in individuals with abnormal homocysteine levels to enable early detection and intervention. Prospective studies with larger, sex-stratified cohorts and full descriptive comparisons of each biochemical parameter are warranted to confirm these associations.

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