

IMPACT OF VITAMIN D STATUS, IRON PROFILES, AND TRACE ELEMENTS IMBALANCES ON CARDIOMETABOLIC RISK FACTORS IN WOMEN WITH SUBCLINICAL HYPOTHYROIDISM

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ABSTRACT:

The main aim of this study was to elucidate the impact of vitamin D, iron profile, and trace elements imbalances on cardiometabolic risk factors in newly diagnosed women with subclinical hypothyroid. Fifty newly diagnosed subclinical hypothyroid (SCHT) females, aged 18-50 years, and 40 healthy females matched in age with patients were enrolled in the present study. Thyroid-stimulating hormone (TSH), Free thyroxine (FT4), Free triiodothyronine (FT3), vitamin D, and serum ferritin were measured using an electrochemiluminescence assay. Lipid profile, serum iron (Fe), and unsaturated iron binding capacity (UIBC) were analyzed by a photometric measuring unit. Serum zinc (Zn), magnesium (Mg), and copper (Cu) were quantified using a colorimetric method. The current study indicated a significant increase in cardiometabolic risk factors such as fasting blood sugar (FBS), lipid profile, Triglyceride-glucose index (TyGi), and Atherogenic index of plasma (AIP) in SCHT, in contrast to the healthy group. Furthermore, a strong association was noted between elevated TSH and lipid profile, fasting blood sugar, AIP, and TyGi. Additionally, a substantial association was indicated between reduced vitamin D, ferritin, magnesium levels, and elevated copper levels with cardiometabolic risk factors. The current study concluded that low vitamin D, ferritin, magnesium levels, and elevated copper levels are linked with cardiometabolic risk factors in females diagnosed with subclinical hypothyroidism. Reduced vitamin D, magnesium levels, disrupted iron metabolism, and elevated serum copper were linked to heightened atherogenic risk and metabolic abnormalities.

KEYWORD: cardiometabolic risk factors, subclinical hypothyroidism, vitamin D, iron profile, trace elements

1. INTRODUCTION

Subclinical hypothyroidism (SCHT) is a biochemical disorder characterized by elevated concentrations of thyroid-stimulating hormone (TSH) in the blood, with normal concentrations of triiodothyronine (T3) and thyroxine (T4) in the peripheral thyroid. The prevalence rate of SCHT has been steadily rising over the past few years, especially among the elderly, and ranges from 5 to 20% in the general population. Subclinical hypothyroidism (SCHT) can present with or without symptoms, depending on the individual. Some of the most common signs of SCHT include mild depression, fatigue, weak muscles, weight gain, and cold sensitivity (Hamad & Raziq, 2020; Wang *et al.*, 2024). An increased risk of cardiovascular disease (CVD), mortality, and coronary artery disease due to coronary diseases is associated with SCHT. Moreover, patients with SCHT in middle age may exhibit cognitive impairment, nonspecific signs such as emotional disturbances and fatigue. Additional cardiovascular risk factors, such as alterations in pulse and atherosclerosis, have been correlated with SCHT (Biondi *et al.*, 2019). CVDs like heart failure, atrial fibrillation, pericardial effusion, mitral valve dysfunction, and atrial tachyarrhythmia are linked with thyroid dysfunction (Lamichhane *et al.*, 2023).

Around 30-60% of the population, including adults and children, suffers from vitamin D deficiency or insufficiency; this has become a significant public health concern worldwide (Gallagher & Rosen, 2023). Vitamin D and thyroid hormones operate through analogous nuclear receptors and are expected to influence each other's effects. Hypothyroidism and hypovitaminosis D have been linked by multiple researchers (Ahi *et al.*, 2020; Appunni *et al.*, 2021). Type 1 diabetes, cardiovascular disease, certain malignancies such as breast cancer, autoimmune disorders, and melancholy have all been linked with a deficiency of vitamin D, an essential nutrient for the body (Hanna *et al.*, 2021; Pál *et al.*, 2023; Giustina *et al.*, 2024).

Metabolism and proper functioning of the thyroid gland depend on trace elements (TE), which are vital structural components of thyroid hormones (Bílek *et al.*, 2020). The thyroid endocrine system can be adversely affected by deficiencies in selenium (Se), copper (Cu), iodine (I), iron (Fe), manganese (Mn), and zinc (Zn). These TE contribute to combating oxidative stress as constituents of enzymes (Hami *et al.*, 2016; Wróblewski *et al.*, 2023). Recent studies have indicated a clear correlation between TE, including Zn, Se, Mn, Cu, and Mg, and cardiovascular risk factors, as these TE can function as both pro-

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oxidants and antioxidants, contingent upon various circumstances (Barragán *et al.*, 2022; Zarmina Khan *et al.*, 2025). Iron is a vital nutrient necessary for numerous physiological functions. Iron deficiency (ID) is a prevalent nutritional problem globally, impacting around two billion individuals, predominantly women of reproductive age and pregnant women (Kumar *et al.*, 2022). The deficiency of iron storage significantly affects the thyroid gland's functionality and hormone production, which is essential for biological processes in the body. Hypothyroidism can lead to iron deficiency anemia by undermining the body's ability to absorb iron (Abdulmawjood & Altamer, 2024). ID frequently occurs in individuals with cardiovascular disease. As many as 60% of individuals with coronary artery disease, and an even greater percentage of those with pulmonary hypertension or heart failure, have ID (Savarese *et al.*, 2023). The primary aim of this study was to elucidate the impact of vitamin D, iron profile, and trace element imbalance on cardiometabolic risk factors in women with subclinical hypothyroidism. Considering the well-documented correlation between vitamin D, iron deficiencies, trace elements imbalance, and various cardiometabolic risk factors.

2. MATERIALS AND METHOD

Study Area, Period, and Design:

This hospital-based quantitative cross-sectional study was conducted from November 2024 to January 2025 at Zakho General Hospital, Zakho City, Kurdistan Region, Iraq.

Study Population and Selection:

A total of 90 female subjects, aged between 18 and 50 years, were enrolled in the study. The cohort included 50 newly diagnosed subclinical hypothyroid female subjects with normal FT3, FT4 levels, and TSH > 4.2 pmol/L as cases, and 40 subjects who were matched with the study group in terms of gender and women of reproductive age as the control group, were admitted to the hospital for a health assessment.

Exclusion criteria for both healthy volunteers and SCHT patients included: medications capable of inducing thyroid dysfunction, diabetes mellitus, pituitary/hypothalamic disorders, liver disorders, renal disorders, dyslipidemia, cardiovascular disease, hypertension, acute or chronic systemic disease, polycystic ovary syndrome, thyroid supplements, medications that might alter iron metabolism and 25-OH vitamin D or thyroid functions, vitamin D, calcium, iron, zinc, magnesium, vitamin B12, or vitamin C supplementation, pregnant, menopause, lactating mothers.

Blood Collection and Biochemical Assessment:

Following an overnight fast of 10 to 12 hours, 5 milliliters of venous blood samples were obtained in the morning between 8:00 and 10:00 AM, placed in a gel tube, and left to stand at room temperature for about thirty minutes. The sample was centrifuged at 5000 rpm for approximately 10 minutes, after which the sera were partitioned into three aliquots for specialized analyses and stored at -80°C in a freezer for biochemical research. Fasting blood sugar (FBS), Triglyceride (TG), Total Cholesterol (TC), High-density lipoprotein cholesterol (HDL-C), TSH, free thyroxine (FT4), free triiodothyronine (FT3), Vit D, iron, unsaturated iron binding capacity (UIBC), and ferritin were analyzed by the immunoassay method using a Cobas 6000 automated analyzer (Roche, HITACHI). Total iron binding

capacity (TIBC) is expressed as TIBC S. Iron + UIBC. Very low-density lipoprotein (VLDL), Total Cholesterol (TC), and atherogenic index of plasma (AIP) were estimated using the Friedwald equation: $VLDL = TG/5$, $LDL = TC - [TG/5 + HDL]$, $AIP = \log(TG/HDL)$. TyG index was calculated using the formula: $TyGi = \ln [TG (mg/dL) \times FBS (mg/dL)/2]$. Serum zinc was measured using a commercially available kit supplied by Spectrum (REF 330 001, Germany) using the colorimetric test with

2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol a red chelates complex, and absorbance was measured at 546nm. Serum copper was quantified using a commercially available kit supplied by Spectrum (REF 232 001, Germany) using the colorimetric test with 4-(3,5-Dibromo-2-pyridylazo)-N-ethyl-sulfopropylaniline a chelate complex and absorbance was measured at 580nm, Magnesium was quantified in serum using a commercially available kit supplied by BioSystems (COD 11797, Spain) using the colorimetric method with XYLIDYL BLUE, these trace elements concentrations were measured using a JENWAY 6700 Visible Spectrophotometer.

Anthropometry And Blood Pressure Measurements:

Body weight in kg was recorded in the morning before eating, without shoes, utilizing a standard weighing scale. At the same time, height was measured with a standard measuring tape in an upright position. $BMI = [weight (kg) / height^2 (m^2)]$ is applied to calculate body mass index (BMI). Following a 5-minute rest period, systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate were assessed using an electronic sphygmomanometer (ORIENTMED).

Statistical analysis:

SPSS Statistics software [IBM, version 26] is implemented to evaluate the statistical analysis. The Shapiro-Wilk test is used to determine the normal distribution of the data; data with normal and non-normal distributions are represented as mean \pm SD. The two-group comparisons (SCHT vs. control) used the Student's t-test and the Mann-Whitney U test for normally and non-normally distributed variables, respectively. The three groups (tertiles of trace elements) were compared using the Kruskal-Wallis test for non-normally distributed variables or one-way analysis of variance (ANOVA) for normally distributed variables. The probable correlation between the analyzed values was investigated using Pearson correlation for normally distributed data and Spearman rank-order correlation for non-normally distributed data. We consider a p-value of less than 0.05 to be statistically significant.

3. RESULTS

Anthropometric and Biochemical Parameters in Control and SCHT Groups:

The comparison of different variables between individuals with SCHT and controls is shown in Table 1. The SCHT group had a mean age of 31.9 ± 7.72 years; whereas, the control group had a marginally higher mean age of 32.15 ± 8.65 years, with a non-significant difference between the SCHT and control groups ($p = 0.885$). The BMI was markedly elevated in SCHT ($p < 0.001$) in contrast to the healthy group. However, both SBP ($p = 0.679$) and DBP ($p = 0.887$) exhibit a non-significant difference between the SCHT and control groups. At the same time, PR was markedly reduced in the SCHT group ($p < 0.001$) in contrast to

the control group. TSH levels were considerably elevated in SCHT ($p < 0.001$) in contrast to the healthy group. Likewise, there were significant differences in levels of FT3 ($p = 0.005$) and FT4 ($p < 0.001$) between the two groups. FBS ($p = 0.026$), TC ($p = 0.001$), TG ($p < 0.001$), LDL-C ($p = 0.004$), VLDL-C ($p < 0.001$), TyGi ($p < 0.001$), AIP ($p < 0.001$), and hsCRP ($p = 0.006$) were considerably higher in the SCHT group in contrast to the

healthy group, while HDL-C was significantly lower in the SCHT group in contrast to the healthy group ($p = 0.019$). Serum iron, Cu, UIBC, and TIBC show no significant difference between the groups. However, Zn ($p = 0.006$), Mg ($p < 0.003$), Ferritin ($p = 0.001$), and vitamin D ($p = 0.003$) were significantly lower in the SCHT group in contrast to the control group.

Table 1: Anthropometric and biochemical parameters in control and SCHT groups.

Variable	SCHT(n=50)	Control(n=40)	P-value
Age (years)	31.9 ± 7.72	32.15 ± 8.65	0.885
BMI (Kg/m ²)	29.71*** ± 9.55	24.06 ± 3.53	< 0.001
SBP (mmHg)	123.24 ± 9.32	122.58 ± 5.72	0.679
DBP (mmHg)	76.58 ± 11.49	77.83 ± 2.64	0.887
PR (pul/min)	86.32*** ± 8.27	94.98 ± 7.19	< 0.001
TSH (μIU/ml)	7.67*** ± 4.04	2.12 ± 0.77	< 0.001
FT3(pmol/L)	5.11** ± 1.01	5.71 ± 0.92	0.005
FT4 (pmol/L)	15.05*** ± 2.42	17.82 ± 2.11	< 0.001
Zn (μg/dl)	94.27** ± 37.57	105.8 ± 32.57	0.006
Mg (mg/dl)	2.62** ± 0.38	2.93 ± 0.52	0.003
Cu (μg/dl)	105.82 ± 33.38	95.04 ± 22.06	0.070
FBS (mg/dL)	100.2* ± 14.57	94 ± 8.71	0.026
TC (mg/dL)	168.9** ± 35.16	145.7 ± 25.36	0.001
TG (mg/dL)	140.2*** ± 79.63	83.12 ± 36.58	< 0.001
HDL-C (mg/dL)	47* ± 15.32	51.22 ± 10.05	0.019
LDL-C (mg/dL)	93.34** ± 29.03	77.36 ± 21.51	0.004
VLDL-C (mg/dL)	28.04*** ± 15.93	16.63 ± 7.32	< 0.001
AIP	0.42*** ± 0.32	0.17 ± 0.23	< 0.001
TyGi	4.69*** ± 0.31	4.43 ± 0.21	< 0.001
Iron (μg/dl)	73.75 ± 35.02	83.22 ± 28.86	0.079
UIBC (μg/dl)	312.72 ± 74.72	290.69 ± 66.98	0.149
TIBC (μg/dl)	386.48 ± 53	373.80 ± 54.08	0.267
Ferritin (ng/ml)	20.63** ± 20.33	33.55 ± 21.42	0.001
hsCRP (mg/L)	3.2** ± 4.42	1.38 ± 1.63	0.006
Vitamin D (ng/ml)	21.31** ± 10.63	28.98 ± 14.68	0.003

Data are presented as mean ± SD for normally and non-normally distributed variables. p-value: <0.05 (*) is significant, <0.01 is high significant (**), $p < 0.001$ (***) statistical very high significant and >0.05 is not significant. SCHT: Subclinical hypothyroidism; BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; PR: Pulse rate; FT3: Free triiodothyronine; FT4: Free thyroxine; TSH: Thyroid stimulating hormone; FBS: Fasting blood sugar; TC: Total cholesterol; TG: Tri glyceride; HDL-C: High density lipoprotein cholesterol; VLDL-C: Very low-density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; AIP: Atherogenic Index of Plasma; TyGi: Triglyceride-glucose index; Zn: Zinc; Mg: Magnesium; Cu: Copper; TIBC: Total iron binding capacity; UIBC: Unsaturated iron binding capacity; hsCRP: highly sensitive C-reactive protein.

Correlation of TSH Level with Several Anthropometric and Biochemical Variables in Studied Subjects:

Table 2 presents a correlation between TSH level and various variables of the studied subjects. Correlation analysis indicated a non-significant association of TSH with age, SBP, DSP, HDL-C, LDL-C, Iron, UIBC, TIBC, and Cu ($p = 0.800$, $p = 0.603$, $p = 0.988$, $p = 0.324$, $p = 0.097$, $p = 0.064$, $p = 0.289$, $p =$

0.428 , $p = 0.097$), respectively. TSH exhibited a significant positive correlation with BMI, FBS, TC, TG, VLDL, AIP, TyGi, and hsCRP ($p = 0.002$, $p = 0.024$, $p = 0.005$, $p = 0.002$, $p = 0.002$, $p = 0.013$, $p = 0.001$, $p = 0.035$), respectively. In contrast, significant negative correlations were detected between TSH and PR ($p < 0.001$), FT3 ($p = 0.001$), FT4 ($p < 0.001$), Zn ($p = 0.001$), Mg ($p = 0.001$), Ferritin ($p < 0.001$), and vitamin D ($p = 0.004$).

Table 2: Correlation between TSH level and various anthropometric and biochemical variables in the studied subjects.

Variable	R	P-value
Age (years)	-0.027	0.800
BMI (Kg/m ²)	0.326**	0.002
SBP (mmHg)	0.056	0.603
DBP (mmHg)	0.002	0.988
PR (pul/min)	-0.429***	< 0.001
FT3(pmol/L)	-0.331**	0.001
FT4 (pmol/L)	-0.560***	< 0.001
Zn (µg/dl)	-0.338**	0.001
Mg (mg/dl)	-0.354**	0.001
Cu (µg/dl)	0.188	0.076
FBS (mg/dl)	0.238*	0.024
TC (mg/dl)	0.295**	0.005
TG (mg/dl)	0.324**	0.002
HDL-C (mg/dl)	-0.107	0.324
LDL-C (mg/dl)	0.176	0.097
VLDL-C (mg/dl)	0.324**	0.002
AIP	0.261*	0.013
TyGi	0.334**	0.001
Iron (µg/dl)	-0.196	0.064
UIBC (µg/dl)	0.113	0.289
TIBC (µg/dl)	0.085	0.428
Ferritin (ng/ml)	-0.383***	< 0.001
hsCRP (mg/L)	0.223*	0.035
Vitamin D (ng/ml)	-0.298**	0.004

r = Spearman's rank correlation coefficient. *Correlation at 0.05 is statistically significant, **Correlation at 0.01 is highly significant, and ***Correlation at 0.001 is very highly significant.

Correlation of Vitamin D Levels and Ferritin Levels with Cardiometabolic Risk Factors:

Table 3 demonstrates the association between Vitamin D and Ferritin levels and cardiometabolic risk factors. Correlation

analysis indicated a significant negative association between Vitamin D and TG, VLDL, and AIP ($p = 0.029$, $p = 0.029$, $p = 0.042$), respectively, in all studied objects. On the other hand, a considerable negative association was found between serum ferritin and TG ($p = 0.048$) and VLDL ($p = 0.048$).

Table 3: Correlation of Vitamin D levels and ferritin levels with cardiometabolic risk factors.

Variable	Ferritin (ng/ml)		Vitamin D (ng/ml)	
	R	P-value	R	P-value
BMI (Kg/m ²)	-0.147	0.166	-0.099	0.355
SBP (mmHg)	-0.194	0.067	0.192	0.070
DBP (mmHg)	0.007	0.945	0.096	0.370

PR (pul/min)	0.176	0.096	0.178	0.094
FBS (mg/dl)	-0.046	0.663	-0.42	0.696
TC (mg/dl)	-0.145	0.174	-0.149	0.162
TG (mg/dl)	-0.209*	0.048	-0.231*	0.029
HDL-C (mg/dl)	0.138	0.196	0.026	0.807
LDL-C (mg/dl)	-0.121	0.251	-0.108	0.311
VLDL-C (mg/dl)	-0.209*	0.048	-0.231*	0.029
AIP	-0.191	0.071	-0.215*	0.042
TyGi	-0.102	0.337	-0.140	0.189
hsCRP (mg/L)	-0.025	0.814	-0.027	0.797

r = Spearman's rank correlation coefficient.

Correlation of serum Zinc, Magnesium, Copper, and iron with cardiometabolic risk factors:

Table 4 shows the association between serum Zn, Mg, Cu, and Fe with cardiometabolic risk factors. Correlation analysis revealed a significant positive association between serum Zinc and PR ($p = 0.008$) and a significant negative association between zinc and BMI ($p = 0.049$) across all studied objects. Furthermore,

a considerable negative association was found between serum Magnesium and BMI ($p = 0.039$), TyGi ($p = 0.047$), and hsCRP ($p = 0.001$). Moreover, a significant positive association was noted between serum copper and TyGi ($p = 0.047$) and hsCRP ($p = 0.013$). On the other side, a considerable negative association was found between serum Iron and BMI ($p = 0.014$) and hsCRP ($p = 0.001$).

Table 4: Correlation of serum Zinc, Magnesium, Copper, and iron with cardiometabolic risk factors.

Variable	Zinc (ng/dl)		Magnesium (mg/dl)		Copper (ng/dl)		Iron (ng/dl)	
	R	P-value	R ² /r	P-value	R ² /r	P-value	r	p-value
BMI(Kg/m ²)	-0.208*	0.049	-0.218*	0.039	0.162	0.128	-0.257*	0.014
DBP (mmHg)	-0.166	0.117	-0.088	0.409	0.081	0.450	-0.111	0.298
SBP (mmHg)	0.124	0.244	-0.001	0.990	-0.048	0.650	-0.044	0.682
PR (pul/min)	0.280**	0.008	0.061	0.567	-0.013	0.904	0.079	0.435
FBS (mg/dl)	-0.111	0.299	-0.067	0.531	0.180	0.090	-0.044	0.683
TC (mg/dl)	0.075	0.481	-0.100	0.346	0.132	0.214	-0.021	0.841
TG (mg/dl)	-0.059	0.580	-0.068	0.524	0.114	0.284	-0.055	0.606
HDL-C (mg/dl)	-0.009	0.932	0.011	0.921	-0.001	0.989	0.146	0.170
LDL-C (mg/dl)	0.073	0.493	-0.092	0.388	0.083	0.435	-0.024	0.824
VLDL-C (mg/dl)	-0.059	0.580	-0.068	0.524	0.114	0.284	-0.055	0.606
AIP	-0.045	0.673	-0.084	0.430	0.125	0.242	-0.097	0.363
TyGi	-0.117	0.274	-0.211*	0.047	0.210*	0.047	-0.106	0.318
hsCRP (mg/dl)	-0.066	0.534	-0.320**	0.001	0.261*	0.013	-0.341**	0.001

r = Spearman's rank correlation coefficient, R² = Pearson correlation coefficient.

Comparison of Cardiometabolic Risk Factors Based on Zn, Mg, Cu, and Fe Tertiles:

A comparison of Cardiometabolic risk factors based on Zn, Mg, Cu, and Fe tertiles is presented in Table 5. Results indicated a significant difference in PR ($p = 0.020$) levels between Zn

tertiles. A decrease in PR levels was noted with a decrease in serum Zn. Furthermore, a considerable difference in TC ($p = 0.032$) levels are found between Mg tertiles. An increase in levels of TC was noted with a decrease in Mg levels. Moreover, a remarkable elevation is found in hsCRP ($p = 0.005$) with decreasing serum Fe.

Table 5: Comparison of Cardiometabolic risk factors based on Zn, Mg, Cu, and Fe tertiles.

Variable	Zinc Tertiles			p-value	Magnesium Tertiles			p-value
	T1 (n=29) < 83.16	T2 (n=31) 83.16-100.74	T3 (n=30) > 100.74		T1 (n=30) < 2.52	T2 (n=31) 2.52 – 2.86	T3 (n=29) > 2.86	
BMI(Kg/m ²)	29.2 (6.76)	26.97 (10.5)	25.49 (5.55)	0.200	28.69 (6.84)	27.36 (10.6)	25.48 (5.22)	0.303
DBP (mmHg)	78.34 (6.52)	78.03 (4.52)	75.03 (12.8)	0.272	78 (7.18)	77.52 (3.66)	75.83 (13.1)	0.610
SBP (mmHg)	122 (8.53)	123.9 (8.21)	122.9 (7.03)	0.661	123.6 (7.88)	122.5 (6.56)	122.8 (9.3)	0.862
PR (pul/min)	86.48* (9.88)	91.26* (7.74)	92.6* (8.34)	0.020	91.3 (9.79)	88.23 (9.04)	91.07 (7.53)	0.326
FBS (mg/dl)	101.4 (17.6)	96.8 (9.74)	94.6 (8.31)	0.129	100.8 (17.5)	94.8 (7.47)	96.84 (10.6)	0.174
TC (mg/dl)	162.3 (31.6)	153 (35.7)	160.9 (32.1)	0.505	170.8* (27.8)	149.1* (30.9)	156.2* (37.3)	0.032
TG (mg/dl)	120.3 (59)	107.6 (75.9)	116.9 (74.9)	0.769	122.7 (60.8)	106.4 (69.9)	115.8 (79.6)	0.664
HDL-C (mg/dl)	49.6 (17.13)	48.4 (10.5)	48.69 (12.2)	0.940	51.62 (16.9)	46.81 (10.2)	48.26 (11.9)	0.357
LDL-C (mg/dl)	87.03 (24.7)	82.95 (28.3)	88.9 (28.3)	0.686	93.77 (22.8)	80.64 (24.6)	84.43 (32.2)	0.151
VLDL-C (mg/dl)	24.07 (11.8)	21.52 (15.2)	23.39 (14.9)	0.769	24.53 (12.2)	21.28 (13.9)	23.16 (15.9)	0.664
AIP	0.35 (0.29)	0.27 (0.31)	0.32 (0.33)	0.619	0.34 (0.29)	0.29 (0.28)	0.31 (0.35)	0.899
TyGi	4.58 (0.28)	4.55 (0.32)	4.59 (0.29)	0.891	4.62 (0.29)	4.55 (0.29)	4.55 (0.33)	0.545
hsCRP (mg/dl)	3.55 (4.81)	1.84 (3.21)	1.84(2.01)	0.105	2.75 (3.84)	3 (4.4)	1.37 (1.81)	0.168

Variable	Copper Tertiles			p-value	Iron Tertiles			p-value
	T1 (n=30) < 84.74	T2 (n=31) 84.74–107.02	T3 (n=29) > 107.02		T1 (n=30) < 59.94	T2 (n=30) 59.94- 87.37	T3 (n=30) > 87.37	
BMI(Kg/m ²)	25.72 (4.69)	27.78 (10.9)	28.1 (6.95)	0.462	28.53 (5.76)	27.79 (11)	25.26 (5.87)	0.252
DBP (mmHg)	76.4 (4.88)	76.6 (13.7)	78.5 (3.73)	0.604	76.27 (8.87)	78.47 (3.84)	76.67 (4.49)	0.587
SBP (mmHg)	122.9 (8.35)	124.5 (7.46)	121.3 (7.77)	0.286	124.6 (8.65)	122.1 (7.48)	122.1 (7.37)	0.392
PR (pul/min)	88.9 (8.18)	92.32 (7.13)	89.1 (10.9)	0.250	88.03 (8.66)	90.5 (9.23)	91.97 (8.6)	0.225
FBS (mg/dl)	97.15 (9.01)	974.7 (8.1)	100.7 (18.3)	0.180	95.97 (8.1)	99.88 (18.3)	96.5 (8.91)	0.435
TC (mg/dl)	156.6 (32.3)	157.8 (36.7)	161.5 (31)	0.842	156.6 (36.1)	155.7 (35.4)	163.6 (27.8)	0.604
TG (mg/dl)	108.2 (55.9)	120 (78.7)	116.2 (74.1)	0.801	117.1 (55.8)	105.3 (76.6)	122.1 (76.5)	0.635
HDL-C (mg/dl)	49.4 (12.8)	47.82 (16.3)	49.5 (10.4)	0.864	46.03 (11.2)	50.26 (15.8)	50.34 (12.6)	0.362
LDL-C (mg/dl)	84.8 (27.5)	85.08 (25.5)	88.96 (28.8)	0.808	86.35 (31)	84.2 (25.3)	88.2 (24.35)	0.835

VLDL-C (mg/dl)	21.63 (11.4)	24 (15.4)	23.25 (14.8)	0.801	23.42 (11.6)	21.05 (15.3)	24.43 (15.3)	0.635
AIP	0.29 (0.3)	0.34 (0.32)	0.31 (0.31)	0.868	0.36 (0.27)	0.27 (0.32)	0.33 (0.32)	0.317
TyGi	4.51 (0.28)	4.57 (0.31)	4.64 (0.29)	0.230	4.58 (0.32)	4.57 (0.32)	4.55 (0.31)	0.878
hsCRP (mg/dl)	1.75 (2.19)	1.92 (1.71)	3.55 (5.5)	0.100	4.05** (5.19)	1.95** (2.56)	1.18** (1.03)	0.005

Results are represented as Mean (SD).

4. DISCUSSION

Cardiovascular disease (CVD) is associated with both subclinical and clinical hypothyroidism, which disrupts normal endothelium function through pathways including inflammation, lipid abnormalities, and oxidative stress (Sami *et al.*, 2024). This study investigates the impact of vitamin D status, iron profiles, and trace elements imbalance on cardiometabolic risk factors. Our study indicated a significant increase in cardiometabolic risk factors such as fasting blood sugar, lipid profile, AIP, TyGi, and hsCRP in SCHT. Furthermore, a strong association was noted between elevating TSH and TC, TG, VLDL-c, fasting blood sugar, AIP, and TyGi. Additionally, a substantial correlation was noted between reduced vitamin D, ferritin, magnesium, iron, and elevated serum copper with cardiometabolic risk factors.

The SCHT group had a notable elevation in BMI relative to the healthy group, aligning with findings of (Mahat *et al.*, 2023). A substantial positive association was determined between TSH and BMI. Research has indicated a positive correlation between BMI and TSH, suggesting that changes in body weight are correlated with thyroid disease (Kirac *et al.*, 2022; Mahat *et al.*, 2023). Both SBP and DBP were found to be nonsignificant between SCHT and the control group, which aligns with those of Luo *et al.* (2022).

The SCHT group showed significantly elevated levels of TG, TC, VLDL-C, and LDL-C and a considerably lower level of HDL-C in contrast to the control group, which is in agreement with the findings of Lamichhane *et al.* (2023) and Liu *et al.* (2023). The precise pathophysiology of dyslipidemia in hypothyroidism remains ambiguous; however, multiple explanations have been suggested. A variety of mechanisms are involved in the accumulation of LDL-c, the emergence of hypertriglyceridemia as a result of reduced lipoprotein lipase activity, and the reduced clearance of cholesterol from the bloodstream due to the liver's impaired capacity to convert cholesterol into bile acids (Cicatiello *et al.*, 2018; Nicolaou & Toumba, 2024). TSH exhibited a substantial positive association with TC, TG, and VLDL-c. Cardiovascular risk variables, including cholesterol levels, arterial blood pressure, and BMI, have been linked to elevated TSH levels in adults, even when they are within the normal range. Certain studies have even linked high-normal TSH levels to the onset of metabolic syndrome (Meisinger *et al.*, 2014; Ramouzi *et al.*, 2024).

AIP is regarded as a significant marker consisting of TG and HDL-C, extensively utilized for quantifying lipid levels and deemed the most effective indicator for assessing CVD and dyslipidemia (Zhu *et al.*, 2018). This study identified significantly higher AIP levels in the SCHT group in contrast to the healthy group. A substantial positive association was identified between TSH and AIP. These findings correspond with research conducted by Lamichhane *et al.* (2023). Elevated TG

and decreased HDL-C compared to normal euthyroid controls cause elevated AIP in SCHT. Subclinical hypothyroid women had an increased risk of CDV compared to euthyroid women, according to their average AIP (Hossain *et al.*, 2021).

A newly proposed surrogate marker of insulin resistance (IR) is the TyGi, which is a product of fasting triglycerides and glucose (Choi *et al.*, 2021). Multiple studies have indicated that elevated TyGi correlates with the onset of cardiometabolic disorders (Kitae *et al.*, 2019; Barzegar *et al.*, 2020). The results showed that the SCHT group had substantially higher TyGi levels than the control group. In addition, TSH and TyGi were discovered to have a very positive correlation (Mahat *et al.*, 2023).

Vitamin D levels were found to be markedly lower in the SCHT group compared to the control group in a recent study. Vitamin D and TSH were also found to have a strong inverse relationship. The results corroborate the findings of the study conducted by Sari and Coşkuner (2022); Mahat *et al.*, 2023). Inadequate intestinal absorption and impaired vitamin D activation may explain why individuals with SCHT have lower vitamin D levels (Talaie *et al.*, 2018). Additionally, vitamin D supplementation improves blood TSH, according to a study conducted on subclinical hypothyroid patients (Safari *et al.*, 2023). Vitamin D insufficiency is linked with cardiometabolic risk factors, including insulin resistance, obesity, dyslipidemia, and hypertension (Milagres *et al.*, 2017; Mousa *et al.*, 2017). The results of this study show that low vitamin D levels are inversely related to high TG and very LDL-c. Vitamin D and AIP were also found to have a negative correlation, suggesting that SCHT patients may be at a higher risk of developing CVD. Findings from our study are in line with previous research showing that low vitamin D levels are linked with an increased risk of CVD and death in individuals with dyslipidemia (Alsamghan *et al.*, 2020; Hu *et al.*, 2024).

Ferritin levels were found to be considerably lower in the SCHT group compared to the control group in the present study. The association between ferritin and TSH was also significantly negative. These results are similar to those found by Sylus *et al.* (2024) and Gupta *et al.* (2024). Reduced ferritin levels, a measure of the body's iron stores, impact thyroid hormone synthesis. Decreased ferritin levels impact thyroid hormone production and the conversion of T4 to its active form, T3. A sufficient amount of iron is needed for this conversion, which primarily occurs in the liver (Krishnamurthy *et al.*, 2023). In contrast to erythroid, researchers found that iron insufficiency was more common in SCHT patients (Swapnika *et al.*, 2024). Additionally, a remarkable negative association was noted between serum ferritin and TG and VLDL-c. Research conducted by Kadoglou *et al.* (2017) found that low ferritin levels in women with no significant chronic disease are correlated with elevated all-cause mortality.

Moreover, the serum zinc levels of the SCHAT group were substantially lower than those of the control group, and a significant negative association was noted between serum Zn and TSH. The study conducted by Patel *et al.*, (2024) agrees with these findings. Zn may impact the conversion of T4 to T3 because of its effect on hepatic deiodinase (DIO) activity; when Zn levels are low, the enzyme's activity decreases (Bryliński *et al.*, 2025). In addition, a considerable positive association between serum zinc and PR was noted, and a decrease in PR levels was noted with a decrease in serum Zn, indicating that low serum zinc could be a risk factor for developing CVD. Prior studies indicate that zinc deficiency can compromise cardiovascular function, whereas sufficient zinc levels correlate with elevated heart rate and heightened sympathetic tone in some contexts (Rosenblum *et al.*, 2020; Hara *et al.*, 2023).

In addition, serum magnesium was shown to decrease notably in the SCHAT group in contrast to the control group, and a significant negative association was noted between serum magnesium and TSH. These results align with those obtained by Athokpham *et al* (2020). Magnesium is crucial for the thyroid's use of iodine and the conversion of dormant T4 into active T3 (Zhou *et al.*, 2022). Moreover, the results revealed a considerable negative association between serum Mg and BMI, TyGi, and hsCRP. These results indicate that low magnesium levels are linked to cardiometabolic risk factors. Magnesium performs numerous physiological activities in the body. These encompass vital functions in sustaining cardiovascular performance, contributing to the regulation of cardiac excitation–contraction coupling, hemostasis, and endothelial function (Fritzen *et al.*, 2023). Numerous studies have associated inadequate magnesium consumption with the occurrence of specific cardiometabolic disorders (Dominguez *et al.*, 2020; Jiao *et al.*, 2022).

Additionally, serum copper was shown to slightly increase in SCHAT in contrast to controls, but the results were statistically nonsignificant between the groups. Research indicates that diminished copper levels may correlate with hyperthyroidism, whilst elevated levels may be associated with hypothyroidism (Zhou *et al.*, 2022). Furthermore, a considerably positive association was noted between serum copper and TyGi and hsCRP these results indicated a link between elevated serum copper and cardiometabolic risk factors. Copper is an essential mineral, and an adequate quantity of copper is necessary to facilitate appropriate physiological functions across multiple systems, including the cardiovascular system (Chen *et al.*, 2023). A study on the women population of China found a significant positive association between serum copper and TC, TG, LDL, and inflammation markers (Chen *et al.*, 2021).

The current study has limitations that must be acknowledged when interpreting the results, specifically its cross-sectional design and the limited sample size (n = 90). Further longitudinal and interventional research is necessary to confirm this correlation and explore its clinical implications.

CONCLUSION

The current study concluded that low vitamin D, ferritin, magnesium levels, and elevated copper levels could be associated with cardiometabolic risk factors in females with newly diagnosed subclinical hypothyroidism. Reduced vitamin D, zinc, and magnesium levels, disrupted iron metabolism, and elevated serum copper were linked to heightened atherogenic risk and

metabolic abnormalities. This study indicates that the surveillance and enhancement of vitamin D, zinc, magnesium, and iron levels may contribute to the management of cardiovascular risk in women with subclinical hypothyroidism. However, because of the cross-sectional nature of this study, further longitudinal and interventional research is required to determine causal relationships and clinical implications.

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Ethical Statement:

The research has been conducted according to ethical principles of the Helsinki Declaration and the local Bioethics Committee at the Medicine College, University of Zakho (NOV2024/UOZE25). Participants were individually informed about the study's purpose. The subjects participated voluntarily, and written and verbal consent was obtained from all the subjects before biochemical measurements were performed.

Author Contribution:

C. H. S., sample collection, methodology, data analysis, and writing. L. Y. M. and M. A. H. conceptualized, and all authors critically reviewed the content of the manuscript. All authors have read and agreed to the published version of the manuscript.

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