original article

Plasma homocysteine concentrations and serum lipid profile as atherosclerotic risk factors in subclinical hypothyroidism

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BACKGROUND AND OBJECTIVES: Because subclinical thyroid dysfunction may be a risk factor for cardiovascular disease, we evaluated the atherosclerosis tendency in subclinical hypothyroid (SCH) patients.

PATIENTS AND METHODS: Fifty-three subclinical hypothyroid patients (serum thyrotropin [TSH] concentrations >4.12 mU/L) were compared with a control group of 50 euthyroid subjects whose age, sex and body mass indices were similar to the patient group. We tested whether serum TSH concentrations were correlated with plasma total homocysteine concentration (tHcy), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglycerides (TG).

RESULTS: There was a significant statistical difference between the patient and control groups for normal free T4 (1.02±0.17 vs. 0.86±0.13, *P*<.001), TSH (1.64±1.02 vs. 6.62±2.61, *P*<.001), TC (185±39 vs. 206±42, *P*=.01), TG (103±54 vs. 132±85, *P*=.04), LDL-C (114±33 vs. 127±36, *P*=.04), and TC/HDL-C (3.81±106 vs. 4.19±1.02, *P*=.04), respectively. No statistically significant difference was found between the two groups for HDL-C, VLDL-C, LDL-C/HDL-C, and tHcy. Serum TSH was significantly correlated with plasma tHcy (r=0.55; *P*=.001), TC (r=0.52; *P*=.001), LDL-C (r=0.49; *P*=.001), TC/HDL-C (r=0.38; *P*=.002) and LDL-C/HDL-C (r=0.36; *P*=.004) across all participants.

CONCLUSION: Our study suggests that the atherogenicity of SCH is not mediated by hyperhomocysteinemia. Associated hyperlipidemia may explain the observed increased risk of coronary artery disease in patients with SCH.

Subclinical hypothyroidism (SCH) and hyperthyroidism represent the earliest stages of thyroid dysfunction.[1] SCH is defined by the finding of elevated serum thyrotropin (TSH) concentrations associated with normal free thyroid hormone levels and a lack of clinical signs and symptoms of hypothyroidism.[2-4] SCH is usually detected during the follow-up of patients with a history of thyroid disease or as a result of biochemical screening for nonspecific symptoms.[5] Whether SCH has any demonstrable effect on serum lipid concentrations has been controversial.[2,3,5-10] Several reports have shown that patients with SCH have a disturbed lipid metabolism, including elevated serum levels of total cholesterol (TC)[7,11-15] and low-density lipoprotein-cholesterol (LDL-C).[7,12-16] Such changes are generally recognised as risk factors for atherosclerosis and coronary heart disease.[16]

Homocysteine (Hcy) is formed during the metabolism of methionine, a sulfur-containing essential amino acid.[17] Many investigators have reported a significant association between hyperhomocysteinemia and cardiovascular disease.[18-23] Elevated plasma total homocysteine (tHcy) levels have been reported in overt hypothyroidism,[24] and have been proposed as an independent risk factors for cardiovascular disease.[25] Whether SCH is a risk factor for premature cardiovascular disease is controversial.[2]

We conducted this study to see whether there is a tendency toward atherosclerosis in SCH. The serum lipid profile and plasma tHcy concentrations of SCH

patients and a euthyroid control group were compared. We also investigated whether there was a correlation between serum TSH and serum lipid profile or serum TSH and plasma tHcy values in SCH patients.

METHODS

We studied 53 SCH patients (47 women and 6 men), aged 40.8±12.1 years and with a mean BMI of 26.2±4.6 kg/m^2 (Table 1). SCH was established by TSH levels >4.12 mU/mL[26] and normal free T3 (FT3) and T4 (FT4) levels in two consecutive measurements. There was no history of thyroidectomy (total or subtotal) or prior exposure to radioiodine or external radiation in any of the patients included in the study. Thirteen of the female patients were post-menopausal but none was receiving hormone replacement therapy. None of the participants was diagnosed with neoplastic, renal, liver disease, diabetes mellitus or familial hypercholesterolemia. Subjects receiving any drugs or who smoked were excluded from the study. Fifty age- and sex-matched healthy individuals were used as controls in baseline measurements. The control group consisted of 43 women (9 postmenopausal) and 7 men. The mean age of the controls was 38.2±10.6 years and the mean BMI was 25.1±5.4 kg/m^2. Informed consent was obtained from all subjects. A local ethics committee approved the study.

Serum samples were collected in the fasting state, immediately put on ice and processed within 30 minutes. Thereafter, they were kept frozen at -20°C. Plasma tHcy concentration was measured by high-performance liquid chromatography (Agilent 1100 series, Germany). The reference interval was 5-14 µmol/L. The intra-assay and inter-assay coefficients of variation for tHcy were 2% and 4.2%, respectively. An upper reference limit from a published study for TSH of 4.12 mU/L,[26] which was accepted for a multicultural population, was used in selecting individuals for the SCH group. Serum TSH, FT3, FT4 concentrations were measured by a chemiluminescent microparticle immunassay on an Architect i2000 analyzer (Abbott, Texas, USA). Reference intervals provided by the manufacturer were TSH 0.35-4.94 mU/L, FT3 1.71-3.71 pg/mL, and serum FT4 0.70-1.48 ng/dL. The sensitivities of the TSH, FT3, FT4 were 0.0025 mU/L, 1 pg/mL, and 0.4 ng/dL, respectively. The intra-assay coefficients of variation for TSH, FT3, and FT4 were 1.7%, 2.7%, and 3.2%, respectively.

Serum TC (reference range, 112-200 mg/dL) and triglyceride (TG) (reference range, 50-179 mg/dL) were determined by enzymatic colorimetric assay on an Aeroset analyzer (Abbott, Texas, USA). High-density lipoprotein cholesterol (HDL-C) (reference range, 28-75 mg/dL) was determined enzymatically in the supernatant after precipitation of other lipoproteins. Very low-density lipoprotein cholesterol (VLDL-C) was calculated using the TG/5 formula and LDL-C was calculated using the Friedewald formula.[27] The intra-assay coefficients of variation for TC, TG, and HDL-C were 0.7% for high concentration, 1.0% for low concentration, 0.5% for high concentration, 0.8% for low concentration, 1.58% for high concentration, 1.7% for normal concentration, and 1.85% for low concentration, respectively. Serum creatinine (reference interval, 0.6-1.3 mg/dL) concentration was measured by the Jaffe method on an Aeroset analyzer (Abbott, Texas, USA). Serum vitamin B12 and folic acid were determined by Access full-automatic chemiluminescent assay (Beckman Coulture, Chaska, USA). Reference intervals were 160-980 pg/mL for vitamin B12 and 0-3 ng/mL for folic acid.

The data for the SCH and control groups were compared by Student's t test and the Wilcoxon signed-rank test. Student's t test was used for comparison of group means with a normal distribution, while a Wilcoxon signed-rank test was used for comparison of means with non-normal distributions. The Spearman rank correlation on the entire data set as well as within groups was used to test whether TSH was correlated with TC, LDL-C, HDL-C, TG, VLDL-C, TC/HDL-C, LDL-C/HDL-C, and tHcy. *P* values less than .05 were considered statistically significant.

RESULTS

SCH patients had significantly lower FT4 (although within the normal range) and higher TSH levels than the control group (Table 1). The mean plasma tHcy levels in patients were not significantly different than in controls (Table 2). Mean serum levels of TC, TG, LDL-C, and the ratio of TC/HDL-C in patients were significantly different from the values in controls. Serum mean levels of VLDL-C, HDL-C, and the ratio of LDL-C/HDL-C were not significantly different from the values in controls. Across all participants TSH was positively correlated with TC, LDL-C, TC/HDL-C, LDL-C/HDL-C, and plasma tHcy concentrations (Table 3). Correlations were not found between serum TSH concentration and serum TG and VLDL-C values.

DISCUSSION

Hypothyroidism is a progressive disorder presenting with different degrees of thyroid failure and metabolic consequences.[28] SCH is defined as an elevation in serum TSH above the upper limit of reference range with

Table 1. Clinical and biochemical characteristics of patients with subclinical hypothyroidism and the control group.

	Control (n=50)	SCH (n=53)	*P* value
Age (years)	38.2±10.7	40.8±12.1	NS
Gender (F:M)	47:3	47:6	NS
Body mass index (kg/m^2)	25.1±5.4	26.6±4.6	NS
Vitamin B12 (pg/mL)	236±99	243±65	NS
Folate (ng/mL)	7.3±2.1	6.8±1.8	NS
Free T3 (pg/mL)	3.03±0.38	2.89±0.44	NS
Free T4 (ng/dL)	1.02±0.17	0.86±0.13	<.001
TSH (mIU/L)	1.64±1.02	6.62±2.61	<.001

Data are mean±standart deviation; NS, not significant; F, female; M, male.

Table 2. Serum concentrations for several risk factors for cardiovascular disease in patients with subclinical hypothyroidism and the control group.

	Control (n=50)	SCH (n=53)	*P* value
Total cholesterol (mg/dL)	185±39	206±42	.01
Triglycerides (mg/dL)	103±54	132±85	.04
LDL-C (mg/dL)	114±33	127±36	.04
VLDL-C (mg/dL)	21±10	26±16	.06
HDL-C (mg/dL)	51±11	51±10	.8
TC/HDL-C	3.81±1.06	4.19±1.02	.04
LDL-C/HDL-C	2.33±0.86	2.58±0.79	.13
Total homocysteine (µmol/L)	9.6±3.1	10.3±3.4	.29

Data are mean±standard deviation; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 3. Spearman correlation coefficients between serum thyrotropin concentration and other parameters in all participants.

	Serum TSH concentration	
	r	*P*
Total cholesterol	0.52	<.001
LDL-C	0.49	<.001
TC/HDL-C	0.38	.002
LDL-C/HDL-C	0.36	.004
Total homocysteine	0.55	<.001

TSH, serum thyrotropin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

a normal serum FT4 concentration.[6]

Cardiovascular diseases are the leading cause of death in industrialized nations.[29] Atherosclerosis is a multifactorial disease. Established risk factors include gender, age, smoking, TC, LDL-C, HDL-C, diabetes,[30] and hypertension.[29] In addition, several case-control, cross-sectional and prospective studies have consistently indicated that hyperhomocysteinemia is an independent risk factor for the progression of vascular disease.[29]

Overt hypothyroidism has been found to be associated with cardiovascular disease.[11] Whether SCH is related to the risk for cardiovascular disease is controversial. It is well known that thyroid hormone has pervasive effects on the transport of the plasma lipoproteins.[31] Cholesterol and LDL typically accumulate in the plasma of patients with hypothyroidism because thyroid hormone stimulates LDL receptor activity.[10,31] Thyroid hormone also influences the transport of TG-rich lipoproteins through its effects on the lipoprotein lipase enzyme system. Hepatic lipase has also been shown to decrease in severe thyroid deficiency and to increase after L-thyroxine administration.[31] Hak et al[11] showed that SCH was present in 10.8% of participants and was associated with a greater age-adjusted prevalence of aortic atherosclerosis (odds ratio, 1.7 [95% CI, 1.1 to 2.6]) and myocardial infarction (odds ratio, 2.3 [CI, 1.3 to 4.0]). Miura et al[32] diagnosed 97 patients with CHD by coronary angiography (CHD group) and compared them with 103 healthy subjects matched for age, sex and body mass index (control group). They observed a significant decrease in serum FT3 and FT4 levels in patients with CHD that was associated with increased serum TSH.

Some studies[10,12,16] suggest that high TSH is associated with deleterious changes in serum lipids, which are considered to be an important cardiovascular risk factor, particularly HDL-C, LDL-C, and the ratio of LDL-C to HDL-C. However, few studies[33,34] have suggested that the majority of patients with SCH did not differ from controls in risk factors for coronary heart disease (e.g. TC, LDL-C, TK/HDL-C, LDL-C/HDL-C). Previous studies of thyroid dysfunction and TG levels have also been conflicting. Compared with individuals with normal thyroid function, TG levels have been reported to be high[6,15] or unchanged[3,7,10,13,14] among those with biochemical evidence of SCH. In many previous clinical studies,[3,6,7,12,13,15,34] HDL-C has been reported to be unchanged among SCH patients.

We demonstrated that mean serum levels of TC, TG, LDL-C, and the ratio of TC/HDL-C in patients with SCH were significantly different from the values

Table 4. Summary of studies comparing plasma total homocysteine levels in patients with subclinical hypothyroidism and control subjects.

Reference	Number of patients and controls		Total homocysteine (μmol/L)		*P* value
	Total (F:M)	Control	Patients	Control	
Lindeman et al[38]	93 (61:32) 19 (13:6)	643 (283:360) 643 (283:360)	12.8±4.1 11.7±2.6	12.5±3.7 12.5±3.7	0.12 0.429
Luboshitzky et al[34]	57 F	34 F	9.0±2.5	9.2±2.6	NS
Christ-Crain et al[29]	63 F	40 F	11.3±2.8	11.0±2.7	NS
Aldasouqui et al[42]	47 (42:5)	50 (46:4)	7.44±0.5	7.22±0.2	0.68
Meek et al[40]	12 (7:5) † 12 (10:2) ‡	§ §	10 (5-37)** 8 (6-15)**	§ §	§ §
Deicher & Vierhapper[39]	37 (31:6)	§	9.9±2.9	§	§
Current study	53 (47:6)	50 (47:3)	9.6±3.1	10.3±3.4	0.29

*Mean±SD, ** Median (range), †Levothyroxine group (baseline measurements), ‡Placebo group(baseline measurements), §No control subjects, NS, No significant difference; F, Female; M, Male.

in controls. Mean serum levels of VLDL-C, HDL-C, and the ratio of LDL-C/HDL-C were not significantly different from the values in controls. TSH was positively correlated with TC, LDL-C, TC/HDL-C, LDL-C/HDL-C, and plasma tHcy concentrations.

Homocysteine is a sulfur-containing intermediate product in the normal metabolism of methionine, an essential amino acid.[22] Numerous retrospective and prospective studies have consistently found a relationship between mild hyperhomocysteinemia (fasting or after oral methionine loading) and cardiovascular disease or all-cause mortality. Starting at a plasma tHcy concentration of approximately 10 μmol/L, an associated risk increase follows a linear dose-response relationship with no specific threshold level.[22] Although 30 years have elapsed since hyperhomocysteinemia (and homocysteinuria) were first associated with an increased risk of atherothrombotic vascular disease, it is only recently that sufficient evidence has mounted to suggest that the association is independent and dose-related, and it remains to be established whether it is causal and modifiable.[18] If a causal relationship does prove to exist between tHcy and cardiovascular disease, the biological mechanism(s) remains to be established.[35] Several mechanism have been proposed to contribute to the vascular toxicity of homocysteine, including platelet aggregation, increased coagulation or reduced thrombolysis, endothelial dysfunction, and effects on the blood vessel wall.[36] Evidence now indicates that hyperhomocysteinemia, which occurs in approximately 5% to 7% of the general population, is an important, independent risk factor for atherosclerosis and thrombotic disease.[37] Lindeman et al[38] point out that thyroid status may be an important determinant of serum/plasma tHcy concentrations. The tHcy concentration appears to be increased in hypothyroidism and decreased in hyperthyroidism. Deicher and Vierhaper[39] reported that patients with SCH did not demonstrate improvement in fasting homocysteine levels after treatment with levothyroxine. Meek and Smallridge[40] reported that levothyroxine treatment with subclinical hypothyroidism did not alter homocysteine levels in the fasting or post-methionine states.

Several studies[29,34,38,41] have been published where subjects with SCH were compared against normal euthyroid subjects to determine if there was a continuum of change in serum tHcy concentrations in those with SCH as opposed to an increase that occurs only when overt hypothyroidism exists. Although there were correlations between serum TSH and tHcy in all participants in these studies, there were no significant differences between cases and controls. Aldasouqi et al[42] found no association between subclinical hypothyroidism and hyperhomocysteinemia. The findings of previous studies, as well as our current study, are summarized in Table 4. The results of our study confirm the findings of the previous studies.

Published retrospective studies demonstrate a stronger association between tHcy and CHD than prospective studies.[18,35,43] A major concern is that tHcy levels may rise after tissue damage; thus, retrospective studies of CHD patients may show elevated tHcy values as a result of, not a cause of, the coronary events.[43] One published report indicated that patients with SCH had homocysteine levels that were higher than those in normal control subjects and improved signifi-

cantly with levothyroxine treatment.[44]

The purpose of our prospective study was to examine the effect of SCH on serum tHcy concentrations after adjusting for differences in gender, age and ethnicity. Additionally, serum folate, vitamin B12, and creatinine concentrations were also adjusted. A comparison had been made of selected CHD risk factors in male and female participants with SCH versus those with control group. In the present study, serum TSH levels were positively correlated with tHcy levels. Our study population consisted of adult patients (mean age of 40 years for the SCH group, of 38 years for the control group) who had previously not taken any drug and did not smoke. Serum TSH levels were greater than 4.12 mU/L and both FT3 and FT4 levels were normal in all participants. Thus, our population was selected more rigoriously than the population in some other studies. There is no consensus on the relationship between SCH and CHD, although many studies have been performed. Only long-term biochemical monitoring will prove the relationship. However, our study contributes information towards establishing an association between SCH and CHD. We have demonstrated higher serum levels of TC, TG, LDL-C in SCH patients. Research from experimental animals, laboratory investigations, epidemiology, and genetic forms of hypercholesterolemia indicate that elevated LDL cholesterol is a major cause of CHD. In addition, recent clinical trials robustly show that LDL-lowering therapy reduces the risk for CHD.[45] For these reasons, focusing on LDL values in SCH patients is important.

In conclusion, in the present study, homocysteine levels were not elevated in the majority of patients with SCH. We found no differences in plasma tHcy concentrations between the SCH group compared with the control group. Our results suggest that the atherogenicity of SCH is not mediated by hyperhomocysteinemia. Associated hyperlipidemia may explain the observed increased risk of coronary artery disease in patients with SCH.

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