

Homocysteine and Fibrinogen Changes with L-thyroxine in Subclinical Hypothyroid Patients

The aim of the present study was to evaluate plasma total homocysteine (Hcys) and serum fibrinogen concentrations in subclinical hypothyroid (SH) and overt hypothyroid patients before and after L-thyroxine (LT4) replacement and to compare them in euthyroid subjects. Fifteen SH and 20 hypothyroid premenopausal women were recruited in the study. We measured fasting plasma levels of Hcys and serum levels of free thyroxine (fT4), free triiodothyronine (fT3), thyrotropin (TSH), folate, vitamin B12, fibrinogen, renal functions, and lipid profiles in patients with SH and overt hypothyroid patients before and after LT4 treatment. Eleven healthy women were included in the study as a control group. Pretreatment Hcys levels were similar in SH and control subjects, whereas mean fibrinogen level of SH patients was higher than that of control subjects ($p < 0.05$). Baseline Hcys ($p < 0.01$) and fibrinogen ($p < 0.001$) levels of the overt hypothyroid patients were significantly higher than those of the healthy subjects, and the pretreatment Hcys levels decreased with LT4 treatment ($p < 0.001$). In conclusion, our data support that SH is not associated with hyperhomocysteinemia and Hcys does not appear to contribute to the increased risk for atherosclerotic disease in patients with SH.

Key Words : Hypothyroidism; Homocysteine and Fibrinogen

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INTRODUCTION

Subclinical hypothyroid (SH) is defined by an elevated serum thyroid-stimulating hormone (TSH) concentration in the presence of normal serum thyroid hormone levels. Subclinical thyroid failure is often asymptomatic; nearly 30% of patients with this condition may have symptoms that are suggestive of thyroid hormone deficiency (1-3). As in overt hypothyroidism, patients with SH also shown to be at high risk for atherosclerosis and cardiovascular disease (4). A high level of homocysteine (Hcys) in plasma has been proposed as an independent risk factor for occlusive cardiovascular disease. The plasma Hcys level is affected by several life-style and physiological factors and is elevated under the condition of impaired folate and cobalamin status and in renal failure (5). There are consistent reports demonstrating that thyroid status is an important determinant of the plasma concentration of Hcys (6, 7).

T4 levels are the determinant of several components of the fibrinolytic system. T4 has an impact on the synthesis and catabolism of proteins, and the final modification of serum levels of these proteins may depend on the severity of the disease, in hypothyroidism. Chadarevian et al. found that plasma levels of fibrinogen was either correlated to plasma levels of T4 or altered in patients displaying normal to low free thy-

roxine (fT4) levels or hypothyroidism (7-9). Therefore, we undertook the present study to investigate the changes of Hcys and fibrinogen levels undergoing subclinical and overt hypothyroid patients before and after L-T4 replacement therapy and compare them in euthyroid subjects.

MATERIALS AND METHODS

Fifteen premenopausal women newly diagnosed with SH (mean age, 41.4 ± 14.1 yr) and 20 overt hypothyroid (mean age, 41.3 ± 11.1 yr) were enrolled in the study. The cause of hypothyroidism was chronic autoimmune thyroiditis with positive anti-TPO antibody. The diagnosis of SH was based on basal serum TSH values between 5-20 mIU/L and normal free triiodothyronine (fT3) and fT4 levels. No patient had received T4 replacement therapy prior to the enrollment in our study. Obese (body mass index [BMI] > 30 kg/m²) subjects, smokers, patients with any major organ or systemic disease and those using alcohol or any medication known to interfere with thyroid hormone, fibrinogen, and Hcys metabolism were excluded from the study. We also excluded women with a history of previous thyroid disease, psychiatric disorder, or anticipated pregnancy. Each group was also compared with age- and weight-matched 11 euthyroid premenopausal

healthy women as a control group.

Blood samples were withdrawn after 12 hr of overnight fasting, at 08.30 a.m., for serum TSH, fT₃, fT₄, total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), creatinine, Hcys, folate, vitamin B₁₂, and fibrinogen. Hcys was measured by high performance liquid chromatography method (HPLC, Los Angeles, CA, U.S.A.). Folate and vitamin B₁₂ levels were determined by using Access Immunoassay system (Sanofi Diagnostics, Pasteur, Paris, France). Fibrinogen levels were determined by using coagulometric method (Multifibren, Dode Behring/BCS, Marburg, Germany). FT₃, fT₄, and TSH were measured by enzyme immunoassay (Roche Diagnostics, Mannheim, Germany). Serum TC, HDL-C and TG were determined enzymatically (Olympus Diagnostica, Lismeehan, Ireland). LDL-cholesterol (LDL-C) was calculated with the Friedewald's formula (LDL-C=TC-[HDL-C+TG/5]). Serum creatinine was measured by an automated enzymatic method (Olympus Diagnostica, Ireland) and creatinine clearance (C_{cr}) was calculated using the Cockcroft and Gault formula: C_{cr} (mL/min)=(140-age [yr]) × weight (kg)/(0.81 × creatinine [μ M/L]). This value was multiplied by 0.85 for women.

Normal ranges in our laboratory are as follows: TSH, 0.27-4.01 μ IU/mL; fT₃, 1.8-4.6 pg/mL; fT₄, 0.93-1.7 ng/dL; TC, 130-200 mg/dL; TG, 25-160 mg/dL; HDL-C, 39-80 mg/dL; LDL-C, <130 mg/dL; Folate, >3 ng/mL; Vitamin B₁₂, 145-

Table 1. Characteristics of subclinical hypothyroid (SH) patients before and after LT4 therapy and controls

	Control subjects (n=11)	SH patients (before treatment) (n=15)	SH patients (after treatment) (n=15)
Age (yr)	39.9±12.5	41.4±14.1	41.4±14.1
BMI (kg/m ²)	23.6±3.9	26.4±3.3	26.2±3.3
Free T ₃ (pg/mL)	2.9±0.6	2.4±0.6	2.7±0.6 [†]
Free T ₄ (ng/dL)	1.4±0.2	1.0±0.1 [†]	1.2±0.2 [†]
TSH (μ IU/mL)	2.2±0.9	15.8±2.4 [‡]	3.4±1.6 [†]
Creatinine clearance (mL/min)	114.4±20.7	114.2±24.8	109.8±10.4
Total cholesterol (mg/dL)	192.0±21.3	228.8±56.6*	203.2±39.4 [‡]
LDL-C (mg/dL)	100.3±11.1**	143.6±45.2 [†]	127.5±36.8 [§]
HDL-C (mg/dL)	49.1±8.7	48.5±15.1	45.3±10.8
Triglyceride (mg/dL)	205.5±52.8	161.0±80.5	152.6±77.2
TC/HDL-C	4.0±0.5	4.9±1.2*	4.7±1.3
Folate (ng/mL)	7.2±1.0	6.8±1.5	7.1±1.5
Vitamin B ₁₂ (pg/mL)	244.2±47.2	245.1±88.8	238.0±51.0
Hcys (μ M/L)	7.9±0.6	9.2±3.3	7.8±2.1
Fibrinogen (g/L)	2.0±0.1**	2.7±1.1*	2.6±0.9

Data are mean ± SD. Comparison between control group and SH patients before treatment (*p<0.05, [†]p<0.01, [‡]p<0.001). Comparison of SH patients before and after treatment ([§]p<0.05, [†]p<0.01, [‡]p<0.001). Comparison between control group and treatment group **p<0.05.

BMI, body mass index; Free T₃, free triiodothyronine; Free T₄, free thyroxine; TSH, thyrotropin; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; TC, total cholesterol; Hcys, homocysteine.

914 pg/mL; fibrinogen, 1.8-3.5 g/L; Hcys, 0-15 μ M/L.

SH and overt hypothyroid patients received replacement therapy with 100 μ g of L-thyroxine daily. Dose titration was adjusted according to the second month's TSH results. They took the medicine early in the morning on empty stomach. Patients were asked not to change their life style (diet or exercise) during the six-month study period. Thyroid function, Hcys, folate, vitamin B₁₂, fibrinogen, lipid profiles, and renal functions were measured again after 6 months of stable euthyroidism. No patients were lost to follow-up. The local ethics committee approved this study, and all the subjects gave written informed consent.

Statistical analyses including parametric and nonparametric tests were done with the statistical package for social sciences software (SPSS, version 10.0). Data are presented as means ± standard deviation. A probability value less than 0.05 was accepted as statistically significant. Paired t-test was used for comparing pre-treatment and post-treatment values of patients. Student t-test was used for comparing patients and control group values. Correlation analyses were performed according to Pearson. Changes of any parameters (delta) with treatment were analyzed, and correlation analyses of delta levels were also done.

Table 2. Baseline data of the overt hypothyroid patients and the control group

	Control subjects (n=11)	Overt hypothyroid patients (before treatment) (n=20)	Overt hypothyroid patients (after treatment) (n=20)
Age (yr)	39.9±12.5	41.3±11.1	41.3±11.1
BMI (kg/m ²)	23.6±3.9	25.8±3.3	25.5±2.7
Free T ₃ (pg/mL)	2.9±0.6	2.0±0.9*	2.4±0.8 [†]
Free T ₄ (ng/dL)	1.4±0.2 [†]	0.6±0.3 [†]	1.1±0.4 [§]
TSH (μ IU/mL)	2.2±0.9**	52.8±21.7 [†]	3.7±1.0 [§]
Creatinine clearance (mL/min)	114.4±20.7	104.0±21.2	107.0±20.3
Total cholesterol (mg/dL)	192.0±21.3	252.2±67.5 [†]	200.1±47.5 [§]
LDL-C (mg/dL)	100.3±11.1 [†]	163.2±49.9 [†]	124.1±36.0 [§]
HDL-C (mg/dL)	49.1±8.7	45.7±10.2	42.5±17.1
Triglyceride (mg/dL)	205.5±52.8	211.3±122.5	185.1±78.3
TC/HDL-C	4.0±0.4 [†]	5.7±1.9 [†]	5.1±1.7 [†]
Folate (ng/mL)	7.2±1.0	6.8±1.6	7.0±1.6
Vitamin B ₁₂ (pg/mL)	244.2±47.2	236.4±102.0	242.0±119.8
Hcys (μ M/L)	7.9±0.6	10.3±3.4*	7.7±2.3 [§]
Fibrinogen (g/L)	2.0±0.1 [†]	3.3±1.2 [†]	3.2±1.3

Data are mean ± SD. Comparison between control group and overt hypothyroid patients before treatment (*p<0.01, [†]p<0.001). Comparison of overt hypothyroid patients before and after treatment ([‡]p<0.05, [§]p<0.001). Comparison between control group and treatment group [†]p<0.05, [‡]p<0.01, [§]p<0.001.

BMI, body mass index; Free T₃, free triiodothyronine; Free T₄, free thyroxine; TSH, thyrotropin; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; TC, total cholesterol; Hcys, homocysteine.

RESULTS

Descriptive characteristics for the control group and the SH patients both during the initial evaluation and after the medical treatment are shown in Table 1. Age and BMI values of the two groups were similar. Before treatment, the mean TSH level was apparently higher in SH patients versus the control subjects (15.8 ± 2.4 vs. 2.2 ± 0.9 $\mu\text{IU/mL}$, $p < 0.001$), and the mean fT_4 level was lower in SH patients than in controls (1.0 ± 0.1 vs. 1.4 ± 0.2 ng/dL ; $p < 0.001$). The fibrinogen level of SH patients was higher than that of control subjects (2.7 ± 1.1 vs. 2.0 ± 0.1 g/L , $p < 0.05$), whereas Hcys level was similar. The mean LDL-C, TC level and TC/HDL-C ratio of SH patients were significantly higher than those of control subjects (143.6 ± 45.2 vs. 100.3 ± 11.1 mg/dL , $p < 0.01$; 228.8 ± 56.6 vs. 192.0 ± 21.3 mg/dL , $p < 0.05$; 4.9 ± 1.2 vs. 4.0 ± 0.4 , $p < 0.05$, respectively).

After treatment of SH patients, the TSH level decreased (from 15.2 ± 2.4 to 3.4 ± 1.6 $\mu\text{IU/mL}$, $p < 0.001$) and fT_3 and fT_4 increased significantly (from 2.4 ± 0.6 to 2.7 ± 0.6 pg/mL , $p < 0.05$; 1.0 ± 0.1 to 1.2 ± 0.2 , $p < 0.001$, respectively). In addition, TC and LDL-C levels decreased significantly with treatment (228.8 ± 56.6 vs. 203.2 ± 39.4 mg/dL , $p < 0.01$; 143.6 ± 45.2 vs. 127.5 ± 36.8 mg/dL , $p < 0.05$, respectively). Neither fibrinogen nor Hcys levels of SH patients changed with treatment. While post-treatment TSH, fT_3 and fT_4 levels of SH patients were similar to those of control subjects, the post-treatment LDL-C level of SH patients was still significantly higher than that of control subjects (127.5 ± 36.8 vs. 100.3 ± 11.1 mg/dL , $p < 0.05$) (Table 1).

The baseline and post-treatment characteristics data of overt hypothyroid patients and the healthy control groups are summarized in Table 2. Age and BMI values of the two groups were similar. The pre-treatment TSH level was significantly higher in overt hypothyroid group than in healthy subjects (52.8 ± 21.7 vs. 2.2 ± 0.9 $\mu\text{IU/mL}$, $p < 0.001$). On the other hand, fT_3 and fT_4 levels were significantly lower (2.0 ± 0.9 vs. 2.9 ± 0.6 pg/mL ; $p < 0.01$; 0.6 ± 0.3 vs. 1.4 ± 0.2 ng/dL , $p < 0.001$, respectively). Before treatment, overt hypothyroid patients had significantly higher Hcys and fibrinogen levels compared to controls (10.3 ± 3.4 vs. 7.9 ± 0.6 $\mu\text{M/L}$, $p < 0.01$; 3.3 ± 1.2 vs. 2.0 ± 0.1 g/L , $p < 0.001$, respectively). In addition, mean TC, LDL-C levels and the TC/HDL-C ratio were significantly higher in overt hypothyroid subjects than in healthy controls (252.2 ± 67.5 vs. 192.0 ± 21.3 mg/dL , $p < 0.001$; 163.2 ± 49.9 vs. 100.3 ± 11.1 mg/dL , $p < 0.001$, 5.7 ± 1.9 vs. 4.0 ± 0.4 , $p < 0.001$, respectively) (Table 2).

After achieving euthyroid state, Hcys levels of overt hypothyroid patients decreased significantly (from 10.3 ± 3.4 to 7.7 ± 2.3 $\mu\text{M/L}$, $p < 0.001$). Furthermore, TC, LDL-C levels, and the TC/HDL-C ratio of overt hypothyroid patients were decreased after gaining euthyroid state (from 252.2 ± 67.5 to 200.1 ± 47.5 mg/dL , $p < 0.001$; from 163.2 ± 49.9 to 124.1 ± 36.0 mg/dL , $p < 0.001$; 5.7 ± 1.9 to 5.1 ± 1.7 , $p < 0.05$,

respectively). Even though all patients' post-treatment TSH, fT_3 , and fT_4 levels were in normal limits, post-treatment TSH level was significantly higher (3.7 ± 1.0 vs. 2.2 ± 0.9 $\mu\text{IU/mL}$, $p < 0.001$) and fT_4 (1.1 ± 0.4 vs. 1.4 ± 0.2 ng/dL , $p < 0.01$) was significantly lower than in control subjects. In addition, post-treatment fibrinogen (3.2 ± 1.3 vs. 2.0 ± 0.1 g/L , $p < 0.01$), LDL-C (124.1 ± 36.0 vs. 100.3 ± 11.1 mg/dL , $p < 0.05$), and the TC/HDL-C ratio (5.1 ± 1.7 vs. 4.0 ± 0.1 , $p < 0.05$) were also significantly higher than in control subjects (Table 2).

Serum vitamin B12, folate, creatinine, and creatinine clearance levels remained unchanged after L-thyroxine treatment either SH or overt hypothyroid patients.

In overt hypothyroid patients, the changes in Hcys levels (ΔHcys) by treatment was negatively correlated with the changes of folate (Δfolate) ($r = -0.49$, $p = 0.025$) and vitamin B12 ($\Delta\text{vitamin B}_{12}$) ($r = -0.45$, $p = 0.044$). In addition, there were positive correlations between ΔHcys and ΔTC ($r = 0.61$, $p = 0.004$), $\Delta\text{LDL-C}$ ($r = 0.625$, $p = 0.003$), and the $\Delta\text{TC}/\text{HDL-C}$ ratio ($r = 0.546$, $p = 0.013$).

Except pre-treatment serum fT_4 and TSH, no significant differences were found between SH and overt hypothyroid patients in any parameters either before or after treatment.

DISCUSSION

Several reports in the literature indicate that hypothyroidism is associated with elevated plasma Hcys concentrations (6, 10, 11). Thyroid status has a profound influence on a variety of biochemical processes, some of which may have secondary effects on the Hcys metabolism. Thyroid hormones markedly affect riboflavin metabolism, mainly by stimulating flavokinase and thereby the synthesis of flavin mononucleotide and flavin adeninedinucleotide (FAD) (12-14). Conceivably, these metabolic changes may affect Hcys metabolism because flavin mononucleotide and FAD serve as cofactors for enzymes involved in the metabolism of vitamin B6, cobalamin, and folate (14). Circulating Hcys concentrations in hypothyroidism can rise through reduced activity of the flavoprotein methylenetetrahydrofolate reductase (MTHFR), an enzyme involved in the catalysis of Hcys and its remethylation to methionine. Hypothyroid individuals can be defective in converting riboflavin to the co-enzyme FAD, and consequently, deficient in MTHFR activity (15).

Increased Hcys levels in hypothyroid patients might contribute to a higher cardiovascular risk (13). It has been shown that thyroid replacement in such patients' results in lowering of the Hcys level. Whether or not individuals with SH also increase their Hcys concentrations, and whether this elevation might help to explain the increased prevalence of atherosclerotic diseases observed in this condition, remain unclear. If an elevation in serum Hcys concentrations with associated atherosclerotic cardiovascular disease could be demonstrated

in individuals with SH, this would provide an added impetus to identify and treat this disorder with thyroid replacement therapy (16-18).

Luboshitzky et al. compared 57 women with SH against 34 healthy controls and found no significant increase in Hcys levels in SH subjects (19). In addition, Atabek et al. investigated Hcys concentrations in adolescent patients with SH. Hcys concentrations showed no statistical difference between patients and controls in their study (20). Furthermore, Deicher et al. measured plasma Hcys levels in newly diagnosed SH patients at baseline and after three months of L-T₄ supplementation. Hcys levels remained unchanged. They proposed that Hcys was not associated with an increased risk for ischemic heart disease in SH patients (21).

Sengul et al. evaluated Hcys levels and the effect of L-thyroxine treatment in SH. After L-thyroxine treatment, Hcys levels reduced significantly. They reported that if Hcys was elevated, treatment of SH with L-thyroxine might decrease the risk of coronary artery disease (22). On the other hand, Perez et al. investigated the impact of euthyroidism restoration on emerging risk factors including Hcys, C-reactive protein, and apolipoprotein B in SH patients. No treatment effect was observed on these emerging risk factors in patients with TSH >10 mIU/L. They proposed that measurement of emerging risk factors did not offer additional arguments for treating patients with a TSH level >10 mIU/L (23). In addition, Ozcan et al. evaluated Hcys levels in SH patients. No difference were found in Hcys levels between patients and control group, and no changes were noted in plasma Hcys concentrations after treatment (24). This result is similar to our results.

Elevated levels of fibrinogen have consistently shown as an independent predictor of initial and recurrent cardiovascular events (25). There are several potential mechanisms by which fibrinogen can promote the development of atherosclerosis and thrombosis (25, 26). It affects the haemostatic system and is the major determinant of plasma viscosity. Fibrinogen is an acute phase reactant and therefore could also be a marker for increased inflammatory activity (27). Cantürk et al. measured fibrinolytic activity in 35 SH patients before and after L-T₄ treatment and found a significantly higher fibrinogen level in SH patients than in healthy controls. However, no significant beneficial effect of LT₄ treatment to fibrinogen levels was seen in patients with SH (3). The result was based on the number of study population and the relatively short period of treatment. Müller et al. investigated various haemostatic variables in 42 women with SH and compared them to 66 euthyroid controls. They found no differences between the groups with respect to fibrinogen (28). In our study, we observed higher fibrinogen levels in SH patients than in control subjects, but fibrinogen levels remained unchanged with L-T₄ treatment as was observed in Cantürk's study.

Hypothyroidism is associated with high cholesterol and lipoprotein levels (29). Treatment of hypothyroid patients

with L-thyroxine normalizes lipid levels (4, 30). The association of SH with changes in serum lipid levels and the effect of T₄ replacement on these changes are still elusive. Arem and Patsch demonstrated that HDL-C, HDL₃-C, and apolipoprotein A-1 were not significantly affected by levothyroxine therapy and there was only a slight trend of increase in HDL₂-C besides a significant reduction in LDL-C during T₄ substitution in SH patients with a mean TSH level of 16.6 μ IU/mL (31). On the other hand, Caron et al. reported lower HDL-C levels in SH patients than in a control group and demonstrated a significant increase in HDL-C levels after levothyroxine therapy (32). However, a controlled trial including 66 women with SH found no significant change in HDL-C (33). Serter et al. found higher pre-treatment serum TC and LDL-C concentrations in SH patients than in control subjects and reduced TC, LDL-C and TC/HDL-C ratio after LT₄ replacement therapy (34). Our results found similar to those from the Serter's study.

In our study, patients with overt hypothyroidism have higher Hcys and fibrinogen levels than healthy subjects. Hcys level of overt hypothyroid patients decreased with LT₄ treatment. There was a negative correlation between Δ Hcys and Δ folate, and Δ vitamin B₁₂ levels in overt hypothyroid patients. Elevated Hcys levels in overt hypothyroidism may be linked to altered folate and vitamin B₁₂ status. In contrast to overt hypothyroidism, only fibrinogen, but not Hcys values, was affected in SH, yet without significant improvement after L-thyroxine therapy. We conclude that SH is not associated with hyperhomocysteinemia and atherosclerosis seen in SH patients might be due to higher fibrinogen and atherogenic lipid profiles.

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