L-Thyroxine Treatment of Patients with Subclinical Hypothyroidism Reduce Inflammation

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Abstract: *Background*: Subclinical hypothyroidism (SCH) has been found to be associated with cardiovascular disease including atherosclerosis which is also called inflammatory disorder. The objective of this study was, to investigate the efficacy of L-thyroxine therapy on inflammation in patients with SCH and to determine whether treatment of SCH would reduce inflammation.

Methods: Twenty patients with thyroid stimulating hormone levels between 5 and 10 mU/L and twenty healthy persons (control) were enrolled to the study. 0.025-0.075 mg/d L-thyroxine therapy was given to the patients. Patients were followed till they became euthyroid. C-reactive protein (CRP), lipid profile and thyroid function tests were evaluated. CRP was determined by a high sensitivity immunoassay.

Results: CRP, total cholesterol and low-density lipoprotein (LDL) were significantly elevated in patients with subclinical hypothyroidism compared to the control group at baseline ($p<0.01$, $p<0.05$, $p<0.001$, respectively). L-thyroxine therapy significantly decreased CRP ($p<0.01$) and low-density lipoprotein in treatment group ($p<0.05$). Nevertheless, no significant change was found in other lipid parameters.

Conclusion: These findings demonstrate that patients with SCH are characterized by inflammatory disorders and higher lipid profile.

Keywords: Subclinical hypothyroidism, atherosclerosis, inflammation, C-reactive protein.

INTRODUCTION

Cardiovascular diseases are recognized as a leading cause of morbidity and mortality in general population [1]. Although the reasons behind the development of cardiovascular diseases are multifactorial, atherosclerosis plays a prominent role. Traditional risk factors such as hypertension, diabetes mellitus, altered lipid profile and non-traditional risk factors such as oxidative stress, inflammation and homocysteinemia, have been shown to be associated with atherosclerotic cardiovascular disease [2-5]. Over the last years; much attention has been paid to the idea that inflammation plays a key role in atherosclerosis. C-reactive protein (CRP) is a sensitive and objective marker of inflammation and may act in the pathogenesis of atherosclerosis *via* several mechanisms [6, 7]. Pasceri *et al.* [8] proposed that CRP directly stimulates the inflammatory reaction of arteriosclerosis by inducing the expression of adhesion molecule in vascular endothelial cells and further hypothesized that CRP could be a treatment target for arteriosclerosis. Furthermore, elevated levels of CRP have been directly related to the risk of cardiovascular disease such as myocardial infarction [9-12].

Subclinical hypothyroidism (SCH) is a metabolic condition characterized by elevations in thyroid-stimulating hormone (TSH) level with normal free T_4 (fT_4) and normal free T_3 (fT_3) [13]. The most common cause of SCH in the community is chronic autoimmune thyroiditis [14]. In addition, the reported prevalence of SCH ranges from 1% to 10% in the general population [15-17]. On the other hand, SCH have also been found to be associated with cardiovascular disease due to atherosclerosis. However, the idea that SCH and thyroid autoimmunity are also risk factors for cardiovascular disease is still controversial [18-21]. Besides, several studies have been published that L-tyroxine treatment of SCH not only causes various effect upon lipid parameters and inflammation but also arrhythmias particularly in elderly patients [22-24].

The aim of this study was to investigate the effect of L-thyroxine therapy on inflammation in patients with SCH and to determine whether treatment of SCH would lessen inflammation.

MATERIALS AND METHODS

Study Protocol

After written informed consent was obtained from all participants, patients who were admitted to endocrinology polyclinic were registered to the study consecutively. Twenty patients with TSH levels between 5 and 10 mU/L with normal fT_4 and normal fT_3 levels (SCH group) and age and sex matched twenty healthy persons with normal serum TSH, fT_4 and fT_3 levels (control group) were enrolled to the study. Patients with SCH were divided into 2 subgroups according to

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their autoimmune markers as well. After an overnight fasting period, all patients underwent full medical assessment and laboratory examinations to rule out the non-thyroidal illness. Patients receiving amiodarone, lithium, anti-thyroid therapy, anti-oxidant and anti-inflammatory drugs, and other potential causes of elevated CRP such as patients with chronic diseases including cardiovascular disease, diabetes mellitus, tobacco habit, periodontal disease and infection were excluded. Patients who had previous history of thyroid disease were also excluded. 0.025 mg/d L-thyroxine therapy was given to the patients with subclinical hypothyroidism. L-thyroxine therapy dose was titrated for patients individually according to their thyrotropine levels. Patients were followed twice a week until they became euthyroid. We did not observe any side effects during the trial.

Laboratory Analyses

Blood samples were drawn before treatment and when patients were euthyroid. The investigators who did the laboratory analyses were blinded either each group. Samples were centrifuged and frozen at –30^{o}C until they were studied. High sensitivity CRP (hs CRP), lipid profile (Total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG), thyroid function tests (TSH, free T_3, and free T_4) and anti-thyroglobulin antibodies (anti-TG), anti-microsomal antibodies (anti-M) were evaluated. hs CRP were determined with nefelometric test (Dade Behring, Marburg GmbH, Germany) by using BN 100 machine (Dade Behring, Liederbach, Germany). Normal range of hs CRP is 0-2 mg/l. The thyroid function tests were measured using one of the luminescence immunoassay methods known as the electrochemiluminescence immunoassay (ECLIA) method. For measurements of fT3, fT4 and TSH, Elecsys fT3 reagent kit-11731386, Elecsys FT4 reagent kit-11731297, Elecsys TSH reagent kit-11731459, and Roche's Elecsys 1010/1010 kit were used, respectively. A Roche modular analytics E170 (Elecsys module) immunoassay analyzer that is compatible with these kits was used for the thyroid function tests. Serum levels of anti-TG and anti-M were measured with ELISA kits (Trinity Biotech Plc, Co Wicklow, Ireland.) by using full automatic Triturus (Grifols Diagnostic, Barcelona, Spain) micro ELISA device. Serum levels of lipids were measured by AU600 autoanalyzer (Olympus Optical Co., Japan).

Statistical Analyses

Statistical analysis was performed using SPSS 10.0. Data were presented as mean ± SEM. Data were normally distributed. To compare values obtained before and after L-thyroxine therapy, paired - t test was used. On the other hand, unpaired t - test was used to examine the difference between SCH and control group. Differences between SCH subgroups were also evaluated with unpaired t - test.

RESULTS

The main characteristics of patients are summarized in Table **1**. There were no significant differences in any clinical parameters (age, gender, BMI) between patients with SCH and control group. Anti-TG and anti-M were all negative in control group. When we compare lipid parameters, only serum LDL and TC levels were significantly higher in SCH group than control group and there were no significant changes in other lipid parameters. Additionally, TSH and hs CRP values were also significantly elevated in patients with SCH in comparison to control group (Table **2**).

Table 1. Main Characteristics of SCH and Control Group

	Control (n = 20)	SCH Group (n = 20)	P
Age (year)	36.2 ± 2.3	37.5 ± 3	NS
Sex (m/f)	1/ 19	1/ 19	NS
Height (cm)	156.7 ± 1.8	158.7 ± 1.4	NS
Weight (kg)	67.4 ± 3	73.4 ± 2.9	NS
BMI (kg/m^2)	27.4 ± 1.1	29.3 ± 1.2	NS
Anti-M (%)	-	60	-
Anti-TG (%)	-	55	-

BMI: Body Mass Index Anti-M: Anti microsomal anticor, Anti-TG: Anti thyroid thyroglobuline anticor,

Table 2. Baseline Laboratory Values of SCH and Control Group

	Control (n = 20)	SCH Group (n = 20)	P
TC (mg/dl)	171.9 ± 6.4	208.3 ± 12.3	**<0.05**
HDL (mg/dl)	39.4 ± 1.9	41.9 ± 1.6	NS
LDL (mg/dl)	106.7 ± 5.1	146.4 ± 8.4	**<0.001**
TG (mg/dl)	124.8 ± 11.8	190.3 ± 25.6	NS
hs CRP (mg/l)	1.8 ± 0.2	4 ± 0.7	**<0.01**
TSH (mU/l)	1.9 ± 0.3	6.7 ± 0.3	**<0.001**

TC: Total cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglyceride, hs CRP: high sensitivity C-reactive protein, TSH: Thyroid stimulant hormone.

In patients receiving L-thyroxine therapy, LDL, hs CRP, TSH serum levels, and BMI were significantly decreased after L-thyroxine therapy. On the other hand, TC, HDL and TG showed no significant changes (Table **3**). No side effect was observed in any patient during L-thyroxine therapy.

Table 3. Laboratory Values of Patients with SCH (Before and After Treatment)

	Before	After	P
TC (mg/dl)	208.3 ± 12.3	192.7 ± 9.5	>0.05
HDL (mg/dl)	41.9 ± 1.6	42.7 ± 2.1	>0.05
LDL (mg/dl)	146.4 ± 8.4	124.2 ± 9.7	<0.05
TG (mg/dl)	190.3 ± 25.6	141.7 ± 20.7	>0.05
hs CRP (mg/l)	4 ± 0.7	2.9 ± 0.5	<0.01
TSH (mU/l)	6.7 ± 0.3	2.5 ± 0.3	<0.001
fT$_3$ (mU/l)	2.6 ± 0.1	3.2 ± 0.1	<0.001
fT$_4$ (mU/l)	1.1 ± 0.04	1.4 ± 0.06	<0.001
Weight (kg)	73.4 ± 2.9	72.6 ± 2.9	<0.05
BMI (kg/m^2)	29.3 ± 1.2	28.9 ± 1.3	<0.05

TC: Total cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglyceride, hs CRP: high sensitivity C-reactive protein, TSH: Thyroid stimulant hormone, fT$_3$: free T_3, fT$_4$: free T_4, BMI: Body Mass Index

CRP values of patients with negative autoimmune markers were higher than patients with positive autoimmune markers and control group. There was no significant difference in CRP serum levels between each subgroup of SCH, but serum CRP levels of each group were significantly higher than control group. CRP serum levels of each SCH subgroup reduced significantly after treatment (Table **4**).

Table 4. CRP Values of SCH Subgroups (Before and After Treatment) and Control Group

	CRP (mg/l) Before	CRP (mg/l) After	p
SCH subgroup 1 (n = 14)	$3.8 \pm 0.7^{*\dagger}$	2.8 ± 0.5	<0.05
SCH subgroup 2 (n = 6)	$4.4 \pm 1.4^{\dagger}$	2.9 ± 1.2	<0.05
Control	1.8 ± 0.2	-	

SCH: subclinical hypothyroid, CRP: C-reactive protein, SCH subgroup 1: anti-TG/M (+), SCH subgroup 2: anti-TG/M (-), $^{*}p > 0.05$ (I *vs* II) and $^{\dagger}p < 0.05$ (I *vs* control, II *vs* control).

DISCUSSION

Hypothyroidism is closely associated with cardiac abnormalities. Patients with elevated serum thyrotropine levels and normal free T_4 and normal T_3, termed SCH, may progress to permanent hypothyroidism. In addition, patients with SCH have more cardiovascular risk factors compared to the normal healthy population and are more likely to develop atherosclerosis and other cardiovascular diseases [25, 26]. Although hypothyroidism is usually associated with higher lipid profile, SCH has a diverse serum lipid profile. Significant elevation of lipid profile in SCH has been reported in some studies [27, 28], but other studies have not supported this results [29, 30]. In the present study, we found significant increases in total cholesterol and low-density lipoprotein cholesterol in patients with SCH before the thyroid hormone supplementation compared with euthyroid controls.

Recent data suggest that inflammation may play a central role in the background and complications of cardiovascular disease, particularly contribute to the development of atherosclerosis [6]. CRP known as a marker of inflammatory state is an independent risk factor for cardiovascular disease. In addition, a recently published report suggested that CRP is more than a marker and may be a mediator of atherosclerosis [7, 31]. In fact, CRP may play a role in the pathogenesis of atherosclerosis through binding to damaged cells and activating complement system, displaying calcium-dependent *in vitro* binding and aggregation of low density lipoprotein (LDL) and very LDL [32, 33]. In our study, CRP values were significantly elevated in patients with SCH than euthyroid controls.

Although CRP level was found as a stronger predictor than the LDL-C level for myocardial infarction, ischemic stroke, coronary revasculature, or death due to cardiovascular causes in healthy women [34], some evidence suggest that lipid lowering models of therapy also reduce inflammation, which may reduce the risk of cardiovascular events, even for individuals with LDL-C levels in the normal range [35]. Nevertheless, because CRP and LDL-C levels appeared to identify somewhat different risk groups, the combined risk assessment was superior to that of either marker alone. In our study, serum levels of CRP and LDL-C reduced significantly after patients became euthyroid with L-thyroxine therapy. The reduction in LDL and CRP serum levels lead us to consider that treatment with L-thyroxine therapy may protect patients from cardiovascular morbidity and mortality due to atherosclerosis.

On the other hand, obese patients are associated with elevated inflammatory cytokines [36]. Additionally, it has been shown that serum levels of CRP and other inflammatory markers were positively correlated with BMI in overweight patients [37]. The study population of the current study was overweight. Although, we did not evaluate the effect of treatment of SCH upon weight of the participants, it is reasonable to expect a weight reduction in SCH patients through a favorable effect of L-thyroxine upon metabolism. This effect subsequently may contribute to a decrease in inflammation as reported above. Moreover, when we compared the CRP serum levels of patients with positive and negative autoimmune markers, we did not observe significant difference in each group. Although the numbers of patients and follow up were insufficient to evaluate this condition, it can be speculated that SCH patients even with negative thyroid autoimmune markers might have inflammatory disorder.

In conclusion, these findings indicate that patients with subclinical hypothyroidism have increased levels of LDL, TC and signs of low-grade inflammation (raised hs CRP levels) and that subclinical hypothyroidism might be a risk factor for development of cardiovascular disease. Besides, although further comprehensive studies are required to explain the relationship between SCH and cardiovascular diseases.

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