CLINICS

CLINICAL SCIENCE

Comparison of pre- and post-levothyroxine high-sensitivity c-reactive protein and fetuin-a levels in subclinical hypothyroidism

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OBJECTIVE: The objective of this trial was to determine the levels of inflammatory markers, high-sensitivity C-reactive protein and fetuin-A pre- and post-levothyroxine treatment in cases of subclinical hypothyroidism.

MATERIALS AND METHODS: A total of 32 patients with a diagnosis of subclinical hypothyroidism and a control group of 30 healthy individuals were tested for high-sensitivity C-reactive protein and fetuin-A, followed by the administration of 50 μg of levothyroxine in the patient group for 3 months. During the post-treatment stage, high-sensitivity C-reactive protein and fetuin-A levels in the patient group were re-assessed and compared with pre-treatment values.

RESULTS: Pre-treatment levels of both high-sensitivity C-reactive protein and fetuin-A were observed to be higher in the patient group than in the control group. The decrease in high-sensitivity C-reactive protein levels during the post-treatment stage was not statistically significant. However, the decrease observed in post-treatment fetuin-A levels was found to be statistically significant.

CONCLUSION: The decrease in fetuin-A levels in subclinical hypothyroidism cases indicates that levothyroxine treatment exerts anti-inflammatory and anti-apoptotic effects. Although the decrease in high-sensitivity C-reactive protein levels was statistically non-significant, it is predicted to reach significance with sustained treatment.

KEYWORDS: Subclinical Hypothyroidism; hs-CRP; Fetuin-A; Levothyroxine.

Bilgir O, Bilgir F, Calan M, Calan OG, Yuksel A. Comparison of pre- and post-levothyroxine high-sensitivity c-reactive protein and fetuin-a levels in subclinical hypothyroidism. Clinics. 2015;70(2):97-101.

Received for publication on September 8, 2014; **First review completed on** October 14, 2014; **Accepted for publication on** December 5, 2014

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INTRODUCTION

Subclinical hypothyroidism (SHO) is a common thyroid disorder and its prevalence in the adult population has been reported to be 1-10%. Furthermore, SHO has been found to be more common in the elderly population (1,2). The diagnosis of SHO is based on a high serum thyroid-stimulating hormone (TSH) level and a normal free thyroxin level. SHO is asymptomatic in general; however, symptoms suggestive of thyroid hormone deficiency may be present in 30% of patients (3–5). In a cross-sectional study of 2336 cases of SHO, the symptoms identified were as follows dry skin in 28% of patients, dysmnesia in 24%, deficiency of judgment in 22%, muscle weakness in 22%, malaise in 18%, muscle cramps in 17%, cold intolerance in 15%, swelling in eyelids in 12%, constipation in 8% and hoarseness in 7% (5). These symptoms were found in 13.7% of the SHO group, in 12.1% of the euthyroid group and in 16.6% of the hypothyroidism group, revealing a statistically significant difference. This finding also showed a correlation between symptoms and thyroid hormone levels in 16.6% of the hypothyroidism group. Diverse symptoms of varying severity levels may develop in cases of SHO, although the disease is theoretically regarded as asymptomatic. In any case, the presence of similar symptoms in euthyroid groups and the lack of detailed, large-scale trials on the treatment response related to this topic have led to the emergence of diverse treatment approaches. Depression, amnesia, deficiency in cognitive functions and various neuromuscular complaints and disorders have been reported in patients with SHO (6–9). In addition, abnormal heart function has been shown related to disordered myocardial contraction and diastolic dysfunction (10–14).

Fetuin-A (α-Heremans-Schmid glycoprotein) is a circulating glycoprotein secreted by the liver into the circulation. Links between fetuin-A and cardiovascular disease, insulin resistance, diabetes mellitus, metabolic syndrome and non-alcoholic fatty liver have been reported (15–19).

Our trial was planned to investigate the levels of the subclinical inflammatory marker high-sensitivity C-reactive

No potential conflict of interest was reported.

DOI: 10.6061/clinics/2015(02)05

Table 1 - Demographic and clinical parameters in SHO patients and controls.

	Subclinical hypothyroidism (before treatment) (n = 32)	Subclinical hypothyroidism (after treatment) (n = 24)	Control group (n = 30)
Age (years)	44.28 ± 10.13	43.50 ± 10.59	40.17 ± 8.29
hs-CRP (mg/L)	5.70 ± 5.85	4.85 ± 4.01	2.06 ± 3.30
Fetuin-A (µg/mL)	410.40 ± 69.59	329.98 ± 166.71	350.30 ± 210.87
FT_3 (pg/mL)	3.02 ± 0.43	2.84 ± 0.45	2.69 ± 0.38
FT_4 (ng/dL)	0.82 ± 0.13	0.83 ± 0.17	0.81 ± 0.11
TSH (mIU/mL)	6.57 ± 2.53	4.64 ± 2.99	2.11 ± 1.26

The results are given as the mean ± SD. A *p*-value of <0.05 was considered significant.

protein (hs-CRP) and the anti-inflammatory, anti-apoptotic protein fetuin-A in SHO cases and in a healthy control group. In addition, the study aimed to show possible alterations in these inflammatory and anti-inflammatory markers following levothyroxine treatment in patients with SHO.

MATERIALS AND METHODS

A total of 32 patients referred to the Outpatient Clinic of the Department of Internal Diseases at İzmir Bozyaka Education and Research Hospital for any reason from 2009-2011 were enrolled in this trial. The selected patients had TSH levels over 5 mIU/mL and normal FT_3 and FT_4 levels during routine laboratory examinations and a diagnosis of SHO. The exclusion criteria were the administration of treatment for any condition, surgery on the thyroid or radioactive iodine treatment and a history of chronic disease; accordingly, the cases not meeting the designated criteria were enrolled. In addition, 30 healthy individuals were enrolled on a voluntary basis to generate a healthy control group. Patients with SHO were treated with levothyroxine at 50 µg/day for 3 months, as appropriate based on the indication and venous blood samples were also collected during the post-treatment stage. The trial was initiated upon approval of the study protocol by the Ethical Committee of İzmir Bozyaka Education and Research Hospital.

To determine TSH, FT_3, FT_4, hs-CRP and fetuin-A levels, 10-mL venous blood samples were obtained from the SHO cases and healthy controls during the pre- and post-treatment stages and placed in non-anticoagulant tubes. The blood samples were kept at room temperature for approximately 30 minutes until the blood was coagulated and then centrifuged at 4000 RPM for 5 minutes. Serum TSH, FT_3 and FT_4 levels were assessed using the chemiluminescence immunoassay method (Immulite 2000 XPi, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

To determine hs-CRP and fetuin-A levels, the serum samples were placed in Eppendorf tubes and kept frozen at -80℃. Following the collection of all samples, the serum samples were thawed and hs-CRP (DRG Instruments GmbH, Marburg, Germany) and fetuin-A (BioVendor, Modrice, Czech Republic) levels were determined using the enzyme-linked immunosorbent assay (ELISA) method according to the manufacturers' protocols.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS; version 11.0 for Windows, Chicago, IL, USA). The data are expressed as the mean ± standard deviation (SD). A two-sided *p*-value<0.05 was considered statistically significant.

A nonparametric Mann-Whitney U test was used to compare the variables between patients and controls and the Wilcoxon signed-rank test was used to compare the variables between treated and untreated SHO patients.

RESULTS

The pre- and post-treatment FT_3, FT_4, TSH, hs-CRP and fetuin-A levels of the patients with SHO and the healthy control group are shown in Table 1. The mean ages of the 32 SHO cases and the 30 healthy controls were determined to be similar.

There was a significant difference in hs-CRP, fetuin-A, FT_3 and TSH levels between pretreatment of SHO group and control group (Figures 1, 2 and 3). After treatment, fetuin-A levels were significantly decreased in the SHO patients. However, no statistically significant difference was observed in hs-CRP levels with treatment. The decrease observed in hs-CRP levels during the post-treatment stage was not statistically significant (Figure 1). In contrast, the decrease observed in post-treatment fetuin-A levels was found to be statistically significant (Figure 2). Comparison of pre- and post-treatment TSH values revealed a significant decrease in post-treatment TSH levels (Figure 3). Comparison of pre- and post-treatment FT_3 and FT_4 values did not reveal a significant difference.

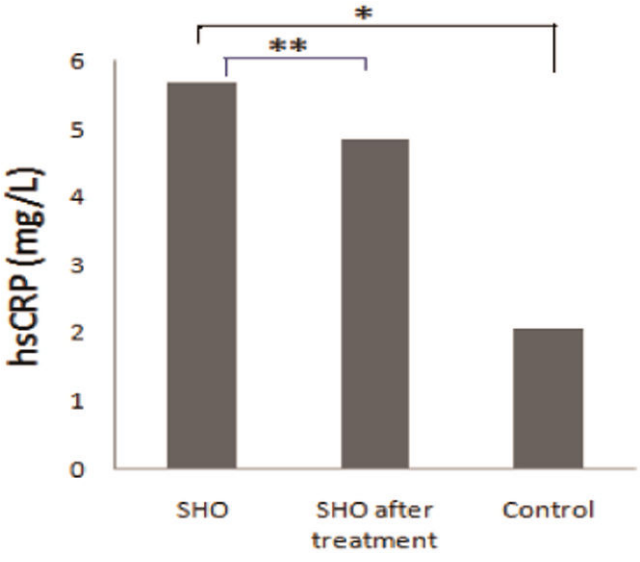

Figure 1 - Comparisons of hs-CRP level in SHO group, SHO patients after treatment and healthy controls. (*$p<0.001$) (**$p = 0.440$)
Abbreviation: SHO, subclinical hypothyroidism.

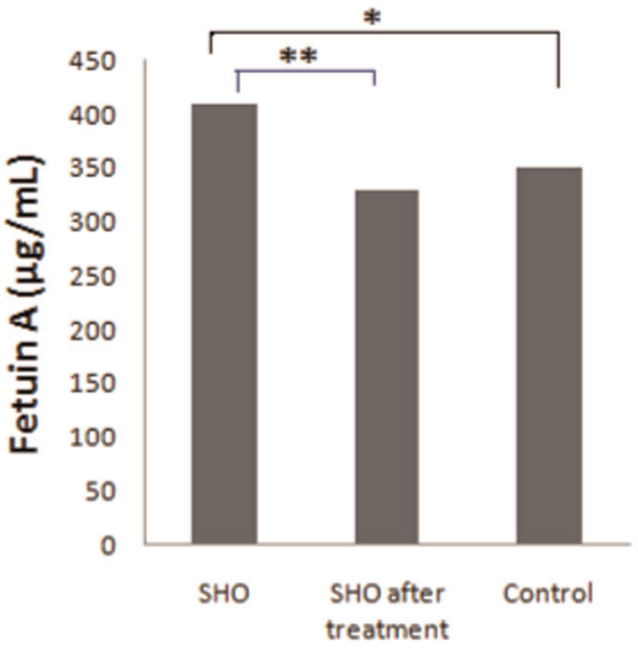

Figure 2 - Comparisons of fetuin A level in SHO group, SHO patients after treatment and healthy controls. (*$p = 0.019$) (**$p = 0.012$)
Abbreviation: SHO, subclinical hypothyroidism.

DISCUSSION

SHO is a common disease affecting 3 million individuals in the United Kingdom and 15 million individuals in the USA. The importance of this disease is based on data indicating its potential to induce an atherogenic lipid profile and endothelial dysfunction. In addition, co-morbidity of SHO and cardiovascular disease and an increase in mortality rates have been reported in several studies. Considering the serious outcomes of such a common disease, the importance of trials that have been and will be performed is increasing.

The acute-phase reactant hs-CRP is known to be an exceptionally sensitive and non-specific marker for inflammation, tissue damage and infection. Elevated hs-CRP levels indicate the presence and severity of inflammation (14). In current clinical practice, serum hs-CRP measurements are used to determine risks and to monitor treatment effectiveness. Hs-CRP is a cardiovascular risk marker, which has been regarded as significant in recent years following the recognition of atherosclerosis as an inflammatory disease. The correlation of SHO and prominent hypothyroidism with coronary heart disease in particular has been shown in a number of trials. However, although hypothyroidism and atherosclerosis are closely related, the correlation between thyroid function tests and hs-CRP levels has varied among different trials.

Currently, hs-CRP is regarded as a predictive marker for myocardial infarction, stroke, peripheral artery disease and sudden cardiac death among individuals with no cardiovascular disease and as a predictive marker for recurrent attacks and mortality in cases of metabolic syndrome (20). Based on previous trials, in terms of cardiovascular risk, individuals with hs-CRP levels <1 mg/L are regarded as low risk; individuals with levels between 1-3 mg/L, as moderate risk; and individuals with levels>3 mg/L, as high risk (21). Recent data suggest that CRP has a direct role in atherogenesis. CRP induces the expression of intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) in endothelial cells and mediates monocyte chemoattractant protein (MCP-1) induction and the phagocytosis of LDL by macrophages. CRP induces the synthesis of tissue factor, a strongly pro-coagulant substance, in monocytes and may bind to activated complement (22). As a result of all of these actions, CRP plays a role in endothelial dysfunction (23). In many trials conducted in diverse groups, a strong correlation was found between CRP levels and cardiovascular disease (24). In one trial performed in a patient group with a history of myocardial infarction, stroke and venous thrombosis and in a control group with no history of the indicated diseases, the patients were followed for 14 years and a group with trough hs-CRP values (<0.55 mg/L) was compared with a group with maximum values (>2.11 mg/L). The results indicated a 2-fold increase in stroke risk, a 3-fold increase in myocardial infarction and a 4-fold increase in symptomatic peripheral vascular disease in the group with maximum values (25). The role of hs-CRP in coronary heart disease was shown in another trial. Basal hs-CRP concentrations were measured in a healthy middle-aged group that was then followed for 8 years and an increase in the SD of hs-CRP levels was shown to increase the risk of coronary heart disease by 50% (26).

In the current trial, levothyroxine was administered in cases of SHO for 3 months and differences in hs-CRP values following treatment (if any) were assessed. Although a decrease was observed in these values, it did not reach a statistically significant level ($p = 0.440$). However, statistically significant decreases may be obtained by increasing the dose of levothyroxine used in treatment, which is expected to further decrease TSH values and by prolonging the treatment duration.

Fetuin-A is a protein with anti-inflammatory and anti-apoptotic effects. Mice with fetuin gene ablation are congenitally prone to calcifications, leading to fatal calcifications in the kidneys, testes, skin, heart and vessels (27). Fetuin-A may inhibit the activity of the insulin receptor tyrosine kinase, which causes insulin resistance. This glycoprotein also regulates bone development and reformation by antagonizing the activity of TGF-β (28). The

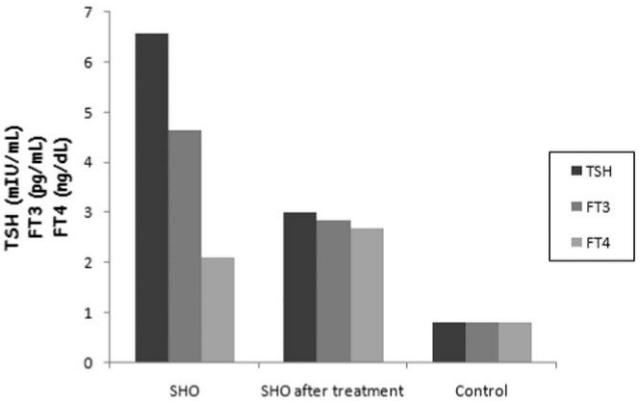

Figure 3 - Comparison of pre-treatment and post-treatment TSH, FT3 and FT4 values of patients with Subclinical Hypothyroidism and healthy control group.
Abbreviation: SHO, subclinical hypothyroidism.

normal serum concentration is 0.5-1 g/L and the levels decrease during inflammation. In addition, an inverse correlation has been reported between serum fetuin-A and CRP (29). Low fetuin-A levels among hemodialysis patients are associated with a high risk of cardiovascular disease and increased mortality. The main activity of fetuin-A is the inhibition of Ca-P accumulation. *In vitro*, this protein has been shown to inhibit the formation of apatites in osteoblast cultures. In addition, fetuin-A displays anti-inflammatory activity by regulating the phagocytosis of apoptotic particles by macrophages (30,31). In one trial, fetuin-A was reported to play a role in the inhibition of calcification in vascular smooth muscle cells (32).

In a trial conducted in adults, a comparison of the fetuin-A levels of healthy controls and a patient group comprising end-stage renal failure and dialysis cases revealed significantly low levels in both groups (33). In another trial conducted in 141 healthy individuals, serum fetuin-A levels and sclerosis of the vessel wall in the carotid artery were shown to be correlated and this correlation was indicated to be significant and independent of other well-known atherogenic factors (34).

Accordingly, in the present study, a statistically significant decrease was found in fetuin-A levels following 3 months of levothyroxine treatment compared with pre-treated values in cases of SHO. Considering that the etiology of the majority of SHO cases is thyroiditis, it is evident that even short-term levothyroxine treatment exerts anti-inflammatory and anti-apoptotic effects in these patients. Hence, even in cases without any alterations in FT_3 and FT_4 levels, thyroid replacement treatment may decrease the need for the management of future cardiovascular problems that would otherwise be expected to develop based on endothelial dysfunction and an atherogenic lipid profile.

■ ACKNOWLEDGMENTS

All of the subjects who contributed to the fulfillment of this work are duly acknowledged.

■ AUTHOR CONTRIBUTIONS

Bilgir O, Bilgir F and Calan M collected the data and contributed to the discussion. Bilgir O, Bilgir F, Calan M, Calan OG and Yuksel A wrote, reviewed and edited the manuscript and contributed to the discussion. Bilgir O is the guarantor of this work and, as such, has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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