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ORIGINAL ARTICLE

Evaluation of the effect of L-thyroxin therapy on endothelial functions in patients with subclinical hypothyroidism

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Abstract Subclinical hypothyroidism (SH) is characterized by normal serum free T4 (FT4), free T3 (FT3) levels and increased serum thyroid-stimulating hormone (TSH) levels. Endothelial dysfunction, which is an early step of atherosclerosis, has been reported in patients with subclinical hypothyroidism. The aim of this study is to evaluate endothelial functions and the effect of L-thyroxin (L-T4) therapy on endothelial functions in SH. Twenty-seven patients with SH and 22 healthy controls were evaluated in terms of endothelial functions, using brachial artery Doppler ultrasonography. After restorating euthyroidism, measurements were repeated. Baseline and nitroglycerin induced diameter (NID) of brachial artery were similar in patients with SH and the control group. Compared to the control group, the patients with SH showed significantly reduced flow-mediated diameter (FMD). Baseline and NID values were significantly higher after LT4 therapy in SH group. FMD also significantly increased after LT4 therapy. Hypothyroidism accelerates atherogenesis through modification of athero-sclerotic risk factors and direct effects on the blood vessels**.** In this study, we observed marked improvement in endothelial functions after L-T4 therapy in SH patients. We suggest that thyroid hormone replacement therapy may help to prevent atherosclerosis in this group of patients.

Keywords Subclinical hypothyroidism · Endothelial function · L-Thyroxin

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Introduction

Overt and subclinical hypothyroidism (SH) are well-known risk factors for atherosclerotic cardiovascular diseases [1, 2]. SH is a disorder characterized by elevated serum thyroid-stimulating hormone (TSH) levels despite normal free thyroid hormone [free T3 (FT3) and free T4 (FT4)] values. SH is mainly caused by Hashimoto's thyroiditis [3, 4]. Patients with SH are characterized by endothelial dysfunction, inflammation and increased prevalence of atherosclerotic lesions and cardiovascular events [5, 6].

Endothelial dysfunction has been reported in patients with overt and subclinical hypothyroidism [7, 8]. As an early stage of atherosclerosis, endothelial dysfunction is associated with increased risk of cardiovascular events [9, 10].

Flow-mediated dilatation (FMD) of the brachial artery, which is a surrogate marker for coronary artery endothelial function, is accepted as an independent predictor of future cardiovascular events [11]. FMD, defined as a change in brachial arterial diameter in response to reactive

hyperemia, is marker of an endothelium-dependent process. Increased flow induces endothelium-dependent dilatation of arteries lined by healthy endothelium [12]. Non-invasive evaluation of flow-mediated endothelium-dependent dilatation by ultrasonography has been used to examine endothelial function in large vessels [7–12].

It was first reported that endothelial dysfunction was present in the obvious hypothyroidism by measurement of FMD [13] which indicates an association between hypothyroidism and atherosclerosis. A reduction of nitric oxide (NO) availability is suggested as the mechanism of endothelial dysfunction in patients with hypothyroidism [5]. It has been demonstrated that thyroid hormone replacement therapy improves endothelial function in patients with hypothyroidism [5–14]. However, there are few studies about the assessment of endothelial functions and the effect of L-thyroxin (LT4) treatment in SH.

We aimed to assess endothelial functions and the effect of LT4 treatment in young adults with SH and without any cardiovascular disease, using brachial artery Doppler ultrasonography (USG).

Methods

Study design and patient population

We prospectively enrolled 27 patients (35.4 ± 11.4 years, 88% female) with newly diagnosed SH and 22 age and sex matched healthy subjects (34.77 ± 8.6 years, 90% women), as controls. Patients with SH were suggested as candidates for L4-therapy due to hypothyroidism symptoms and high serum levels of total cholesterol and anti-thyroid antibodies. SH was diagnosed as increased serum level of TSH and normal FT3 and FT4 levels. Normal reference levels of the thyroid panel were considered as, TSH: 0.27–4.20 mIU/ml, FT3: 1.80–4.60 pg/ml, FT4: 0.93–1.70 ng/dl according to the standards of the biochemistry laboratory of our clinic. SH was defined as having a TSH level above 4.20 mIU/ml and FT4 value within normal range. Exclusion criteria of the study were pregnancy, impaired liver or renal function, hypertension, heart failure, ischemic or valvular heart disease, respiratory diseases, diabetes mellitus, psychological or neurological disorders, malignancy, smoking, and use of the drugs that may influence the heart rhythm or the level of thyroid hormones.

All the SH patients were symptomatic, and were in sinus rhythm. Physical examinations, medical history, and electrocardiograms were found to be normal. Blood samples were collected from all patients after a 8–12-h fasting period. Height, weight, waist, and hip circumference were also measured.

Oral L-thyroxin was initiated at a daily dose of 0.5 μg/kg at baseline and the patients were under closer follow-up until euthyroid levels were provided. Brachial ultrasonography examination was performed before L-T4 therapy and 4–6 weeks after the restoration of euthyroidism.

Study protocol was approved by local Ethics Committee of our institute and a detailed written informed consent was obtained from each patient. The study has carried out according to the "Declaration of Helsinki".

Brachial ultrasonography

The noninvasive determination of endothelial dysfunction was performed according to the method described by Celermajer et al. [12]. Imaging studies of the brachial artery were performed using a high-resolution ultrasound machine (GE Vingmed, Vivid 7, Horten, Norway) equipped with a 7.5-MHz linear-array transducer. All vasoactive medications were withheld for 24 h before the procedure. The subjects remained at rest in the supine position for at least 15 min before the examination started. Subject's right arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. The brachial artery was imaged in the longitudinal plane. A segment of the artery showing anterior and posterior intimal interfaces between the lumen and the vessel wall was selected for 2-D gray scale imaging. All ultrasound images were recorded on S-VHS videotape for subsequent blinded analysis. Recordings of both B-mode and pulsed Doppler spectral curve were taken at rest, during reactive hyperemia (endothelium-dependent vasodilatation), and following the sublingual application of isosorbide dinitrate (endothelium-independent vasodilatation). After baseline measurements, a sphygmomanometer cuff, placed around the right upper arm proximal to the imaged artery segment, was inflated to the pressure of 240 mmHg for 4.5 min to occlude arterial flow. To verify that suprasystolic compression of the brachial artery caused adequate increase in blood flow, flow velocity was measured at rest and within 15 s after cuff deflation. Blood flow, pressure, and end-diastolic diameter were recorded at 30-s intervals for 300 s after cuff release and at 6, 8, and 10 min until recovery to baseline values. After reestablishing of baseline conditions 15–20 min later, measurements of arterial diameter and flow velocity were repeated, followed by sublingual isosorbide dinitrate administration at a dose of 5 mg to assess endothelium-independent vasodilatation. Four minutes later, measurements of arterial diameter and flow velocity were repeated. The arterial diameter was measured in millimeters as the distance between the anterior wall media-adventitial interface ("m" line) and the posterior wall intima-lumen interface at end-diastole, coincident with the R wave on the

continuously recorded electrocardiogram at two sites along the artery. The maximum FMD and nitroglycerin induced diameter (NID) diameters were calculated as the average of the three consecutive maximum diameter measurements after hyperemia and nitroglycerin, respectively.

Biochemical analysis

FT3, FT4, TSH level, serum total cholesterol and triglyceride, LDL and HDL cholesterol levels were measured from all patients. Thyroid function tests were repeated until the restoration of the euthyroidism. FT3, FT4, TSH, anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-TG) levels were measured using Roche Eleycsys Modular Analytics E170 (Roche Diagnostics GmbH, Mannheim, Germany) autoanalyser that works with electrochemiluminescence immunoassay (ECLIA) method. Abbott Aeroset (Chicago, IL, USA) was used to measure total cholesterol, triglyceride, and LDL-C enzymatically and HDL-C with immunoinhibition method.

Statistical analysis

The data were evaluated using descriptive statistics (mean, standard deviation). Qualitative data were compared using chi-square test. Pre-treatment and post-treatment parameters were compared using Wilcoxon ranking test because of the failure of the establishment of parametric test conditions. For the evaluation of the correlation, Spearman Correlation test was used because of the failure of the establishment of parametric test conditions. The results were considered significant when the P value was less than 0.05. Statistical analyses were done using Epi Info Version 3.5.1.

Results

Table 1 summarizes the baseline clinical and demographic characteristics of the patients and the controls. Total cholesterol levels of the patients with SH was significantly higher when compared to the control group ($P = 0.025$). The patients with SH and healthy control group were similar in terms of clinical and other demographic characteristics. Compared to the control group, the patients with SH showed significantly lower serum FT4 level ($P < 0.001$) and a significantly higher serum TSH level ($P < 0.001$) (Table 1).

The patients with subclinical hypothyroidism and healthy control group were similar in terms of baseline diameter and nitrate-induced diameter of brachial artery. Compared to the control group, the patients with SH showed significantly reduced FMD ($P < 0.001$) but there was no correlation between FMD and TSH level (Table 2).

Serum total cholesterol level of the patient group markedly decreased after LT4 therapy ($P = 0.021$). The baseline and nitrate-induced diameters of brachial artery were significantly higher after LT4 treatment in SH group ($P = 0.005$ and $P = 0.001$, respectively). FMD value markedly increased after L4 treatment in patients with SH

Table 1 Baseline clinical and demographic characteristics of the patients and the controls

Parameters	Patients with subclinical hypothyroidism ($n = 27$)	Healthy controls ($n = 22$)	P value
Age (years)	35.4 ± 11.4	34.77 ± 8.6	0.680
Gender (women/men)	27/3	22/2	0.816
BMI (kg/m^2)	27.61 ± 5.47	26.09 ± 3.09	0.282
Waist/hip ratio	0.80 ± 0.07	0.82 ± 0.07	0.239
SBP (mmHg)	115.7 ± 7.39	113 ± 8	0.013
DBP (mmHg)	69.3 ± 5.79	71.55 ± 8.31	0.118
Heart rate (beats/min)	69.7 ± 3.48	69.67 ± 8.06	0.707
Total cholesterol (mg/dl)	188.11 ± 38.26	167.91 ± 21.65	0.025
LDL cholesterol (mg/dl)	113.43 ± 38.56	100.55 ± 18.93	0.135
HDL cholesterol (mg/dl)	50.11 ± 10.69	50.05 ± 10.9	0.983
Triglyceride (mg/dl)	106.89 ± 55.11	85.27 ± 38.02	0.125
Free T3 (pg/ml)	3.25 ± 0.57	3.11 ± 0.27	0.271
Free T4 (ng/dl)	1.12 ± 0.18	1.33 ± 0.19	**<0.001**
TSH (m IU/ml)	7.09 ± 2.36	2.02 ± 1.04	**<0.001**
Anti-TPO (IU/ml)	123.43 ± 167.72	–	
Anti-TG (IU/ml)	153.14 ± 208.78	–	
Duration of euthyroidism (weeks)	10.85 ± 3.70	–	

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *TSH* thyroid-stimulating hormone, *anti-TPO* anti thyroid peroxidase antibody, *anti-TG* anti-thyroglobulin antibody

The significant values were made in bold

Table 2 Baseline brachial artery USG parameters of the patients and the controls

Parameters	Patients with subclinical hypothyroidism ($n = 27$)	Healthy controls ($n = 22$)	P value
BD (cm)	3.09 ± 0.71	3.07 ± 0.54	0.717
FMD (cm)	3.25 ± 0.71	3.75 ± 0.53	**<0.001**
NID (cm)	3.54 ± 0.78	3.77 ± 0.58	0.381

BD baseline diameter of brachial artery, *FMD* flow-mediated diameter, *NID* nitroglycerin induced diameter, *cm* centimeters

The significant value is made in bold

Table 3 The effect of thyroid hormone replacement treatment on serum lipid profile and endothelial functions

Parameters	Before the thyroid hormone replacement ($n = 27$)	After the thyroid hormone replacement ($n = 27$)	P value
Free T3 (pg/ml)	3.25 ± 0.57	3.16 ± 0.33	0.259
Free T4 (ng/dl)	1.12 ± 0.19	1.22 ± 0.16	**0.001**
TSH (m IU/ml)	7.01 ± 2.36	3.16 ± 0.33	**<0.001**
Total cholesterol (mg/dl)	188.11 ± 38.26	166.85 ± 20.75	**0.021**
LDL cholesterol (mg/dl)	113.43 ± 38.56	98.8 ± 9.05	0.15
HDL cholesterol (mg/dl)	50.11 ± 10.69	49.05 ± 9.2	0.99
Triglyceride (mg/dl)	106.89 ± 55.11	84.30 ± 35.01	0.11
Brachial ultrasonography parameters			
BD (cm)	3.09 ± 0.71	3.43 ± 0.46	**0.005**
FMD (cm)	3.25 ± 0.71	3.70 ± 0.55	**0.001**
NID (cm)	3.54 ± 0.78	4.04 ± 0.52	**0.001**

TSH thyroid-stimulating hormone, *BD* baseline diameter of brachial artery, *FMD* flow-mediated diameter, *NID* nitroglycerin induced diameter, *cm* centimeters

The significant values were made in bold

($P = 0.001$) (Table 3). We also observed a significant improvement in clinical symptoms of the patients due to hypothyroidism, at the end of the therapy.

Discussion

In this study, we aimed to evaluate endothelial functions and the effect of LT4 treatment on endothelial functions in SH. In the previous studies, endothelial dysfunction was demonstrated in obvious hypothyroidism by using brachial artery Doppler USG [13]. Recently, impairment in endothelial functions in patients with SH has been demonstrated in some studies [5–8]. Lekakis et al. [13] were the first to demonstrate the relationship between SH and FMD. However, there are few studies evaluating the effect of LT4 treatment on endothelial functions by brachial artery Doppler ultrasonography in SH [14–16]. In this study, we demonstrated that endothelial functions was significantly impaired in patients with SH compared to the control group ($P < 0.001$). Additionally, we found that after LT4 treatment, FMD value significantly increased ($P = 0.001$), indicating marked improvement in endothelial functions. Taddei et al. [5] observed that patients with SH are characterized with endothelial dysfunction, as a result of the reduction in NO availability and it can be improved by LT4 therapy, supporting our results. Marfella et al. [17] suggested an association between SH and inflammatory activity in atherosclerotic plaque instability. They also revealed that LT4 therapy helps to elicit improvement in plaque stabilization. This study was important in demonstrating the cellular and molecular effects of LT4 therapy on the atherosclerotic process. In contrast to these studies, Duman et al. [18] observed no improvement in FMD after LT4 treatment in patients with SH. In some studies, TSH levels have been found to be inversely correlated with FMD value in SH patients [19, 20]. In our study, we did not find significant correlation between serum TSH levels and FMD value in our patient group.

In this study, there was no significant difference between NID values of the patients and the control group, similar to some studies in literature [16–20]. However, we demonstrated that NID significantly increased after LT4 treatment in patients with SH. Adrees et al. [15] also found that NID markedly improved after LT4 treatment, supporting our results. In contrast, Razvi et al. [16], and Duman et al. [18] did not document improvement in NID after LT4 treatment.

Endothelium plays an important role in maintaining vascular function, through the production of several substances. Endothelial dysfunction is identified as an early marker of atherosclerosis and helps to predict cardiovascular events before they become overt [21].

SH causes endothelial dysfunction, low-grade inflammation, and increases prevalence of atherosclerotic lesions and cardiovascular events [5]. Thyroid hormone has antiatherosclerotic effects and hypothyroidism accelerates atherogenesis through modification of atherosclerotic risk factors and direct effects on the blood vessels [22]. TSH induces tumor necrosis factor-alpha production by bone-marrow cells [23]. Taddei et al. [24] demonstrated increase in serum levels of high-sensitive C-reactive protein (CRP) and interleukin-6 (IL-6) in SH patients suggesting an inflammatory process. Endothelial dysfunction in SH is partially dependent on the increase of serum lipid levels. In our study, total cholesterol level of the patient group was significantly higher than the control group and all our patients had thyroiditis; so endothelial dysfunction in SH patients may be the concomitant effect of hyperlipidemia and autoimmunity. As endothelial dysfunction is accepted

as an early sign of atherosclerosis, LT4 treatment may be sufficient to prevent future cardiovascular events, before symptoms due to hypothyroidism develop. Our results hypothesize an inhibitory effect of LT4 therapy on atherosclerosis in SH. Nevertheless, there is still no clear data, demonstrating the absolute benefit of treatment of SH patients and thyroid replacement therapy is not expected to change the natural history of the disease. In addition, despite its potential beneficial effects; especially on the symptoms and lipid profile, thyroid hormone replacement therapy may have some risks; such as osteopenia and atrial fibrillation [25]. Current evidence is insufficient to confirm the absolute beneficial effect of LT4 therapy of SH patients. Larger, controlled studies that investigate the long-term effects of the therapy on cardiac events are warranted.

Limitations of our study

The limiting points of our study may be the small number of our patient group and the exclusion of coronary artery disease without performing a coronary angiography. Long-term studies are required to confirm the benefits of LT4 treatment in SH and on cardiovascular mortality and morbidity.

Conflict of interest None declared.

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