Increased Serum Chitotriosidase Activity following Restoration of Euthyroidism in Patients with Subclinical Hypothyroidism

Muhammed Erdal¹, Mustafa Sahin², Kenan Saglam¹, Adnan Hasimi¹, Gokhan Uckaya², Mustafa Yalcin Yarpuz¹, Abdullah Taslipinar², Hossein Gharib⁴ and Mustafa Kutlu²

Abstract

Objective Whether to treat subclinical hypothyroidism (SH) remains controversial. Serum chitotriosidase activity, a marker of activated macrophages, predicts new cardiovascular events. Chitotriosidase activity (ChT) is a new cardiovascular risk marker and is independent of C-reactive protein. The purpose of this study was to determine ChT levels in SH and to examine the effect of levothyroxine replacement on ChT.

Subjects and Methods A cohort of 60 patients with subclinical hypothyroidism and 62 healthy controls were enrolled in this study. Serum total and LDL cholesterol, total homocysteine (t-Hyc), highly sensitive C-reactive protein (hsCRP) levels and serum ChT in patients with subclinical hypothyroidism at baseline and after achieving euthyroid state by levothyroxine were assessed.

Results Pretreatment levels of TSH (10.06±5.09 vs. 2.08±0.95 mIU/L, p<0.05), and free T4 (0.94±0.21 vs. 1.35±0.26 ng/dl, p<0.05) were significantly higher than controls while total cholesterol, LDL cholesterol, t-Hyc, ChT and hsCRP levels were not different. ChT levels significantly increased after replacement therapy (137.2±14.18 vs. 156.88±13.10 nmol/mL/h, p<0.05). T-Hyc and hsCRP levels were not significantly different after treatment with levothyroxine therapy even in this subgroup of patients. None of the other biochemical risk factors improved after euthyroidism in patients with SH with average dose of 85±30 μg/day when compared to pretreatment levels.

Conclusion We conclude that clinical management of subclinical hypothyroidism does not decrease the serum hsCRP or t-Hyc levels but does increase the serum ChT levels. The clinical significance of this increment should be studied in further studies.

Key words: subclinical hypothyroidism, levothyroxine replacement, chitotriosidase, highly sensitive CRP, homocysteine, cardiovascular risk factors

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Introduction

Subclinical hypothyroidism (SH) is a condition diagnosed when the serum thyroid stimulating hormone (TSH) level is above the reference value (0.45-4.5 mIU/L) and the free thyroid hormones are within normal levels (1, 2). The prevalence of SH has been reported to be between 4% and 10% of adult population (3-5). Whether to treat SH remains a dilemma (6, 7). The decision to treat patients with SH is mainly based on the assessment of cardiac morbidity and mortality in patients with mild disease.

The relationship between SH and serum lipids remains controversial (8, 9). Most studies on treatment with T4 have dealt only with surrogate markers. It is also uncertain whether SH is associated with increased cardiac risk mark-
ers (10). Four double-blind placebo randomized controlled trials found that replacement therapy may have a beneficial effect on lipid profile (11-13). Replacement therapy does not appear to affect lipoprotein a, homocysteine (t-Hyc) or C-reactive protein (CRP) (10). T4 replacement did not affect CRP levels in a double-blind placebo-controlled study in patients with SH (14). In a recent study, replacement therapy with L-thyroxine to normalize TSH did not modify t-Hyc levels in the fasting or postmethionine states in 24 patients with mild hypothyroidism (TSH levels between 5 and 10 mIU/L) (15). T-Hyc levels were unaffected by the treatment of SH in three double-blind placebo-controlled studies (14, 15).

There is no report in the literature which shows thyroid replacement therapy increases cardiac risk factors. We know that in some patients, however, increased metabolic demands on the heart can cause a clinical demasking of a previously compensated preexisting ischemic heart disease (16), Keating et al (17) investigated the effects of T4 on angina pectoris in 1,503 hypothyroid patients. Thirty-eight percent improved with the treatment, 46% showed no change, and 16% had more symptoms. Thirty-five patients developed overt chest pain after the start of therapy. Indeed, SH appeared to exert a protective cardiovascular effect in patients older than 85 years (18).

Human chitotriosidase (ChT) is a recently described fully active chitinase expressed by activated macrophages (19). ChT was first discovered in plasma of patients suffering from Gaucher disease (20). In circulation exclusively the 50-kDa enzyme is present. A 24-bp insertion occurs in exon 10 of the ChT gene that prevents formation of active enzyme pan ethnically (21). Recently, Boot et al (22) reported that ChT activity is elevated up to 55 fold in extracts of atherosclerotic tissue, showing a clear connection between ChT expression and lipid-laden macrophages inside human atherosclerotic vessel wall, as in GD. Serum ChT activity was shown to be related to the severity of the atherosclerotic lesions, suggesting a possible role as a marker of atherosclerotic extension. Patients with atherothrombotic stroke and ischemic heart disease were reported to have significantly higher ChT activities than the control group (23). In addition, the increase in serum ChT activity was found to be age dependent. This phenomenon could be explained by the ongoing accumulation of lipid-laden macrophages during the gradual progression of atherosclerosis in relation to age. Also it was shown that serum ChT activity predicts the risk of new cardiovascular events. This new cardiovascular risk marker is independent of CRP and, when combined, the prediction of the risk of new cardiovascular events and the identification of a lower risk group seem to improve (24).

Modulation of the immune response by thyroid hormones has been well recognized for years. It is unclear whether they play a significant role in T lymphocyte development and cell-mediated immunity (25). Thus, the aim of this study was to assess the impact of correcting SH with levothyroxine on biochemical risk factors (such as ChT, sCRP and t-Hyc) of cardiovascular diseases.

### Materials and Methods

#### Study subjects

Sixty autoimmune thyroiditis patients with subclinical hypothyroidism (55 women, 5 men; mean age 42.28±12.65 years, mean BMI 26.25±4.12 kg/m²) were examined and followed-up in the outpatient clinic of Department of Endocrinology and Metabolism, Gulhane School of Medicine, Ankara. After an overnight fast, all patients underwent full medical assessment and laboratory examinations to rule out non-thyroidal illnesses. The exclusion criteria were as follows: coronary heart disease, pituitary/hypothalamic disorders or other non-thyroidal diseases. None were receiving vitamins, lipid-lowering drugs, or other medications known to interfere with homocysteine metabolism, lipid profile, or thyroid function. Sixty-two healthy matched controls (55 women, 8 men; mean age 39.43±13.02 years, mean BMI 25.03±5.04 kg/m²) were voluntarily enrolled in the study; physical examination and venous blood samplings were performed for the same parameters as patients. All participants were informed and written consent was obtained. SH was defined as an elevated TSH concentration (>5 mIU/L) in the presence of normal thyroxine levels in two determinations (26).

#### Study design

Venous blood samples were withdrawn from the brachial vein after 12 hours of overnight fasting, between 08:00 and 09:00 a.m. All patients were treated with L-T4 (Levothyroxine, Abdi Ibrahim Co., Istanbul, Turkey) starting from the dose of 50 μg/day. TSH was measured every 4-6 weeks to adjust L-T4 dose. The mean L-T4 dose required to restore euthyroidism was 75±30 μg/day. A reevaluation was performed with venous blood sampling at least four months after restoration of euthyroidism.

#### Methods

Fasting serum samples were immediately put on ice and kept frozen at -70°C until analyses were performed. Serum TSH, free thyroxine (f-T4), free triiodothyronine (f-T3) levels (Immulite, 2000 autoanalyzer by BioDPC, Los Angeles, CA, USA) and total cholesterol, triglyceride, and high-density lipoprotein cholesterol levels (Olympus AU2700 auto analyzer, Hamburg, Germany) were determined by using commercially available methods. LDL cholesterol was calculated by the Friedwald’s formula. t-Hyc levels were determined using high pressure liquid chromatography; normal range was 5-12 mmol/L with a 0.4-5% intraassay coefficient of variation (CV). Highly sensitive (hs) CRP was determined by using a highly sensitive latex-based immunoassay (Dade Behring, Newark, DE, USA), normal ranges were 0-1 mg/L with 0.9-2.1% interassay CV. Vitamin B12 and folate levels were assayed in serum by using a com mer-
Table 1. Comparison of Clinical and Laboratory Parameters between Patients with Subclinical Hypothyroidism and the Control Group

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=60)</th>
<th>Control (n=62)</th>
<th>p</th>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>55</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td></td>
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<td>&gt;0.05</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>47</td>
<td>51</td>
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</tr>
<tr>
<td>Body Mass Index</td>
<td>26.25±4.12</td>
<td>25.03±5.04</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Age</td>
<td>42.28±12.65</td>
<td>39.43±13.02</td>
<td></td>
</tr>
<tr>
<td>Chitotriosidase</td>
<td>137.2±14.18</td>
<td>100.14±21.52</td>
<td>&gt;0.05</td>
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<tr>
<td>TSH</td>
<td>10.06±5.09</td>
<td>1.81±0.95</td>
<td>&lt;0.05</td>
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<tr>
<td>T4</td>
<td>0.94±0.21</td>
<td>1.21±0.24</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T3</td>
<td>2.83±0.55</td>
<td>3.20±0.63</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>9.92±2.22</td>
<td>9.71±6.90</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>203.67±42.74</td>
<td>189.21±45.74</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>155.72±109.2</td>
<td>147.53±89.21</td>
<td>&gt;0.05</td>
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<tr>
<td>HDL</td>
<td>45.62±11.89</td>
<td>48.87±13.85</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LDL</td>
<td>127.50±32.47</td>
<td>121.50±34.63</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>hsCRP</td>
<td>1.90±1.26</td>
<td>1.79±0.72</td>
<td>&gt;0.05</td>
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</table>

Blood samples were obtained in an EDTA-containing tube for ChT activity measurement. Plasma and packed cells were separated for centrifugation at 1,500 g for 10 minutes and frozen for ChT activity determination. ChT activity was measured according to the method described previously by Hollak et al (20). Briefly, 5 μL of plasma was incubated with 100 μL of 4-methylumbelliferyl-β-D-N,N’-N’-triacetylchitotriosidase (Sigma M-5639; Sigma-Aldrich ChemieGmbH, Taukirchen, Germany) in McIlvain’s phosphate-citrate buffer; pH=5.2, for 1 hour at 37.0 °C in the dark. The reaction was terminated by adding 120 μL 0.5 mol/L Na2CO3 -NaHCO3 buffer, pH=10.7. In the quantitative method, the fluorescence of 4 methylumbelliferon was read in a Microfluor 2® plate by a fluorimeter (BIO-TEK SynergyHT; Biotek Instruments Inc., Winooski, VT) (excitation 360, emission 450 nm).

The ChT activity was expressed as nanomols of substrate hydrolyzed per milliliter per hour (nmol/mL/h). Reference range of plasma ChT (4-195 nmol/mL/h) was used as described by Guo et al (27).

Statistics

Database management and all statistical analyses were performed by using SPSS for windows (version 15.0, SPSS, Chicago, IL, USA). Results are expressed as mean ± SD. P values of <0.05 were considered significant. As some variables including Cht were not distributed normally as checked by histograms and Kolmogorov-Smirnoff test, non-parametric testing for further analysis was employed. Differences were tested with the non-parametric Wilcoxon’s test for paired observations; Mann-Whitney test for unpaired observations or Kruskal Wallis test where appropriate. Two tailed student’s t-test and ANOVA test were used for parametric variables. Correlation analyses were determined by calculating the Spearman’s r coefficient. A p value <0.05 was considered to indicate statistical significance in all analyses.

Results

The main findings of controls and patients are given in Table 1. There were no significant difference in sex, body...
Table 2. Analyses of Laboratory Parameters before and after Levothyroxine Replacement

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Posttreatment</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Chitotriosidase</td>
<td>137.2±14.18</td>
<td>156.88±13.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSH</td>
<td>10.06±5.09</td>
<td>2.08±1.30</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T4</td>
<td>0.94±0.21</td>
<td>2.99±0.65</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T3</td>
<td>2.83±0.55</td>
<td>3.00±0.65</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>9.92±2.22</td>
<td>9.36±1.90</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>203.67±42.74</td>
<td>201.03±38.99</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>155.72±109.2</td>
<td>138.70±81.44</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HDL</td>
<td>45.62±11.89</td>
<td>46.78±12.71</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LDL</td>
<td>127.50±32.47</td>
<td>126.67±35.14</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>hsCRP</td>
<td>1.90±1.26</td>
<td>1.91±1.07</td>
<td>&gt;0.05</td>
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</table>

mass index, presence of menopause and age between patients and the control group. Pretreatment levels of TSH (10.06±5.09 vs. 2.08±0.95 mIU/L, p<0.05) were higher, and those of free T4 (0.94±0.21 vs. 1.21±0.24 ng/dL, p<0.05) were significantly lower than controls while total cholesterol, LDL cholesterol, t-Hyc, ChT and hsCRP levels were not different.

By definition, all patients presented with increased TSH levels (>5 mIU/L, range; 5.8-27 mIU/L) and f-T4 and f-T3 within the references range (0.8-1.9 ng/dL and 1.6-4.7 pg/ml, respectively). Euthyroidism was restored in all subjects and its mean duration appeared as 18.2 ± 4.4 weeks throughout the study. No patient had vitamin B12 or folate deficiency.

While TSH levels were significantly reduced, f-T4 levels were significantly increased from pre- to post-treatment levels (both p<0.001) (Table 2). No significant changes were observed in BMI, vitamin B12 and folate levels by the end of study.

The change between pre- and post-treatment total cholesterol levels did not reach statistical significance (203.67±42.74 vs. 201.03±38.99, p>0.05). Although a slight decrement was observed in LDL cholesterol levels, it did not change significantly after treatment (127.50±32.47 vs. 126.67±35.14 mg/dl, p>0.05) in all patients. HDL and triglyceride levels, did not change significantly after treatment and were not different from controls, either (p>0.05 in both comparisons) (Table 2).

The effects of L-T4 treatment on other emerging cardiovascular risk factors are shown in Table 2. After treatment, mean Hcy in patients with subclinical hypothyroidism (9.92±2.22 vs. 9.36±1.90 μmol/L, p>0.05) did not decrease significantly. Also, mean hsCRP (1.90±1.26 vs. 1.91±1.07 mg/dL, p>0.05) did not decrease significantly.

ChT levels were significantly increased after replacement therapy in patients with subclinical hypothyroidism (137.2±14.18 vs. 156.88±13.10 nmol/mL/h, p<0.05). Post-therapy ChT levels also significantly differed from ChT levels of control group (p<0.05) (Fig. 1).

ChT increment did not differ according to smoking and menopause status (data not included).

There was a significant correlation between ChT levels, or ChT change during therapy, and other laboratory parameters. Basal ChT levels in subclinical hypothyroidism were greater than the control group (p>0.05).

Discussion

The main finding of the present study is that restoration of euthyroidism did not significantly modify LDL cholesterol, t-Hyc, and hsCRP levels, which are known as cardiovascular risk factors, in patients with SH. However, ChT levels were significantly increased after euthyroidism, which is a new cardiovascular risk marker.

There is still doubt whether or not replacement therapy lowers serum lipid levels in patients with SH (10). Homocysteine levels were unaffected by the treatment of SH in double-blind placebo-controlled studies (14, 15). The present study did not result in t-Hyc after restoration of euthyroidism, regardless of baseline TSH or t-Hyc levels. In our study, having no change in hsCRP levels after treatment is still valid and in line with the findings of a double-blind placebo-controlled study that investigated the impact of L-T4 treatment on hsCRP (14).

We found no significant difference in chitotriosidase activity between patients with subclinical hypothyroidism and the control group. However, there was a significant increase in chitotriosidase levels after restoration of euthyroidism in subclinical hypothyroid patients. The reason for this increment in chitotriosidase levels after thyroid hormone replacement therapy may be related to the TNF alpha or IL-2 receptor alpha increase caused by thyroid hormone (28, 29)
or to another mechanism whereby thyroid hormone increases macrophage activation (30). Thyroid hormones are known to affect cell-mediated immune response (31). ChT is viewed as a component of innate immunity (32). Thyroxine may increase the cellular immune response. More detailed studies are needed into this.

Among elderly patients, the mean annual mortality rate was lower in those with subclinical hypothyroidism compared to both those with low and normal TSH levels in one study (33). Also in another recent report, SH was associated with a significantly better outcome in patients with acute stroke (34). Chitotriosidase was found to be directly correlated with stroke severity independently of preexisting inflammatory or infectious conditions (35).

More clinical trials are needed to assess whether T4 replacement reduces the risk of coronary heart disease in subjects with subclinical thyroid disease.

In conclusion, based on the present findings, thyroid replacement therapy may increase the ChT level, which may in turn increase cardiac morbidity. Particularly, in patients with stroke or angina ChT may be checked in those taking thyroid replacement therapy. The clinical significance of this increment must be studied further. More clinical trials are needed to assess whether or not T4 replacement reduces the risk of coronary heart disease in subjects with subclinical thyroid disease.

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