The Effect of Hormone Replacement Treatment on Thrombin-Activatable Fibrinolysis Inhibitor Activity Levels in Patients with Hashimoto Thyroiditis

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Abstract

Background  Hypothyroid patients have increased risk of cardiovascular diseases, and several mechanisms have been considered responsible in these patients. Although, a few studies demonstrated fibrinolytic system changes in hypothyroid patients, there is no study demonstrating TAFI activity in hypothyroid Hashimoto’s thyroiditis patients. The aim of this study was to evaluate TAFI activity status and the effect of L-thyroxin hormone replacement treatment on fibrinolytic system in this patient group.

Methods  Thirty patients with hypothyroid Hashimoto thyroiditis (all were female and the mean age was 44.3±14.6 years, ranging between 17-68 years) were enrolled to study. Their TSH levels were high (27.2±5.2 mU/L) and Free T3 and Free T4 hormone levels were below than normal. In this study, euthyroid 20 healthy volunteers (mean age 32.5±4.9 years, range 26-42 years) were adopted. L-thyroxin treatment before and after TAFI activity levels were measured in patients.

Results  In the control group, TAFI activity levels were 9.6±0.4 μg/mL. In patients with L-thyroxin before and after treatment there were high levels of TAFI activity value of 14.2±0.9 and 12.9±0.8 μg/mL, respectively. In the patient group, after L-thyroxin treatment TAFI activity levels were decreased but they were not statistically significant (p=0.187). When compared to the control group, high levels of TAFI activity were observed in the patient group (p<0.0001).

Conclusion  Our data demonstrated that in Hashimoto thyroiditis, patients have high levels of TAFI activity compared to controls. A high level of TAFI activity suggests fibrinolytic deficit or thrombotic tendency in hypothyroid patients and this deficit is persistent after L-thyroxine replacement.

Key words: Hashimoto’s thyroiditis, thrombin-activatable fibrinolysis inhibitor activity

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Introduction

Hypothyroidism is a frequent endocrine disorder in the general population, especially in women. Hashimoto thyroiditis is the most common cause of hypothyroidism; it is characterized by gradual thyroid failure due to autoimmune-mediated destruction of the thyroid gland. It was demonstrated that hypothyroid patients have an increased risk of atherosclerosis and myocardial infarction (1, 2) and several mechanisms have been proposed in hypothyroid patients. High levels of cholesterol and triglyceride (3), hyperhomocysteinemia (4), immune-complex-mediated vascular damage (5), hemostatic profile changes (1) and recently, endothelial dysfunctions were detected in hypothyroid patients (6). There were some studies in which coagulation parameters were altered in hypothyroid patients, however, the underlying mechanisms are not understood well.

Thrombin plays a critical role in coagulation and the fibrinolytic system (7). It not only activates coagulation pro-
teins, it also inhibits fibrinolysis by activating TAFI (thrombin-activatable fibrinolysis inhibitor). Activated TAFI, TAFIa, down-regulates fibrinolysis by removal of C-terminal arginine and lysine residues from fibrin (8-10). These residues are important for binding and activating plasminogen. Thus, TAFI leads to a potent inhibitor of tissue plasminogen activator-induced fibrinolysis (11).

A few studies have demonstrated fibrinolytic system changes in hypothyroid patients (12, 13). Only one study showed elevated plasma levels of TAFI antigen both in overt and sub clinical hypothyroidism, which may be associated with hypofibrinolysis and an elevated risk of thrombosis (14). There is no study demonstrating TAFI activity in hypothyroid Hashimoto’s thyroiditis patients. The aim of this study was to evaluate TAFI activity status and the effect of hormone replacement treatment on fibrinolytic system in hypothyroid Hashimoto thyroiditis patients.

Materials and Methods

Subjects

Thirty patients with clinically hypothyroid Hashimoto thyroiditis (all were female and the mean age was 44.3±14.6 years, ranging between 17-68 years) were enrolled to study. Their TSH levels were high (27.2±5.2 mU/L) and free thyroid hormone (Free T3 and Free T4) levels were below normal. All patients had a high serum concentration of antibodies (thyroid peroxidase antibody: anti-TPO, thyroglobulin antibody: anti-Tg) against one or more thyroid antigens, and diffuse thyroid hyperplasia was proven scintigraphically and sonographically. Age- and sex-matched (mean age was 32.5±4.9 years, ranging between 26-42 years) euthyroid 20 healthy volunteers, mainly staff members, their relatives or friends were adopted. Informed consent was obtained in all subjects and the study was approved by the local ethics committee. Body mass index of patients and controls were similar (28.2±6.5 vs 28.0±4.6 kg/m², respectively). Exclusion criteria were history of atherosclerotic or thromboembolic disease, familial hyperlipidemia, diabetes, morbid obesity and severe systemic diseases. Oral contraceptive and lipid lowering drug users were also excluded.

Methods

For patients, before treatment with L-thyroxin (mean dose is 125 microgram/day), fasting blood samples were taken during hypothyroid condition and after L-thyroxin 125 microgram/day treatment, euthyroidism was obtained after 55±17 days and blood samples were drawn again between 8:00 AM and 9:00 AM from the antecubital vein into a 3.2% sodium citrate containing tube. The tube was immediately centrifuged at 4,500xg for 5 minutes at +4°C to obtain platelet-poor plasma. Plasma was stored at -80°C for future use of TAFI activity measurement. Blood samples from twenty controls were also taken and stored at -80°C for future use of TAFI activity measurement. Serum samples were obtained for thyroid function tests and lipid profile.

Hormone measurements

Serum TSH (reference range 0.1-4.0 mU/L) serum fT3 (reference range 2.5-3.9 pg/mL) and fT4 levels (reference range 0.61-1.12 ng/dL) were measured by a chemiluminescent immunoassay method. (Access Hypersensitive hTSH, Access Free T3 assay and Access Free T4 assay kits were purchased from Beckman Coulter Inc., Carlsbad, CA, USA).

An immunoprecipitation method (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA) was used to detect anti-Tg and anti-TPO antibody levels; 0-40 IU/mL and 0-35 IU/mL were considered as normal, respectively.

TAFI functional activity assay

TAFI functional activity was assayed using ACTICHROME® TAFI activity kit (American Diagnostica Inc., Greenwich, CT). TAFI levels were determined by first incubating the plasma with TAFI activation reagent. Then activating stop reagent was added for to halt the activation step. Next, the TAFI developer containing substrate was added to diluted (1:25) plasma samples and enzymatic reaction was started. The reaction was stopped by the addition of sulfuric acid and it was read at 490 nm. Plasma that was not activated (not incubated with the TAFI activation reagent) was assayed concordantly as a control. The difference in absorbance between the activated and non-activated plasma was calculated as an amount of TAFI activity.

Statistical analysis

All data are presented as means ± standard deviation (SD). Paired sample t-test was used to evaluate treatment differences in the patient group and the independent sample t-test was used to compare the patient group and control group. Analysis of covariance (ANCOVA) was used for TAFI activity. Values of p<0.05 were considered as statistically significant. Data were analyzed using SPSS for windows (version 14.0, SPSS Inc., Chicago, IL, USA).

Results

All 30 patients were clinically hypothyroid Hashimoto thyroiditis. 20 controls were euthyroid. Before and after treatment with L thyroxin, characteristics of patients were given in Table 1.

When values of Hashimoto thyroiditis patients were compared before and after treatment, they had low thyroid hormone levels (p<0.0001), high TSH (p<0.0001), and elevated total cholesterol and LDL cholesterol levels (p<0.0001); positivity for at least one autoantibody against thyroid gland was observed. High levels of TAFI activity were observed in the patient group (p<0.0001) when compared to those of the control group. After L-thyroxin replacement treatment for 55±17 days, euthyroidism was obtained and lipid parameters were normalized. In the control group, TAFI activity levels were 9.6±0.4 μg/mL. When compared to the control group,
Table 1. L-thyroxin, Blood Pressure, Thyroid Function Tests, and Lipid Profile of Hashimoto Thyroiditis Patients before and after Treatment.

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.2 ± 6.5</td>
<td>28.0 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110 ± 11</td>
<td>120 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70 ± 10</td>
<td>80 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>TSH (0.1-4.0 mU/L)</td>
<td>27.2 ± 5.2</td>
<td>0.6 ± 0.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>fT3 (2.5-3.9 pg/mL)</td>
<td>2.1 ± 0.8</td>
<td>3.0 ± 0.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>fT4 (0.61-1.12 ng/dL)</td>
<td>0.9 ± 0.39</td>
<td>1.6 ± 0.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total cholesterol (&lt; 200 mg/dL)</td>
<td>240 ± 57</td>
<td>198 ± 35</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LDL- cholesterol (&lt; 130 mg/dL)</td>
<td>152 ± 43</td>
<td>120 ± 24</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HDL- cholesterol (&gt; 65 mg/dL)</td>
<td>55 ± 12</td>
<td>58 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol /HDLD ( &lt;5)</td>
<td>4.4 ± 1.4</td>
<td>3.3 ± 0.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Triglycerides (&lt; 150 mg/dL)</td>
<td>132 ± 71</td>
<td>113 ± 57</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2. When Compared to Control Group, TAFI Activity Levels were High and Statistically Significant (p<0.0001) in Hashimoto Thyroiditis Patients before and after L-thyroxin Treatment.

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFI activity (ugram/mL)</td>
<td>14.2 ± 0.9***</td>
<td>12.9 ±0.8***</td>
<td>9.6± 0.4</td>
</tr>
</tbody>
</table>

Figure 1. TAFI activity levels in Hashimoto thyroiditis patients before and after L-thyroxin treatment.

Discussion

Hashimoto’s thyroiditis is the most common autoimmune disease of thyroid gland and the most common cause of hypothyroidism. Impaired coagulation and fibrinolytic activity might be responsible in the pathogenesis of atherosclerosis and thrombosis formation in hypothyroidism.

In hypothyroidism, an increased risk of cardiovascular disease is described (2). Muller et al (1) demonstrated that, hypothyroid patients have an increased prevalence of coronary artery disease because of increased FVII activity. However, FVII antigen levels is not increased. Recently, the duration of reactive hyperemia, as a marker of endothelial dys-
function, is decreased in hypothyroid patients. Since endothelial dysfunction is a factor leading to atherosclerosis, this defect predisposes patients to cardiovascular disease (6). In patients with well-controlled hypothyroidism by L-thyroxine therapy the reactive hyperemia time has been found to be close to normal and it does not differ significantly when compared to controls.

Impaired fibrinolytic activity is associated with the later onset of myocardial infarction, stroke, and cardiovascular death (15). Circulating t-PA antigen was a predictor of coronary events during 13- and 16-years follow-ups (16, 17). High levels of PAI-1 and fibrinogen were associated with future coronary heart disease (18). In addition, t-PA and tPA/PAI-1 complex were found to be an independent predictor for stroke (19).

In hypothyroid patients decreased fibrinolytic activity has been reported. Plasma AT and PAI-1 levels were significantly increased in these patients when compared with the control group (20). In sub-clinical hypothyroid patients, the overall fibrinolytic activity was investigated and found to be lower than control group (13). Recently, new markers of fibrinolysis inhibitor, Thrombin Activatable Fibrinolysis Inhibitor (TAFI) have been described. It is a major determinant of clot lysis time (21). In coronary artery disease, men require coronary artery bypass grafting (CABG) because of stable angina pectoris; TAFI antigen levels are found to be high, demonstrating that increased TAFI levels carry a risk for coronary artery disease (22). However, after myocardial infarction, decreased TAFI antigen levels were indicative of a protective effect against infarction (23).

On the other hand, functional TAFI was based on the activation of TAFI with thrombin-thrombomodulin complex and the generation of TAFI activity was measured. High levels of functional TAFI in plasma were a significant risk of acute coronary artery disease (24). Also, high functional TAFI levels were found to be associated with an increased risk of first ischemic stroke (25).

Recently, there has only been one study showing elevated plasma levels of TAFI antigen both in overt and sub-clinical hypothyroidism, which may be associated with hypofibrinolysis and an elevated risk of thrombosis (14). According to this study, normalization of thyroid state by L-thyroxin replacement seems to be effective in the lowering of TAFI antigen levels in hypothyroidism. But, TAFI activity levels were not considered in this study.

Our data demonstrated that after L-thyroxine replacement treatment, TAFI activity levels were decreased but they were not statistically significant. It has been reported that after L-thyroxine replacement treatment, TAFI antigen levels are decreased significantly. Our data support that a high level of TAFI activity demonstrates thrombotic tendency and it continues and is independent of L-thyroxine replacement in Hashimoto thyroiditis. A high level of TAFI activity suggested fibrinolytic deficit in hypothyroid patients and this deficit was persistent after L-thyroxine replacement. High levels of TAFI activity may partly explain the predisposition of patients to cardiovascular disease. It may worthy to explore whether or long-term and/or a higher dose of replacement treatment of L-thyroxine may normalize TAFI activity.

References


