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NOTE

Global Fibrinolytic Capacity in Patients with Subclinical Hypothyroidism

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Abstract. Subclinical hypothyroidism (SH) represents the earliest stages of hypothyroidism but the benefits of detecting and treating SH are not well known. The aim of this study was to evaluate the alterations in global fibrinolytic capacity (GFC), which indicates the overall fibrinolytic activity, in patients with SH. The study group comprised of 15 patients with SH and 15 healthy controls. The GFC was significantly lower in patients with SH than in control group ($p<0.002$). This result suggests a relative hypercoagulable state in SH.

Key words: Subclinical hypothyroidism, Global fibrinolytic capacity, Hypothyroidism, Fibrinolytic system
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THE development of sensitive assay for thyroid stimulating hormone (TSH) has led to the discovery that many patients have an elevation in TSH above the upper limit of the reference range with a normal serum free thyroxine (fT4), a condition defined as subclinical hypothyroidism (SH). SH represents an early stage of thyroid dysfunction that will commonly progress to overt hypothyroidism [1, 2]. The prevalence of SH has been reported to be 4–10% in general population [3]. Although most patients with SH, have few signs or symptoms of thyroid hormone deficiency [4], the patients with SH were shown to be at high risk for atherosclerosis and cardiovascular disease, as well as overt hypothyroidism [5, 6]. On the other hand, the clinical importance and necessity for diagnosis and treatment of SH has yet to be clearly established.

Various abnormalities of the haemostatic markers concerning coagulation and fibrinolytic system are risk factors for cardiovascular diseases and thromboembolism [7, 8]. The reported data about the haemostatic abnormalities among overt hypothyroid patients differ widely, such as alterations of circulating coagulation proteins and impaired fibrinolytic activity [9–11]. However, there are few studies which investigate the fibrinolytic activity in patients with SH. A recent study reported that the hypofibrinolytic and hypercoagulable state play an important role in the development of atherosclerosis in patients with SH [12].

The global fibrinolytic capacity (GFC) is a new technique to evaluate the overall fibrinolytic activity such as tissue plasminogen activator (t-PA), prourinary type plasminogen activator, urinary type plasminogen activator, alpha 2 anti-plasmin, histidine-rich glycoproteins, plasminogen activator inhibitor-1 (PAI-1), plasminogen, activated C protein, and factor XII [13]. The aim of this study was to investigate the variations of GFC in women with SH.

Materials and Methods

The study group comprised of 15 pre-menopausal patients with SH (age range 20–40 years), defined as a serum TSH concentration above the upper limit of the reference associated with normal serum fT4 and fT3 concentrations, and 15 healthy pre-menopausal controls (age range 23–42 years) who attended our out-

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patient clinic. Nine patients had autoimmune thyroiditis and six patients with nodular goiter had developed SH after subtotal thyroidectomy. Antithyroid peroxidase (anti-TPO) and anti-thyroglobulin antibodies (anti-Tg) were positive in all patients with autoimmune thyroiditis. None of the participants had received thyroid hormone replacement therapy. Exclusion criteria included such factors as overt obesity (≥30 kg/m^2), smoking, alcohol consumption, diabetes mellitus, cardiac, renal, and other systemic diseases and patients on drugs affecting haemostasis and thyroid function (*e.g.* diuretics, β-blockers, anti-hyperlipidemic agents, anticoagulant drugs, antihistamines, and corticosteroids). The study was approved by the local Ethics Committee and all patients gave their informed consent to participate in the study.

Physical examination included systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurement with subject sitting after a 10-min rest using mercury sphygmomanometer. Body weight and height were measured in subjects without shoes and wearing light clothing by using a portable calibrated scale. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Blood samples were collected from an antecubital vein without the use of a tourniquet, between 08:30 and 09:00 a.m. after an overnight fast to avoid the differences of diurnal variation. For GFC, a venous blood sample was collected into vacutainer tubes containing 0.129 mol/L trisodium citrate (1 : 9 dilution). Samples were centrifuged within 15 minutes at 2500 g and the supernatant plasma samples were transferred into polypropylene tubes at –80°C until assays for determination of GFC. Plasma GFC levels were measured by the semiquantitative macrolatex agglutination technique (STA-Liatest D-Di, Diagnostica Stago, France). The principle of evaluation of GFC in plasma is the measurement of D-dimer generated from a standardized fibrin amount in the presence of a constant and limited amount of exogenous t-PA. Generated D-dimer which reflects GFC was also measured by the semi-quantitative macrolatex agglutination technique (STA-Liatest D-Di, Diagnostica Stago, France). For this purpose, a standardized fibrin tablet (containing silica and completely lacking plasminogen) is introduced into a 200 µL plasma sample supplemented with a constant and limited amount of t-PA. The mixture is then incubated for 1 h at 37°. Fibrinolysis is stopped by introducing 50 µl of an excess of aprotinin and the generated D-dimer was measured by STA-Liatest D-Dimer again and the generated D-dimer in the GFC test was calculated [13]. Spectrophotometric method (Beckman Coulter LX20) was used to measure triglyceride, cholesterol and high density lipoprotein-cholesterol (HDL-C) levels. Low density lipoprotein-cholesterol (LDL-C) level was calculated according to Friedewald formula [14]. TSH (third generation, normal range 0.4–4 µIU/mL), fT4 (normal range 0.8–1.9 ng/dL), free triiodothyronine (fT3, normal range 1.8–4.2 pg/mL) levels were measured by immunometric assay method, chemiluminescence immunoassay method, and competitive immunoassay method, respectively (DPC, Immulite 2000, Los Angeles, CA). Serum levels of anti-Tg and anti-TPO antibodies were measured by immunometric assay method (DPC, Immulite 2000, Los Angeles, CA).

Statistical analysis

Statistical analyses were done with the SPSS (Statistical Package for Social Sciences, release 10.0: SPSS, Chicago, IL) for Windows. Mann-Whitney U test was applied to evaluate differences in continuous variables between patients and control subjects. Correlations between variables were assessed using Pearson correlation analysis. Data were expressed as the mean ± SD. A value of $p<0.05$ was considered as statistically significant.

Results

The biochemical characteristics of the patients and controls are shown in Table 1. No significant differences in the means of age (31.0 ± 7.6 vs 31.2 ± 6.4

Table 1. The characteristics of all participants

	Subclinical Hypothyroid Group	Control Group	P
Cholesterol (mmol/L)	4.54 ± 0.87	4.32 ± 0.42	*
Triglyceride (mmol/L)	1.38 ± 0.58	0.62 ± 0.35	0.001
HDL-C (mmol/L)	1.41 ± 0.19	1.39 ± 0.20	*
LDL-C (mmol/L)	3.03 ± 0.67	2.64 ± 0.34	*
fT3 (pg/mL)	2.8 ± 0.5	2.5 ± 0.6	*
fT4 (ng/dL)	1.3 ± 0.2	1.4 ± 0.2	*
TSH (µIU/mL)	7.3 ± 2.1	1.4 ± 0.8	<0.001
D-dimer (µg/mL)	0.29 ± 0.22	0.18 ± 0.11	*
GFC (µg/mL)	8.5 ± 6.5	17.3 ± 5.8	0.002

*p>0.05

year olds; p>0.05), BMI (24.2 ± 3.2 vs 26.6 ± 3.3 kg/m^2; p>0.05), or systolic (119.2 ± 9.5 vs 117.5 ± 7.5 mmHg; p>0.05) and diastolic blood pressure (78.8 ± 7.1 vs 78.3 ± 3.9 mmHg; p>0.05) were found between SH and control groups. The mean level of serum triglyceride was found to be significantly higher in patients with SH than in the control group (p<0.001). There was no statistically significant difference in levels of other lipid parameters between the two groups (p>0.05) (Table 1).

The mean plasma level of GFC was significantly lower in patients with SH than in control group (p<0.002; Fig. 1), although there was no statistically significant difference in the mean plasma levels of initial D-dimer between the two groups (p>0.05; Table 1). In the patients with SH, the plasma GFC level was not correlated with age, BMI, blood pressure, or the levels of serum lipid parameters (p>0.05). We did not find any correlation between the levels of GFC and fT4 (Fig. 2), fT3 (Fig. 3), TSH (Fig. 4) in patients with SH.

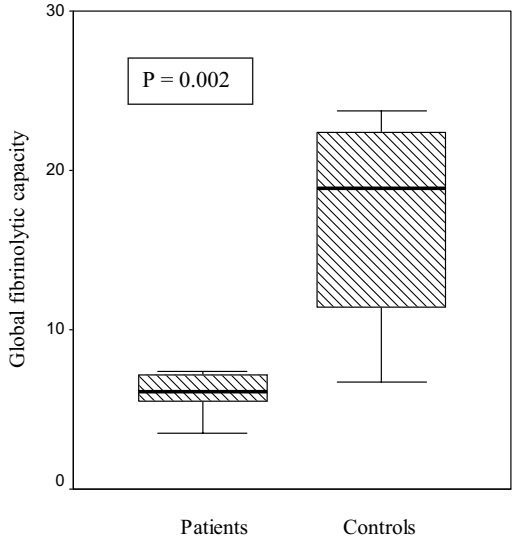


Fig. 1. The differences of the global fibrinolytic capacity between patients with SH and controls.

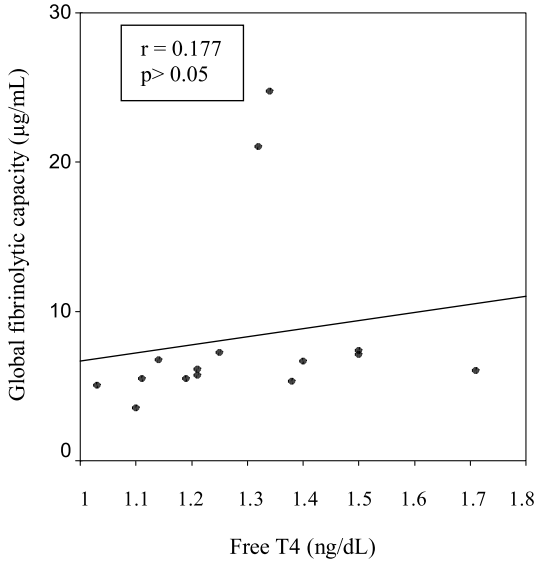


Fig. 2. Relationship between fT4 and global fibrinolytic capacity in patients with SH.

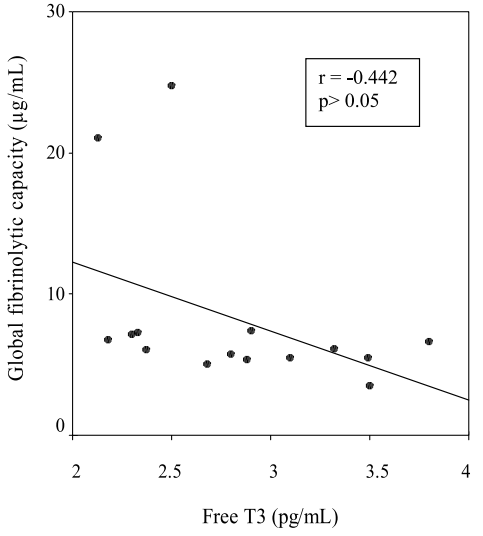


Fig. 3. Relationship between fT3 and global fibrinolytic capacity in patients with SH.

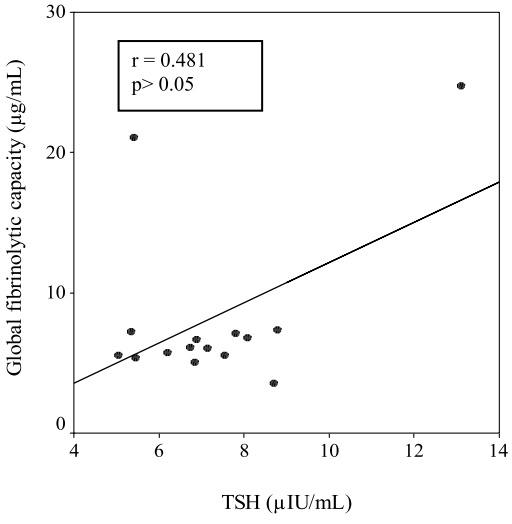


Fig. 4. Relationship between TSH and global fibrinolytic capacity in patients with SH.

Discussion

The mechanism of how low levels of thyroid hormones may lead to atherosclerosis and its complications is still unknown. Impaired coagulation and fibrinolytic functions might be partly responsible in the pathogenesis of atherosclerosis in hypothyroidism. It is generally accepted that the risk of atherosclerotic diseases is increased in disorders of coagulation and/or fibrinolytic system such as alterations of t-PA antigen, PAI-1, D-dimer, or plasmin-antiplasmin complex levels or as delayed clot lysis [7, 8]. Plasma fibrinolytic activity is an important defense mechanism against fibrin accumulation after thrombin has been generated. Fibrinolytic activity can be measured by various methods, such as the dilute blood clot lysis time, the fibrin agarose plate method, the euglobulin clot lysis time, measurements of t-PA, PAI-1 and t-PA/PAI-1 complexes levels, and by the specific assay for t-PA activity [15]. The whole fibrinolytic potential of the plasma could be evaluated by GFC assay which reflects the amount of generated D-dimer when the fibrinolysis of a freeze-dried fibrin clot is stopped by introducing aprotinin [13, 16]. The reported abnormalities of coagulation factors in overt hypothyroid patients differ widely and the pathogenesis of these abnormalities of coagulation factors is unknown. Several studies have suggested that there were hypocoagulable [11] or hypercoagulable states [17, 18] in overt hypothyroidism. Chadarevian *et al.* [19] reported recently that fibrinolytic activity decreased in patients with moderate hypothyroidism, conversely increased in patients with severe hypothyroidism. On the other hand, Erem *et al.* [9] suggested that fibrinolytic activity decreased in patients with overt hypothyroidism. Regarding SH, the potential association between thyroid function and abnormalities of fibrinolytic activity and coagulation system has received little attention. There are conflicting reports about this relationship. Müller *et al.* [20] showed that there was no significant difference of PAI-1 and t-PA levels between SH and control subjects. However, Cantürk *et al.* [12] showed that increased levels fibrinogen, PAI-1, and factor VII, and decreased levels of antithrombin III activity occurred in patients with SH. In the present study in which whole activity of fibrinolytic system was evaluated, we found that GFC levels were significantly lower in the SH group than in the healthy control group. To the best of our knowledge, there have not been any previous reports about the plasma levels of GFC in patients with SH. Our findings were in concurrence with Cantürk *et al.* [12] that suggested hypercoagulable and hypofibrinolytic state might play a role in developing atherosclerotic complications in patients with SH.

It has been reported that GFC is not only an indicator of fibrinolytic activity but also is inversely related to traditional cardiovascular risk factors such as obesity, hyperlipidemia and hyperglycemia, and positively correlated to HDL-C in both pro-atherosclerotic disease and healthy populations [16, 21]. We investigated whether the alteration of GFC in patients with SH could be related to cardiovascular risk factors. Contrary to expectation, we did not find any association between GFC levels and cardiovascular risk factors such as BMI and lipid parameters in the SH group. This discrepancy could be partly explained by whether the study participants had these cardiovascular risk factors. The serum lipid profiles except triglyceride, glucose levels and BMI of our patients were all in normal range. In terms of the relationship among fibrinolytic activity markers, thyroid hormones and TSH, Chadarevian *et al.* [19] reported that fT4 was significantly associated with fibrinogen, PAI-1, and alpha 2 antiplasmin. Moreover, Cantürk *et al.* [12] also reported that there was no correlation between haemostatic parameters and TSH levels. Considering the relationship between GFC and thyroid hormones and TSH, we did not find any association between GFC and thyroid hormone levels in SH group. We speculated that numerous factors may affect GFC level in SH; for instance, decreased GFC could be attributed to alterations of any of the proteins involved in fibrinolysis. Several mechanisms could be responsible for the fibrinolytic alteration in hypothyroidism, such as reduced catecolamine receptor density leading to an increase in PAI-1 level [22], or the direct effect of thyroid hormones on either synthesis or catabolism of protein [23].

Finally, our results suggest that women with SH tend to have a hypercoagulable state as reflected by the decreased GFC. Decreased fibrinolytic activity could contribute to increase the risk of thromboembolic complications in SH. Our findings suggest that SH should not be regarded as a benign condition.

Study limitations

This study has several limitations. First, we evalu-

ated only 15 patients, a smaller number than evaluated in other studies. Second, our analyses are based on a single base-line determination that may not reflect the patient's status over long periods.

Acknowledgment

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