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Original Article

The Effect of Thyroid Dysfunction on Tissue Factor Level and Activity in Rats

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Abstract

Introduction: Tissue factor (TF) is a cellular element that initiates the coagulation cascade. Hypothyroidism and hyperthyroidism are the most common thyroid dysfunctions and several coagulation and hemostatic abnormalities occur in thyroid disorders. The study aims to investigate the effects of thyroid dysfunction on TF activity in the tissues, such as brain, liver, and kidney tissues, and on TF levels in sera of rats.

Methods: Thirty rats were divided equally into 3 groups: 1. Controls, 2. Hypothyroid group, 3. Hyperthyroid group. Methimazole (75 mg/100 g diet) was added to the diet of the hypothyroid group, and L-thyroxine (0.4 mg/100 g diet) was added to the diet of the hyperthyroid group to obtain experimental groups. Controls were fed with standard chow. TF activities were determined in brain, liver and kidney tissues, while TF levels were investigated in sera.

Results: In brain, liver, and kidney tissues, significant decreases were observed in TF activities of both hypothyroid and hyperthyroid rats compared to the controls. Sera TF levels increased significantly in both hypo- and hyperthyroid rats than those of controls.

Conclusion: Coagulation abnormalities can be developed and coronary artery diseases might be triggered by hypo- and hyperthyroidism.

Keywords: Tissue factor, hypothyroid, hyperthyroid, coagulation, thrombosis

1. Introduction

Tissue factor (TF), also known as Factor III (FIII), is a cellular element that initiates the coagulation cascade in physiological and also pathological conditions. It is a membrane-embedded protein in contact with the extracellular and intracellular environment (1). Several tissues, blood, and body fluids have TF activity and following vascular damage, TF performs its physiological function in the circulation. Under physiological conditions, TF, found in the vascular system and in case of damage,

contacts the epithelial surface and is activated with calcium to initiate the coagulation mechanism (2). TF-initiated coagulation has a crucial role in the pathophysiology of diseases and it has been revealed that there are changes in TF activity in many diseases, such as diabetes, hyperlipidemia, and atherosclerosis (3-5). Thrombosis, a primary cause of morbidity, can be developed by the presence of blood or plasma-derived TF in the circulation, and the increase in TF expression is considered as a factor causing atherosclerosis (6).

Thyroid hormones play an important role in the repair after injury in several tissues and organs. There may be a common mechanism of repair in the organism which can be regulated by thyroid hormone because thyroid hormone is effective in the regulation of DNA repair following the damage (7). Hypothyroidism and hyperthyroidism are the most common thyroid dysfunctions worldwide and these disorders affect the regulation of blood cells. Besides, several coagulation and hemostatic abnormalities occur in thyroid disorders; hypothyroidism patients are at risk for developing hemorrhage, atherosclerosis, and cardiovascular diseases. In addition to that, hyperthyroidism patients display a tendency to develop thrombotic complications (8). Especially altered free thyroxine levels lead to thrombotic tendency due to impaired dysfunction of coagulation factors in blood circulation (9).

In accordance with this information, the study aims to investigate the effects of thyroid dysfunction on TF activity in the brain, liver, and kidney tissues and TF levels in sera of rats.

2. Methods

2.1. Study group

Thirty Wistar Albino rats were divided equally into 3 groups, as follows: 1. Controls, 2. Hypothyroid group, 3. Hyperthyroid group, Methimazole (75 mg/100 g diet) was added to the diet of the hypothyroid group, and L-thyroxine (0.4 mg/100 g diet) was added to the diet of the hyperthyroid group to obtain experimental groups for 3 months. Controls were fed with standard chow during the study. All animals were sacrificed, and blood and tissues, such as the brain, liver, and kidney were taken from animals at the end of the study. The blood was centrifugated and sera were taken, also the tissues were homogenized with saline (0.9% NaCl), and 10% (w/v) homogenates were done for biochemical analyses.

2.2. Biochemical analysis

Homogenates made from brain, liver and kidney were used for the analysis of TF activity, and also TF levels were determined in the sera of the rats. TF activity was done according to Quick's one-stage method (10). Pooled plasma collected from healthy subjects used for the determination of TF activity and it was performed by mixing 0.1 mL tissue homogenate with 0.1 mL of 0.02 M $CaCl_2$, with the clotting reaction being started on addition of 0.1 ml of plasma. All reagents were brought to the reaction temperature (37 ◦C) before mixture. TF levels were determined by using a commercially available enzyme-linked immunosorbent assay (catalog no: E90524Ra).

2.3. Statistical analysis

Statistical analysis of the results was carried out using GraphPad Prism 5.0 (GraphPad Software, USA). One way ANOVA, Kruskal Wallis and Post-hoc Dunn tests were used for the comparison of the groups and a $p < 0.05$ value was defined as significant.

3. Results

Brain, liver, and kidney TF activity and sera TF levels of the rats were shown in Table 1. The clotting time is inversely proportional to the TF activity. In brain, liver, and kidney tissues, significant decreases were observed in TF activities of both hypothyroid ($p<0.05$, $p<0.001$, $p>0.001$, respectively) and hyperthyroid rats ($p<0.05$, $p<0.01$, $p<0.001$, respectively) compared to the controls. Also, sera TF levels increased significantly in both hypothyroid and hyperthyroid rats than those of controls ($p<0.05$, $p<0.001$).

Table 1. Brain, liver and kidney TF activity and sera TF level of the rats

		Controls	Hypothyroid	Hyperthyroid
TF activity (sec.)	Brain	34,03±3,38	39,41±3,04*	39,03±4,20*
	Liver	96,86±2,20	103,50±1,75***	101,6±2,03**
	Kidney	39,02±1,06	48,46±1,76***	44,33±1,41***
TF level (pg/mL)	Sera	47,97±0,51	113,00±1,15***	86,36±0,50*

Values are given as mean±standard deviation. TF: Tissue factor, *p<0.05, **p<0.01, ***p<0.001 significant compared to control group.

4. Discussion

Thyroid dysfunction and thyroid autoimmunity may lead to several hemostatic disorders, such as thromboembolism, hemorrhage, and some laboratory abnormalities, affecting physiologic hemostasis (11). Excessive or insufficient release of thyroid hormones affects thrombocyte function, and regulation of coagulation factors. Also, changed blood viscosity may be effective in the emergence of coagulopathies caused by thyroid disease (12). High levels of procoagulant factors create a prethrombotic environment in the blood, and this situation increases the hemostatic risk aspect of atherosclerosis, and thrombin formation accelerates. Although the relationship between thyroid insufficiency and the hemostatic system has been studied for many years, it is still not well understood.

There are conflicting results regarding hypothyroidism; a tendency to bleed has been reported in some hypothyroid cases, on the contrary, a tendency to the development of atherosclerosis, its associated complications or thrombus are also mentioned (13-17). However, to generalize, hypothyroid and coagulation-related studies are in the direction of hypercoagulability. Studies have reported that fibrinogen and D-dimer are increased in hypothyroidism and this situation tends to cause hemostatic hypercoagulation in hypothyroidism, and such cases are at risk of cardiovascular disease (18,19). Although similar findings to fibrinogen deficiency were observed in the decrease in TF, none of these effects were determined in factor XI deficiency (20). In addition, it was reported that thrombus formation accelerated when TF is encountered, especially in the damage of vessels with high flow rates such as the carotid artery (21).

Thrombocytes are important components of coagulation. Hyperthyroidism may cause a disruption in thrombocytes, thus it is a risk factor for thrombosis. Besides, increased fT4 level is associated with elevated concentrations of Von Willebrand factor (VWF), which promotes platelet aggregation and FVIII (22). Also, a hyperdynamic circulatory in a hyperthyroidism state can stimulate the endothelium to disruptions in endothelial function, and due to increased synthesis of the endothelial proteins via a thyroid hormone-responsive element, a tendency to endothelial dysfunction can be developed (8,23). Considering the previous results, it can be suggested that thyroid hormones may affect the regulation of TF activity and/or inhibition from the endothelium.

5. Conclusion

In the present study, TF activities in brain, liver, and kidney tissues of the hypothyroid and hyperthyroid groups decreased significantly than those of controls and showed different values from each other, which may be a result of TF having tissue-specific functions. Since TF activity is inversely proportional to the clotting time, the lengthening of the clotting time is a manifestation of decreased TF activity. Under normal conditions, endothelial cells do not express TF, but in the case of endothelial dysfunction, TF appears on the cell surface. TF is also a potent cofactor and receptor for circulating FVII. The extrinsic and intrinsic systems are stimulated after TF comes into contact with blood. Thrombin, formed by TF activation, activates the platelets by stimulating them and accelerates the

adhesion, secretion, and aggregation of platelets. In this case, atherothrombosis and hypercoagulation are both triggers and consequences of each other. We found the decreased activity of TF and increased levels of TF in both hypothyroid and hyperthyroid groups. These results can be a compensation mechanism of the organism for the irregularities in the coagulation. Also, it may be a hemostatic mechanism to protect these organs. This situation may indicate a tendency to hypercoagulation in thyroid dysfunction. In this case, we can conclude that coagulation abnormalities can be developed and coronary artery diseases might be triggered by hypo- and hyperthyroidism.

Conflict of Interest: Authors state that there are no conflicts of interest in the manuscript, including financial, consultant, institutional, and other relationships that might lead to bias or a conflict of interest.

Ethics approval: The study was approved by the Ethics Committee of the Marmara University (71. 2009. mar).

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