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The Effects of Experimental Hypothyroidism on Hemorheology and Plasma Fibrinogen Concentration

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Effects of hypothyroid on hemorheology of patients had widely attracted the attention of researchers during last decade. The present study has been planned with the purpose to determine the effects of experimental hypothyroidism on hemorheological parameters and fibrinogen concentration. To induce experimental hypothyroid methimazole (75 mg/100 g) was added to the fodder of an experimental group rats for 20 d. After experimental duration, plasma and blood viscosity, hematocrit (Hct), hemoglobin, erythrocyte rigidity index, and plasma fibrinogen concentration values of both the control and the experimental group animals were determined and evaluated. The serum T_3 and T_4 levels of the experimental group were found lower ($p < 0.001$) but TSH level higher ($p < 0.001$) than that of the control group. Plasma viscosity and fibrinogen concentration of hypothyroid group were found significantly higher than controls ($p < 0.01$). Hematocrit and hemoglobin values were also found lower in the experimental group than the control group animals ($p < 0.01$). However, there was no significant difference found in blood viscosity at the original Hct value but there was a significant increase at standard Hct value ($p < 0.01$). There was also no change in erythrocyte rigidity index between control and experimental groups. According to these results it may be said that in hypothyroidism, increased fibrinogen concentration may alter the rheological structure of blood by inducing increase in plasma viscosity.

Key Words: Hypothyroidism; viscosity; fibrinogen; erythrocyte rigidity.

Introduction

Effects of thyroid hormones even on the cellular level in the cardiovascular system are well known. Higher risk of atherosclerosis and ischemic hearth diseases in hypothyroid patients has been proved *(1,2)*. Deformation of vascular structure and narrowness are important factors, which may alter the rheology of blood. Therefore, it has to be answered if alterations in hemorheological parameters are directly related to deficiency of thyroid hormones or changes in plasma protein concentrations (such as fibrinogen) in hypothyroidism lead to the hemorheological changes. It was reported that moderate hypothyroidism, which were consistently shown to be at high risk for cardiovascular diseases, have decreased fibrinolytic activity. Homeostatic abnormalities are observed in moderate hypothyroidism *(3,4)*.

Previous studies mostly have been concerned only on the effects of hypothyroidism on fibrinolytic system or blood viscosity. There are a limited amount of studies present on both rheology of blood and fibrinogen as a component of fibrinolytic system together *(4–6)*.

Therefore, this study has been planned to study the changes in hemorheological parameters and fibrinogen concentration that are known to be effective parameters in hemorheology in experimental hypothyroidism.

Results

The results of both groups from all measured parameters are given in Table 1. Statistically significant decreases in serum T_3, T_4 levels were seen in the experimental group compared to the controls ($p < 0.001$). The TSH level was found significantly higher in experimentals than that of the controls ($p < 0.001$). Plasma viscosity and plasma fibrinogen concentrations were found to be higher in hypothyroid group than control group animals ($p < 0.01$). Blood viscosity values at original Hct value has showed no significant difference but at standard Hct values blood viscosity was found statistically higher in the experimental group than that of the control group rats ($p < 0.01$). RBC rigidity index also did not change in both groups. Hct and Hb values were found to be lower in the experimental group than that of the control group ($p < 0.01$). Statistically significant negative correlations were found between T_4 and fibrinogen concentration ($r = –0.58$, $p < 0.05$) and T_4 and plasma viscosity ($r = –0.85$, $p < 0.001$). When stepwise multiple regression analysis was applied, a significant correlation was deter-

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Table 1
Rheological Parameters and Fibrinogen Concentrations of Control and Hypothyroid Group Rats

Parameter	Control (n = 10)	Hypothyroid (n = 10)
T_3, ng/100 mL	69.9 ± 14.8	26.9 ± 4.47***
T_4, µg/100 mL	5.4 ± 0.64	1.01 ± 0.12***
TSH, µIU/mL	0.03 ± 0.01	0.13 ± 0.05***
Hematocrit, %	40.0 ± 4.4	33.5 ± 0.97**
Hemoglobin, g/100 mL	12.3 ± 1.82	9.48 ± 1.22**
Fibrinogen, mg/100 mL	192.2 ± 30.4	234.7 ± 33.4**
Plasma viscosity, mPa·s	1.03 ± 0.04	1.10 ± 0.05**
Blood viscosity , mPa·s original hematocrit	2.71 ± 0.29	2.60 ± 0.26
Blood viscosity, mPa·s standard hematocrit Hct = 40%	2.78 ± 0.24	3.17 ± 0.26**
Rigidity index standard hematocrit Hct = 40%	2.89 ± 0.34	2.75 ± 0.35

Data are the means ± SD.
**$p < 0.01$, ***$p < 0.001$.

mined between fibrinogen concentration and plasma viscosity ($r = 0.76$, $p < 0.027$).

Discussion

In this study after methimazole application to the experimental rats, hypothyroidism occurred. Production of the hypothyroidism has been proved by the measurement of the T_3, T_4, and TSH levels of the experimental and the control groups. Statistically significant decreased T_3, T_4 and increased TSH values in experimental group were taken as the indicator of the primary hypothyroidism ($p < 0.001$).

It is proposed that the blood rheology is directly affected by thyroid hormones. However, results of various researches show differences related to the subject. Költringer et al. *(7)*, who worked on effects of hypothyroidism on blood rheology in human, in a series of 338 persons (age: 21–72 yr, 199 F, 139 M) investigated, 110 patients (75 F, 35 M) suffered from hypothyroidism, while 228 (124 F, 104 M) were healty subjects, even though they had found no difference in Hb, Hct, and mean corpuscular volume (MCV) values between control and experimental groups, but there was a significant increase in blood viscosity at both original and corrected (40%) Hct values. But in the same study there was no difference detected in plasma viscosity between two groups. Larsson et al. *(8)*, who examined the detection of variations in blood and plasma viscosity in hypothyroid patients, found that after L-thyroxine therapy the increased blood and plasma viscosities were corrected to the same level of controls. Kossler et al. *(9)* in their study in which they produced experimental hypothyroidism in rats, explained the increased plasma viscosity by an increase in plasma protein concentrations. When the results of Kossler et al. are considered, it has been seen that while there was no significant increase in albumin, γ globulin, and β globulin a significant increase was found in total plasma protein concentration.

In our study, in the hypothyroid group plasma viscosity was found significantly higher than the control group values ($p < 0.01$) (Table 1). It is known that plasma viscosity is affected by plasma protein concentrations *(10,11)*. Therefore, it is expected that the increase in the fibrinogen concentration affects the plasma viscosity as determined by our results (Table 1). The physicochemical or rheological approach states that the contribution of individual plasma proteins and lipoproteins to plasma viscosity depends on their concentration, molecular weight, rigidity, and asymmetrical shape. Based on this principle it has been suggested that the asymmetric fibrinogen has a stronger effect on viscosity than large globular immunoglobulins, which, in turn, have a stronger effect than the relatively small (relative molecular mass) globular protein albumin *(12,13)*. Therefore, it seems that the statistically significant change in our results may be based on the explanation given above. Even though Müller et al. *(14)* detected a slightly increase in fibrinogen concentration, the increase was not statistically significant in female hypothyroid patients who carry vascular and thrombotic risk. Chadarevian et al. *(3)* found negative correlation between free T_4 and fibrinogen concentrations in the study of the relation between thyroid hormones and fibrinogen concentration. The same group in another study had studied the fibrinolytic system in moderate and severe hypothyroid patients and found positive correlation between free T_4 and fibrinogen concentration *(4)*.

According to our results there is a negative correlation between the blood T_4 level and the plasma fibrinogen concentration ($r = -0.58$, $p < 0.05$). On the other hand, in our results there is also negative significant correlation between the blood T_4 level and the plasma viscosity ($r = -0.85$, $p < 0.001$). This result shows that, in deficiency of thyroid hormones, observed increase in plasma viscosity is possibly due to the increase in the plasma fibrinogen concentration. Although the major determinants of blood viscosity are hematocrit and plasma viscosity, plasma lipids and total serum protein have significant effects on blood viscosity. Hyperlipidemia with increase of serum cholesterol and triglyceride is also a common finding in hypothyroidism *(15, 16)*. There are studies that show a decrease in serum triglyceride levels in experimental hypothyroidism *(17–19)*. Therefore, in this study, fibrinogen concentration was handled as a parameter that probably affects the blood rheology.

In the hypothryoid group of our study Hb and Hct values were found lower than that of the control group values (Table 1). In the results of previous studies performed in

our department, it has been determined that Hct and Hb values in hypothyroid animals were found lower than the value of control rats *(20)*. In the present study, in the hypothyroid animals there was no statistically significant difference found in blood viscosity between the experimental and the control group animals but there was a statistically significant increase found in blood viscosity at standard Hct in the experimental group when compared to controls. Decreased Hct and Hb are balanced by increased fibrinogen related to increased plasma viscosity and the result is no observed change in blood viscosity. According to our results blood viscosity did not change in the experimental group when compared to controls; on the other hand, as an effective parameter on blood viscosity, erythrocyte rigidity also showed no difference between two groups. No change in the erythrocyte rigidity index in our results can be explained by the early phase of hypothyroidism. In early phase there may be no significant effect in hypothyroidism that reflects any change in erythrocyte membrane and blood rheology.

According to results of our study it may be concluded that in the early phase of hypothyroidism, changes in hemorheology are only seen as an increase in plasma viscosity. Such an increase may be dependent on increased fibrinogen concentration.

Materials and Methods

In present study Spraque–Dawley type albino female rats weighting 160–200 g were used in both control and experimental group animals. Both control (n = 10) and experimental group (n = 10) animals were kept in the same physical conditions during the experimental period and were fed with standard fodder and tap water. To constitute hypothyroidism, methimazole (75 mg/100 g fodder) was given to experimental group animals for 20 d *(17)*. At the end of 20 d, the animals were sacrificed under the anesthesia and blood samples were drawn to measure thyroid stimulant hormone (TSH), triiodothyronine (T_3), thyroxine (T_4) hormone levels, hematocrit (% Hct), hemoglobin (Hb), blood viscosity, plasma viscosity, and plasma fibrinogen concentration and erythrocyte deformability. Our protocol and methods were approved by the Animal Care and Use Committee of Laboratory Animal Service of the Istanbul University, Turkey. Serum levels of T_3, T_4 and TSH were determined by the radioimmunoassay (RIA) method (Diagnostic Products Corporation). The coat-A-count procedure is a solid-phase radioimmunoassay where ^{125}I-labeled T_3, T_4, and TSH compete for a fixed time with T_3, T_4, and TSH in the sample for antibody sites. This reaction takes place in the presence of blocking agents that serve to liberate bound triiodothyronine from carrier proteins; hence, the assay measures total T_3, T_4, and TSH, because both free and protein-bound T_3, T_4, and TSH from the sample are able to compete with radiolabeled T_3, T_4, and TSH for antibody sites. Radioactivity counting was performed in a gamma counter (Searle, Nuclear Chicago Division, model 1185). Hematocrit was measured by the microhematocrit centrifuge method. Blood viscosity was measured at original and standard Hct values. For standardization plasma of the animal either has been added or removed from the blood and % Hct was adjusted to 40% *(21)*. Erythrocyte deformability was described as erythrocyte rigidity index (RBC index). The ability of RBCs to deform was represented as a RBC rigidity index, which is the inverse value of the RBC deformability. The RBC rigidity index was calculated according to the ratio:

$$\text{RBC rigidity index} = \frac{\text{Asymptotic blood viscosity}}{\text{Plasma viscosity}}$$

where asymptotic blood viscosity was measured for standart hematocrit of Hct = 40% *(22)*. Asymptotic viscosity is a function of solid fraction only and is independent of particle size and shear rate. It corresponds to a microstructure in which there is no agglomeration between solid particles *(23)*. An increase in the rigidity index signifies a decrease in the RBC deformability *(24)*.

Both plasma and blood viscosity were measured in Harkness viscometer (Coulter Electronics Ltd, Ser. No: 6083, England) and evaluated in relation to distilled water at 37°C *(25)*. The plasma fibrinogen concentration was determined according to Ratnoff and Menzie method in spectrophotometer (UV-160 A, Shimadzu) at 520 nm *(26)*.

Statistical analysis was done by the Mann–Whitney U test. Data were expressed as means ± SD. Correlation analysis were performed by Pearson–Bravis and stepwise multiple regression analysis method between experimental and control groups. Differences between groups were considered significant at the $p < 0.05$ level.

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