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Investigation of hemorheology in patients with hyperthyroidism via blood viscosity, erythrocyte deformability and aggregation

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Abstract

Objective Hyperthyroidism's impact on cardiovascular, hematopoietic systems and metabolism might lead to hemorheological changes. This study aimed to investigate the changes in hemorheological properties via erythrocyte deformability and aggregation, whole blood viscosity (WBV) and plasma viscosity (PV) in hyperthyroid patients. The effect of anti-thyroid treatment on hemorheology was also studied.

Material methods Thirty-six patients with overt hyperthyroidism, 19 patients with subclinical hyperthyroidism and 66 controls were included in the study. Hematocrit, erythrocyte deformability and aggregation, plasma and whole blood viscosity values were measured before treatment. Hemorheological parameters of the patients were compared with the control. Before and after treatment results of overt hyperthyroidism were analyzed. Methimazole was given as anti-thyroid treatment. Deformability and aggregation measurements were conducted using a laser ektacytometer (LORRCA) while viscosity measurements were performed with a cone-plate viscometer (Brookfield DV-III).

Results The maximum elongation index (EImax) decreased significantly from 0.664 (0.01) pre-treatment to 0.657 (0.01) post-treatment ($p=0.04$). The aggregation index was significantly higher in both the subclinical hyperthyroidism group [68.05 (7.66), $p=0.001$] and the overt hyperthyroidism group [66.78 (8.815), $p=0.001$] compared to the control group. Additionally, the aggregation half-time was significantly shorter in the subclinical hyperthyroidism group [1.9 (1.21–2.27), $p=0.001$] and the overt hyperthyroidism group [1.91 (1.43–2.46), $p=0.001$] relative to the control group.

Conclusion The hemorheological status of patients was influenced by excessive thyroid hormones in both subclinical and overt hyperthyroidism groups. Additionally, anti-thyroid therapy with methimazole may play a role in the observed decrease in the maximum elongation index following treatment.

Keywords Erythrocyte deformability, Aggregation, Plasma viscosity, Whole blood viscosity, Hyperthyroidism, Hemorheology

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Introduction

Hyperthyroidism is a condition in which thyroid gland is overly active and produces excessive amount of thyroid hormones [1–3]. Hyperthyroidism can be further classified into two main groups based on clinical presentation and hormone levels: overt and subclinical hyperthyroidism. Overt hyperthyroidism is characterized by elevated free T3 (fT3) and free T4 (fT4) levels accompanied by suppressed TSH levels. On the other hand, in subclinical hyperthyroidism fT3 and fT4 levels are within the normal range while TSH is slightly suppressed [3, 4]

The most common causes of hyperthyroidism are Graves' disease, toxic multinodular goiter, and toxic adenoma. Graves' disease is an autoimmune disorder caused by the production of thyroid-stimulating immunoglobulins (TSIs), which target the thyroid-stimulating hormone (TSH) receptor on thyroid follicular cells. These autoantibodies mimic the action of TSH, leading to excessive stimulation of the TSH receptor. As a result, there is increased synthesis and secretion of thyroid hormones (thyroxine and triiodothyronine), along with thyroid gland hypertrophy. This hyperactivity of the thyroid gland causes the clinical features of hyperthyroidism, including tachycardia, weight loss, and heat intolerance. Additionally, orbital fibroblast stimulation by TSIs can result in Graves' ophthalmopathy, a hallmark extrathyroidal manifestation of the disease. In contrast, toxic multinodular goiter and toxic adenoma are conditions in which thyroid nodules autonomously produce and secrete excessive amounts of thyroid hormones, independent of TSH regulation [5, 6].

Thyroid dysfunctions are known to cause hematological changes that, in turn, effect the hemorheological status. However, the pathophysiology of this aspect is still being investigated. Thyroid hormones play a role in regulating erythropoiesis through their interaction with thyroid hormone receptors located on hematopoietic stem cells. [7]. Hyperthyroid states often exhibit elevated erythrocyte counts and hematocrit (Hct) percentages. These changes may contribute to increased whole blood viscosity (WBV) and alterations in erythrocyte aggregation (EA) behavior.

There are studies indicating elevation in intracellular sodium (Na) concentration of erythrocytes in hyperthyroid patients [8, 9]. Hyperthyroidism's effect on erythrocyte ion channels on the cell membrane could lead to changes in erythrocyte volume, as sodium has the ability to attract water. This would ultimately impact erythrocyte deformability by altering the volume and internal viscosity of the erythrocytes.

In a group of patients with subclinical hyperthyroidism, plasma fibrinogen concentrations have been found to be increased [10]. Shih et al. demonstrated that T3 hormone effectively promotes the synthesis of fibrinogen and certain coagulation factors [11]. Elevated levels of fibrinogen and coagulation factors contribute to hypercoagulation, as well as increased plasma viscosity (PV), whole blood viscosity, and erythrocyte aggregation. Furthermore, previous studies conducted by various researchers have reported that patients with hyperthyroidism exhibit endothelial dysfunction and decreased fibrinolytic activity. [12–14]. Taking these factors into account, impaired hemorheological status could contribute to an increased risk of thromboembolic events.

Excessive thyroid hormones may adversely affect hemorheological properties, including viscosity of the blood and/or rheological behavior of erythrocytes. Such hemorheological impairments, in combination with the aforementioned hemodynamic alterations, can lead to disruption of tissue oxygenation in hyperthyroid patients. [14–16].

Moreover, in a hypermetabolic state, there is an increased oxygen demand by the tissues, leading to an elevation in erythrocyte count and erythrocyte mass. Both of these changes have an impact on hemorheological parameters such as whole blood viscosity and erythrocyte aggregation. Therefore, when evaluating rheological status of the patients with hyperthyroidism, it is crucial to consider these multiple factors contributing to impaired rheology.

In light of this information, the purpose of this study was to investigate the impact of excess thyroid hormones on various rheological properties of blood, including plasma and whole blood viscosity, erythrocyte deformability, and aggregation. Patients with hyperthyroidism are prone to disturbances in microcirculation and tissue oxygenation, attributed to reduced fibrinolytic activity and a hypercoagulable state. We hypothesize that these patients may also experience alterations in their hemorheological properties that derived from behaviors of the erythrocytes and viscosity of the blood, alongside diminished fibrinolytic activity. As a result, disruptions in microcirculation expected to be more pronounced in this population. We aimed to enhance our understanding of the pathophysiology of the condition from a hemorheological perspective by exploring the effects of erythrocyte behavior in hyperthyroid patients. To the best of our knowledge, this study represents the first attempt to evaluate the hemorheological status in human subjects with hyperthyroidism through the assessment of blood and plasma viscosity, erythrocyte deformability, and aggregation.

Material and Methods

Patient selection and sample preparation

This study was conducted at the internal medicine and endocrinology clinics of University of Health Sciences Haydarpasa Numune Training and Research Hospital. A total of fifty-five recently diagnosed hyperthyroid patients, who had not been administered any anti-thyroid medication, were included in this study. Thirty-six of them were in the overt hyperthyroidism group and nineteen were included in the subclinical hyperthyroidism group according to their clinical presentation and thyroid hormone levels. The overt hyperthyroidism (OH) group comprised patients with suppressed TSH and increased T3 and/or T4 levels, while the subclinical hyperthyroidism (SH) group included patients with suppressed TSH but T3 and T4 levels were within the normal ranges. Sixty-six healthy individuals, who were compatible with the patient groups in terms of sex and age, were included in the control group. Demographic data and clinical characteristics of the study population are presented in Table 1. Ethical approval was obtained from University of Health Sciences, Hamidiye Clinical Research Local Ethics Committee (date of approval: 13/02/2020 reference number of approval: 2020.02.13–09). The measurements in this study have been performed in accordance with the Declaration of Helsinki. Prior to blood sampling, participants were thoroughly briefed about the study procedures to ensure their understanding. Written consent was obtained from all volunteers, including those in the patient and control groups. Participants were instructed to fast for 8 h before blood collection.

Blood samples for hemorheological parameters were collected from the antecubital vein into 10 ml K2-EDTA tubes. Hemorheological measurements were conducted within three hours of blood sampling in accordance with the guidelines for hemorheological studies [17]. Before erythrocyte aggregation measurements, hematocrit was adjusted to 40% using autologous plasma. If a hct exceeding 40% was detected, an appropriate amount of plasma from separately centrifuged whole blood was added to achieve a 40% level. Conversely, if the hematocrit was below 40%, sufficient amount of whole blood was retained to allow erythrocytes to settle at the bottom of the tube. Subsequently, an adequate volume of plasma was removed from the whole blood to adjust it to a 40% hct level. Later the tube was gently inverted a few times to prepare the whole blood for aggregation measurements.

Hormonal parameters and complete blood counts of the patients were obtained from hospital records.

Study design

Patients were selected based on predefined inclusion and exclusion criteria. The inclusion criteria comprised individuals between the ages of 18 and 65 who had recently been diagnosed and had not received any prior antithyroid treatment. The exclusion criteria encompassed patients with other endocrinological or hematological disorders, any form of malignancy, viral, bacterial, or fungal infections, chronic inflammatory conditions, or those currently using antithyroid medication. Blood samples were collected from all patients at the onset of the diagnosis. Patients in subclinical hyperthyroidism group did not receive any anti-thyroid treatment. In the overt hyperthyroidism group, patients received Methimazole (MMZ) as anti-thyroid treatment and post-treatment measurements were conducted after achieving an euthyroid state, as indicated by the hormone panel, within a period of 30 to 45 days. The results were statistically compared among the groups to see the effects of excessive thyroid hormone on hemorheological parameters. Furthermore, the results of pre- and post-treatment measurements for the overt hyperthyroidism group were statistically compared to observe any possible alterations in hemorheological parameters in response to anti-thyroid treatment.

Hemorheological measurements

Hemorheological measurements were performed at 37 °C within three hours of blood sampling. To measure the hematocrit, a blood sample was transferred from K2-EDTA tubes to heparinized capillary tubes. The samples were then centrifuged at 10,000 rpm (g = 11.180) using a hematocrit centrifuge device (NT 715, Nüve, Ankara) for five minutes. The hematocrit value was noted as a percentage.

Table 1 Demographic data of the groups

Demographic Data	*Control Group (n = 66)*	*SH Group (n = 19)*	*OH Group (n = 36)*
Age-Mean (SD)	37.68 (10.82)	41.73 (13.73)	41.97 (14.84)
Age (Min–Max)	20–58	23–62	21–65
Female/Male ratio	39/27	14/5	19/17

OH Overt hyperthyroidism; SH, Subclinical hyperthyroidism

Measurement of erythrocyte deformability

Erythrocyte deformability (ED) measurements were conducted using a laser ektacytometer (LORRCA, RR Mechatronics, Hoorn, The Netherlands). The method was described in detail by Hardeman et al. previously [18]. In summary, 25 µlt. of whole blood sample diluted in 5 ml of isotonic medium (4% polyvinylpyrrolidone 360 solution; MW 360 kD; Sigma P 5288; St. Louis, MI). The suspension was then subjected to shear stress in a Couette system. This Couette system is formed of a glass cup and a concentric bob that fits the cup with a gap of 0.3 mm between them. A laser beam aimed at the sample generated a diffraction pattern of sheared erythrocytes, which was captured by a camera and analyzed using computer software. The Elongation Index was calculated based on the obtained diffraction pattern. The Elongation Index is formulated as the difference in length of the long (L) and short (S) axis of the pattern divided by the sum of the two [(L-S)/(L + S)]. Elongation Indices were determined under nine different shear stresses ranging from 0.3 to 30 Pa. The software gives a maximum elongation index value (EImax) from the calculated Elongation Indices (EI) and the shear stress value required to achieve half of the maximum elongation index (SS1/2).

Measurement of erythrocyte aggregation

Erythrocyte aggregation was also measured by LORRCA following previously described methods [19, 20]. Prior to measurement, the blood sample with Htc of 40% was oxygenated for fifteen minutes. The aggregation-disaggregation behavior of the erythrocytes was analyzed by software based on the laser back-scatter ratio from the sheared sample. Initially, high shear (500 s^{-1}) was applied first to observe the disaggregation behavior, followed by an abrupt stop and then low shear to observe the formation of rouleaux structures and three-dimensional aggregates. The computer software evaluated the amount of laser back-scatter during each phase and generated an aggregation index (AI) to quantify the extent of aggregation, as well as the aggregation half-time (t ½). These parameters were calculated based on the principle that less light is backscattered from aggregating red cells.

Whole blood viscosity and plasma viscosity measurements

Plasma and whole blood viscosity were measured using a cone-on-plate Rotational Viscometer (Brookfield DV-III, Brookfield, Middleboro, MA, USA). This device consists of a cylindric container and a disc concentric with the container, which has a cone-shaped tip (cone-plate model). The sample was sheared in the cone-on-plate Couette system, whereby the resistance generated by the sample induced torque in the spring connected to the cone-on-plate system. Measurements were conducted at 37 °C using a CP-40Z spindle, with shear rates of 37.5, 75, 150, 300, and 450 s^{-1} for WBV, and 450 s^{-1} for PV.

Statistical analysis

Statistical analyses were conducted using SPSS 16.0 (Statistical Package for Social Sciences). The normality of the data was assessed using the Shapiro-Wilks test. For normally distributed data, ANOVA and post-hoc Tukey tests were used to compare groups, and the Student's t-test was employed for comparison of pre- and post-treatment measurements. To compare groups with non-normally distributed data, the Kruskal–Wallis-H test and the post-hoc Tamhane test were utilized. The Wilcoxon test was applied for pre- and post-treatment comparisons. Correlation analysis was performed using the Pearson correlation test for normally distributed data and the Spearman correlation test for non-normally distributed data. *P* values less than 0.05 were accepted as statistically significant. The sample size was determined by an a-priori power analysis using G*power 3.1.9.7, with an alpha level of 0.05, a power of 85% (1-β = 0.85), and an expected large effect size according to Cohen's equation [21].

Results

Of the initial 36 patients in the OH group, six did not participate in the post-treatment follow-up. Consequently, for comparisons with the SH and control groups, the OH group comprised 36 patients, while pre- and post-treatment comparisons within the OH group were based on 30 patients. Demographic data for the groups are presented in Table 1, while Table 2 displays complete blood count parameters and thyroid-related blood work for the patient groups.

Erythrocyte deformability was assessed using the parameters including elongation index (EI) at nine different shear stress levels (0.3; 0.53; 0.95; 1.69; 3; 5.33; 9.49; 16.87; 30 Pa), maximum elongation index (EImax), and shear stress at half of the maximum elongation index (SS ½). The EI values of patient groups remained lower than the control group as the applied shear stress increased, though this difference was not statistically significant (Table 3). The EImax and SS ½ values did not show a significant difference between the three groups. However, after treatment, the EImax of the overt hyperthyroidism group was significantly decreased compared to the before treatment value ($p = 0.04$), (Table 4). Additionally, SS ½ did not show a statistical difference between before and after treatment measurements.

The aggregation properties of erythrocytes were analyzed through Aggregation Index (AI) and aggregation half-time (t1/2). AI provides an indication of the extent and kinetics of the aggregation, while t1/2 reveals the aggregation velocity. Statistically significant decrease

Table 2 Complete blood count and thyroid-related parameters of the groups

Blood Count Parameters **(Reference Range)**	***Control Group (n = 30)*** **Mean (SD)**	***SH Group*** **(n = *19*)** **Mean (SD)**	***OH Group*** **(n = *36*)** **Mean (SD)**	***P value***
RBC *(3.5–5 × 10^6 µ/L)*	4.9 (0.62)	5.53 (3.29)	5.29 (1.24)	P1:0.79 P2:0.97 P3:0.97
Hemoglobin *(11–15 gr/dL)*	14.46 (1.87)	15.53 (10.54)	13.4 (2.24)	P1:0.85 P2:0.95 P3:0.56
Hematocrit *(%36–47)*	43.66 (5.89)	42.78 (18.04)	41.57 (5.82)	P1:0.81 P2:0.94 P3:0.94
MCV *(80–100 fL)*	88.93 (2.95)	77.71 (18.1)	83.2 (10.48)	P1:0.16 P2:0.68 P3:0.35
MCH *(27–34 pg)*	29.48 (0.79)	27.78 (3.41)	27.66 (3.35)	P1:0.08 P2:0.99 P3:0.48
MCHC *(30–36 gr/dL)*	32.12 (1.29)	32.78 (1.15)	33.05 (1.42)	P1:0.40 P2:0.88 P3:0.23
RDW *(%11–16)*	13.25 (0.56)	14.67 (4.27)	13.46 (1.52)	P1:0.72 P2:0.26 P3:0.66
RDW-SD *(35–56 fL)*	40.86 (2.08)	42.83 (12.02)	38.80 (2.55)	P1:0.26 P2:0.19 P3:0.92
Platelet *(100–400 × 10^3 µL)*	240.67 (25.03)	270 (77.49)	282.14 (80.9)	P1:0.92 P2:0.99 P3:0.85
WBC *(4–10 × 10^3 µL)*	6.02 (1.154)	7.94 (1.87)	7.30 (1.76)	P1:0.89 P2:0.30 P3:0.54
TSH *(0.35- 4.94uIU/mL)*	1.70 (0.55)	0.05 (0.09)	0.04 (0.08)	P1:0.72 P2:0.26 P3:0.66
Free T_4 *(0.7- 1.48 ng/dL)*	1.09 (0.16)	1.16 (0.21)	1.57 (0.71)	P1:0.26 P2:0.19 P3:0.92
Free T_3 *(1.71- 3.71 pg/mL)*	-	3.52 (0.86)	8.45 (7.43)	
Anti TPO *(< 5.60 IU/mL)*	-	33.59 (62.35)	28.57 (43.85)	
TSI *(0.00- 14.00 U/L)*	-	4.02 (1.52)	19.12 (23.45)	
TR-Ab *(< 1.5 IU/L)*	-	0.5 (0.4)	1.96 (2.71)	
Anti-thyroglobulin *(< 4.1 IU/mL)*	-	41.13 (68.86)	20.07 (40.74)	

OH Overt hyperthyroidism, *SH* Subclinical hyperthyroidism, *RBC* Red blood cells, *MCV* Mean corpuscular volume, *MCH* Mean corpuscular hemoglobin, *MCHC* Mean corpuscular hemoglobin concentration, *RDW* Red cell distribution width, *RDW-SD* Red cell distribution width-standard deviation, *WBC* white blood cells, *TSH* thyroid stimulating hormone, *Anti-TPO* Anti-thyroid peroxidase antibody, *TSI* Thyroid stimulating immunoglobulin, *TR-Ab* Thyroid receptor antibody

P1: OH vs Control group; P2: OH vs SH; P3: SH vs Control group. Data are presented as "Mean (SD)"

in t1/2 was observed in both patient groups compared to the control group (p values for both groups = 0.001). AI demonstrated a significant increase in both patient groups compared to the control group ($p = 0.001$). Aggregation parameters did not show significant differences between before and after treatment measurements in the OH group (Table 4).

Table 5 presents the whole blood viscosity and plasma viscosity of the groups at native hematocrit. The overt hyperthyroidism group exhibited a noteworthy increase

Table 3 Elongation indices of the groups at 9 different shear stress levels

	Control (n = 66)	***SH (n = 19)***	***OH (n = 36)***	***OH* (pre-tr.) (n = *30*)**	***OH* (post-tr.) (n = *30*)**
EI-1 (at 0.3 Pa)	0.04 (0.01)	0.06 (0.02)	0.05 (0.02)	0.05 (0.02)	0.06 (0.02)
EI-2 (at 0.53 Pa)	0.11 (0.02)	0.13 (0.02)	0.12 (0.02)	0.12 (0.02)	0.12 (0.02)
EI-3 (at 0.95 Pa)	0.20 (0.02)	0.21 (0.02)	0.20 (0.02)	0.20 (0.02)	0.21 (0.02)
EI-4 (at 1.69 Pa)	0.29 (0.02)	0.30 (0.02)	0.29 (0.02)	0.29 (0.02)	0.29 (0.02)
EI-5 (at 3 Pa)	0.39 (0.01)	0.39 (0.02)	0.38 (0.02)	0.39 (0.01)	0.39 (0.02)
EI-6 (at 5.33 Pa)	0.47 (0.01)	0.47 (0.02)	0.46 (0.01)	0.46 (0.01)	0.46 (0.01)
EI-7 (at 9.49 Pa)	0.53 (0.01)	0.53 (0.01)	0.52 (0.01)	0.52 (0.01)	0.52 (0.01)
EI-8 (at 16.87 Pa)	0.57 (0.01)	0.57 (0.01)	0.57 (0.01)	0.57 (0.01)	0.57 (0.01)
EI-9 (at 30 Pa)	0.60 (0.01)	0.60 (0.01)	0.60 (0.01)	0.60 (0.01)	0.60 (0.009)

EI Elongation index, *OH* Overt hyperthyroidism, *SH* Subclinical hyperthyroidism. Nine step applied shear stress levels are 0.3; 0.53; 0.95; 1.69; 3; 5.33; 9.49; 16.87; 30 Pa, respectively. Data are presented as Mean (SD). The differences among the groups were not statistically significant at all shear stress levels ($p > 0.05$)

Table 4 Percentage of hematocrit and parameters of erythrocyte deformability and aggregation

	***Control* (n = *66*)**	***SH* (n = *19*)**	***OH* (n = *36*)**	***OH* (pre-tr.) (n = *30*)**	***OH* (post-tr.) (n = *30*)**	***p Value***
Hct (%) Mean (SD)	40.59 (3.99)	39.68 (4.46)	42.23 (4.05)	42.43 (4.25)	43.4 (4.53)	P1:0.08 P2:0.04 P3:0.67 P4:0.04
EI_{max} Mean (SD)	0.65 (0.01)	0.66 (0.01)	0.66 (0.01)	0.66 (0.01)	0.65 (0.01)	P1:0.80 P2:0.49 P3:0.20 **P4: 0.04**
$SS_{1/2}$ (Pa) Median (IQR)	1.28 (1.17–1.44)	1.38 (1.08–1.58)	1.42 (1.2–1.65)	1.39 (1.15–1.69)	1.49 (1.31–1.58)	P1:0.11* P2:0.91* P3:0.65* P4:0.23^{+}
AI (%) Mean (SD)	58.95 (9.68)	68.05 (7.66)	66.78 (8.81)	67 (8.61)	64.6 (8.0)	**P1 < 0.001** P2 = 0.87 **P3 = 0.001** P4 = 0.08
$t_{1/2}$ (sec) Median (IQR)	2.49 (1.85–3.79)	1.9 (1.21–2.27)	1.91(1.43–2.46)	1.83 (1.38–2.4)	1.99 (1.69–2.46)	**P1 < 0.001*** P2 = 0.84* **P3 < 0.001*** P4 = 0.12 **+**

Hct Hematocrit, *Elmax* Maximum elongation index, *SS1/2* Shear stress at one-half of maximum elongation, *AI* aggregation index, *t1/2* aggregation half-time, *OH* Overt hyperthyroidism, *SH* Subclinical hyperthyroidism, *pre-tr.* pre-treatment, *post-tr.* post-treatment

P1: OH vs Control group; P2: OH vs SH; P3: SH vs Control group; P4: Difference between pre- and post-treatment results of OH group. (*Kruskal-Walli-H test, + Wilcoxon test. $SS_{1/2}$ and $t_{1/2}$values are given as median and interquartile range as 25%-75%)

in both PV and WBV at all shear rates when compared to the control group ($p < 0.02$). Conversely, in the subclinical hyperthyroidism group, only the PV demonstrated a significant increase compared to the control group ($p = 0.008$). Notably, the PV and WBV values in the overt hyperthyroidism group did not exhibit a significant difference between pre- and post-treatment measurements. Furthermore, a positive correlation emerged between specific complete blood count parameters (Hct, Hgb, MCHC, RBC) and the WBV of pre-treatment values

Table 5 Parameters of whole blood and plasma viscosity

		Control (n = *66*)	*SH* (n = *19*)	*OH* (n = *36*)	*OH* (pre-tr.) (n = *30*)	*OH* (post-tr.) (n = *30*)	*P value*
PV (cP) *(shear rate: 450 s^{-})*	1.3 (0.09)		1.42 (0.146)	1.42 (0.16)	1.40 (0.14)	1.35 (0.13)	**P1 = 0.001** P2 = 0.99 **P3 = 0.008** P4 = 0.12
WBV (cP) *(shear rate: 37.5 s^{-})*	5.16 (1.24)		4.99 (0.76)	5.87 (1.33)	5.56 (1.07)	5.79 (1.46)	**P1 = 0.04** **P2 = 0.01** P3 = 0.86 **P4 < 0.001**
WBV (cP) *(shear rate: 75 s^{-})*	4.47 (0.76)		4.48 (0.6)	4.96 (1.15)	4.83 (0.83)	4.83 (1.08)	**P1 = 0.02** P2 = 0.13 P3 = 0.99 P4 = 0.99
WBV (cP) *(shear rate: 150 s^{-})*	3.99 (0.57)		4.05 (0.48)	4.39 (0.77)	4.35 (0.75)	4.30 (0.91)	**P1 = 0.01** P2 = 0.14 P3 = 0.94 P4 = 0.69
WBV (cP) *(shear rate: 300 s^{-})*	3.65 (0.48)		3.73 (0.44)	3.97 (0.63)	3.96 (0.61)	3.87 (0.69)	**P1 = 0.01** P2 = 0.25 P3 = 0.84 P4 = 0.29
WBV (cP) *(shear rate: 450 s^{-})*	3.50 (0.44)		3.72 (0.58)	3.82 (0.59)	3.81 (0.58)	3.54 (0.85)	**P1 = 0.009** P2 = 0.76 P3 = 0.24 P4 = 0.11

OH Overt hyperthyroidism, *SH* Subclinical hyperthyroidism, *PV* Plasma viscosity, *WBV* Whole blood viscosity

P1: OH vs Control group; P2: OH vs SH; P3: SH vs Control group; P4: Difference between pre- and post-treatment results of OH group. Data are presented as Mean (SD)

in the overt hyperthyroidism group (p values < 0.005) (Table 6). This correlation aligns with the discussions in the corresponding section, providing support for the elucidation of the elevated WBV.

Discussion

The main findings of this study include: a notable reduction in erythrocyte deformability in overt hyperthyroidism group following treatment with Methimazole, an increased tendency to form aggregates in patient groups compared to the control, elevated levels of whole blood and plasma viscosity in the OH group when contrasted with the control group, and increased plasma viscosity in SH group compared to the control group.

Erythrocyte deformability is an essential factor for delivering oxygen to tissues, as it is one of the major determinants of red cell survival in the circulatory system. As erythrocytes pass through the small capillaries, their ability to change shape and adapt to the conditions enables them to transport oxygen. In our study, we observed no significant difference in erythrocyte deformability among the OH and SH patient groups and the control group. There are limited studies for direct comparison, and conflicting results exist. Levi et al. reported a significant decrease in erythrocyte deformability in an experimental hyperthyroidism model compared to the control group, which contradicts our results [22]. This decline is attributed to the increased mean corpuscular hemoglobin concentration (MCHC) of the experimental group, which indicates an elevated internal viscosity of erythrocytes and plays a crucial role in their deformability. In contrast, Özdemir et al. presented results that were consistent with our study, indicating no significant difference between the experimental and control groups in terms of erythrocyte deformability. [23]. Notably, our study demonstrates dissimilarities to both of these studies as we conducted experiments using human blood samples and employed the ektacytometer method, which is considered more reliable than the methods utilized in prior studies. Variations in methodologies and sample characteristics may account for the discrepancies in results.

Pre- and post-treatment results revealed a significant decrease in erythrocyte deformability after treatment with Methimazole (MMZ) ($p = 0.04$ for EImax). All patients in the OH group were administered MMZ as anti-thyroid treatment, and post-treatment measurements were conducted after observing an euthyroid state in the blood work. MMZ is known to cause agranulocytosis. Several mechanisms underlie Methimazole-induced agranulocytosis, including its suppressive effect on the common progenitor cell series (CPCS) [24–26]. This

Table 6 Correlation between hemogram and hemorheology parameters of Overt Hyperthyroidism group (pre-treatment values)

	HCT	EImax	$SS_{1/2}$	AI	$T_{1/2}$	PV	WBV1	WBV2	WBV3	WBV4	WBV5	RBC	HGB	MCV	MCH	MCHC	RDW	RDW_SD	PLT	WBC
HCT	1.00	-0.15	0.15	0.12	-0.11	0.27	0.17	0.62	0.69	0.75	0.76	0.60	0.78	0.03	0.12	0.32	-0.24	-0.31	-0.29	0.14
EI_{max}	-0.15	1.00	-0.30	0.17	-0.22	0.14	0.00	-0.12	-0.04	0.02	0.03	-0.17	0.18	0.29	0.32	0.32	-0.39	-0.07	-0.17	-0.09
$SS_{1/2}$	0.15	-0.30	1.00	0.01	0.02	0.03	0.32	0.45	0.19	0.12	0.10	0.15	-0.12	-0.36	-0.35	-0.18	0.24	-0.28	0.00	-0.02
AI	0.12	0.17	0.01	1.00	-0.99	0.46	0.30	0.23	0.22	0.26	0.28	-0.15	0.13	0.21	0.18	0.06	-0.27	-0.11	0.50	0.38
$T_{1/2}$	-0.11	-0.22	0.02	-0.99	1.00	-0.52	-0.30	-0.23	-0.23	-0.27	-0.29	0.11	-0.13	-0.16	-0.13	-0.05	0.24	0.13	-0.50	-0.34
PV	0.27	0.14	0.03	0.46	-0.52	1.00	0.09	0.53	0.67	0.70	0.68	0.29	0.41	0.27	0.27	0.23	-0.35	-0.20	0.17	0.38
WBV1	0.17	0.00	0.32	0.30	-0.30	0.09	1.00	0.24	0.06	0.06	0.08	-0.06	0.09	-0.12	-0.03	0.16	-0.12	-0.39	-0.05	-0.08
WBV2	0.62	-0.12	0.45	0.23	-0.23	0.53	0.24	1.00	0.89	0.87	0.86	0.50	0.62	0.03	0.17	0.43	-0.27	-0.35	-0.24	0.09
WBV3	0.69	-0.04	0.19	0.22	-0.23	0.67	0.06	0.89	1.00	0.97	0.95	0.72	0.72	0.15	0.24	0.39	-0.32	-0.24	-0.29	0.25
WBV4	0.75	0.02	0.12	0.26	-0.27	0.70	0.06	0.87	0.97	1.00	1.00	0.63	0.82	0.17	0.28	0.47	-0.37	-0.28	-0.27	0.25
WBV5	0.76	0.03	0.10	0.28	-0.29	0.68	0.08	0.86	0.95	1.00	1.00	0.59	0.84	0.18	0.30	0.49	-0.39	-0.28	-0.26	0.25
RBC	0.60	-0.17	0.15	-0.15	0.11	0.29	-0.06	0.50	0.72	0.63	0.59	1.00	0.89	-0.69	0.09	0.03	0.04	0.06	-0.18	0.26
HGB	0.78	0.18	-0.12	0.13	-0.13	0.41	0.09	0.62	0.72	0.82	0.84	0.89	1.00	-0.50	0.43	0.28	-0.15	0.08	-0.22	0.26
MCV	0.03	0.29	-0.36	0.21	-0.16	0.27	-0.12	0.03	0.15	0.17	0.18	-0.69	-0.50	1.00	0.51	0.33	-0.37	-0.02	-0.06	-0.15
MCH	0.12	0.32	-0.35	0.18	-0.13	0.27	-0.03	0.17	0.24	0.28	0.30	0.09	0.43	0.51	1.00	0.70	-0.56	0.06	-0.32	0.12
MCHC	0.32	0.32	-0.18	0.06	-0.05	0.23	0.16	0.43	0.39	0.47	0.49	0.03	0.28	0.33	0.70	1.00	-0.43	-0.08	-0.34	0.17
RDW	-0.24	-0.39	0.24	-0.27	0.24	-0.35	-0.12	-0.27	-0.32	-0.37	-0.39	0.04	-0.15	-0.37	-0.56	-0.43	1.00	0.80	0.10	-0.02
RDW_SD	-0.31	-0.07	-0.28	-0.11	0.13	-0.20	-0.39	-0.35	-0.24	-0.28	-0.28	0.06	0.08	-0.02	0.06	-0.08	0.80	1.00	-0.14	0.02
PLT	-0.29	-0.17	0.00	0.50	-0.50	0.17	-0.05	-0.24	-0.29	-0.27	-0.26	-0.18	-0.22	-0.06	-0.32	-0.34	0.10	-0.14	1.00	0.29
WBC	0.14	-0.09	-0.02	0.38	-0.34	0.38	-0.08	0.09	0.25	0.25	0.25	0.26	0.26	-0.15	0.12	0.17	-0.02	0.02	0.29	1.00
-1.00	0	1.00																		

HCT Hematocrit, EI_{max} Maximum elongation index, $SS_{1/2}$ Shear stress at one-half of maximum elongation, *AI* Aggregation index, $t_{1/2}$ Aggregation half-time, *PV* Plasma viscosity, *WBV1* Whole blood viscosity at 37.5 s^{-} shear rate,*WBV2* Whole blood viscosity at 75 s^{-}shear rate, *WBV3* Whole blood viscosity at 150 s^{-} shear rate, *WBV4* Whole blood viscosity at 300 s^{-} shear rate, *WBV5* Whole blood viscosity at 450 s^{-} shear rate, *RBC* Red blood cells, *HGB* Hemoglobin, *MCV* Mean corpuscular volume, *MCH* Mean corpuscular hemoglobin, *MCHC* Mean corpuscular hemoglobin concentration, *RDW* Red cell distribution width, *RDW-SD* Red cell distribution width-standard deviation, *PLT* platelet, *WBC* white blood cells

suppression extends to the erythroid cell series, although not significantly enough to induce anemia. However, it may lead to an increased prevalence of older erythrocytes in peripheral circulation. Hence, the decrease in EImax after the treatment might be the result of MMZ's myelosuppressive impact.

Aggregation of erythrocytes is a reversible cluster formation of red cells at low shear rates. When the shear rate increases, erythrocytes disaggregate and flow freely through the bloodstream. Aggregation behavior of erythrocytes is mostly influenced by plasma proteins, morphological and membrane properties of the erythrocytes, and other macromolecular content of the plasma [27]. In our study, both patient groups exhibited notable differences in aggregation parameters compared to the control group, indicating a greater tendency to form aggregates. Specifically, AI was significantly elevated in both patient groups compared to the control group accompanied by a significant decrease in $t_{1/2}$ ($p=0.001$). Remarkably, a strong negative correlation was observed between AI and $t_{1/2}$ ($r=$-0.99, $p<0.001$). AI measures the extent of aggregate formation, with higher values indicating more pronounced aggregation. Conversely, $t_{1/2}$ represents the time required for aggregates to form, with lower values indicating faster aggregation kinetics. Our results suggest a shorter duration of aggregation in patient groups compared to control, indicating that the aggregates formed more rapidly. The simultaneous increase in AI suggests an overall increase in the tendency for erythrocyte aggregation. This pattern of results indicates an increased tendency to form aggregates in patient groups and a shift towards more rapid erythrocyte aggregation [19, 20, 28].

We observed an elevation in PV in both patient groups compared to control. There are several possible explanations for the increased plasma viscosity in OH and SH groups of our study. The most common one, as reported by Özdemir et al., is the increase in plasma protein concentrations. Their results indicate increased plasma protein levels and increased PV in experimental hyperthyroidism model [23]. Thyroid hormones induce an increase in the concentrations of a number of proteins derived from liver and/or endothelium by stimulating the gene expression. Fibrinogen, albumin, alpha and gamma globulins, Von Willebrand Factor, Factors IX, VIII, are among these proteins [29–31]. Additionally, elevated acute phase reactants in hyperthyroidism could lead to an increase in PV [32].

Additionally, a significant correlation was found between the aggregation parameters and plasma viscosity. AI showed positive correlation with the PV ($r=0.46$, $p=0.004$) while t1/2 was negatively correlated with PV ($r=$-0.52, $p=0.001$). These correlations may indicate that elevated plasma viscosity could originate from the same factors as those contributing to an increased tendency for aggregation, more likely related to alterations in plasma protein content, which are commonly observed

in hyperthyroid patients. It is known that the concentrations of plasma proteins, particularly fibrinogen, increase in hyperthyroid patients and this is the main cause of increased PV [23, 29, 33]. Although specific protein concentrations were not quantified in our study, observed correlation between plasma viscosity and aggregation parameters led us to infer a potential relationship. The elevated PV observed in our results may be attributed to heightened plasma protein content.

An improvement in plasma viscosity is anticipated following antithyroid treatment. However, this improvement may not be immediately detected upon achieving a euthyroid state based on blood work. Similarly, in our study, after treatment, plasma viscosity did not show any significant difference compared to the before treatment results ($p=0.12$). Multiple measurements with intervals after the treatment could be of help in detecting an improvement in plasma viscosity.

Whole blood viscosity at 5 different shear rates (37.5; 75; 150; 300; 450 s^{-1}) was found to be increased in overt hyperthyroidism group compared to the control, whereas the subclinical hyperthyroidism group did not show any significant difference (Table 5). Increased whole blood viscosity can be explained by the increase in plasma viscosity, although, as Larsson et al. reported, WBV could also increase without any changes in plasma viscosity [34]. The authors mentioned that in hyperthyroid patients, due to elevated intracellular sodium concentrations there is retention of water in the erythrocytes, and this leads to structural changes by increasing the erythrocyte volume. Erythrocytes' deformability and aggregation behaviors are influenced by these structural changes, resulting in an elevation in WBV. In our study, aggregation parameters of OH group differed significantly, in support of an increased tendency for aggregation compared to the control, which can be pointed out as one of the reasons for increased WBV.

Correlation analysis of OH group showed a positive correlation between the viscosity at all shear rates and some of the complete blood count parameters such as RBC, Hgb, Hct and MCHC ($p<0.005$). These correlations are expected and demonstrate the reliability of the methods used in our study.

Elevated WBV could be related to the changes in lipid profile and electrolyte levels. It has been reported that increases in WBV and PV are positively correlated with the elevated levels of HDL, LDL, triglycerides, Na, K and Ca. According to the authors of the study, altered concentrations of plasma content cause erythrocytes to form aggregates more easily, resulting in increased WBV [35, 36]. Our findings did not demonstrate a significant difference in the lipid panel parameters or electrolytes across the groups. Although the aggregation and viscosity parameters of OH group showed a significant difference compared to the control group, before and after treatment measurements were not significantly different. An explanation for this may be that the possible changes in hemorheological parameters cannot be detected in the blood immediately after euthyroid state was observed in blood work.

Presented study has several limitations that should be acknowledged. Firstly, the relatively small study population may limit the generalizability of the findings. Secondly, the study was conducted at a single center, which may reduce the relevance of these findings to other populations. Thirdly, the study did not include patients with thyrotoxicosis, which could have provided additional insights into hemorheological changes. Despite these limitations, our findings contain statistically significant results and contribute valuable information to the understanding of hemorheological alterations in hyperthyroid patients and may help illuminate existing gray areas in this field.

Conclusion

In conclusion, our study suggests that hemorheological parameters are affected in hyperthyroid patients, leading to an impaired microcirculation and thus, possibly reduced tissue oxygenation. Increased metabolic activity, elevated plasma proteins and lipids, decreased fibrinolytic activity, and a hypercoagulative state of this patient group are likely contributing factors for the alternations in hemorheological parameters. Although our results did not show a significant difference after the treatment, apart from erythrocyte deformability, we believe there would be an improvement with the correction of thyroid function. Multiple measurements after attaining euthyroidism should be conducted in order to detect any improvements. Furthermore, considering comorbidities such as hyperlipidemia, congestive cardiac failure, diabetes mellitus, and metabolic syndrome which can cause further impairments in microcirculation, evaluation of hemorheological status in hyperthyroid patients is essential to ensure adequate tissue oxygenation.

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Authors' contribution
SEC: conceived and planned the experiments, carried out the experiment, interpreted the data, wrote the manuscript YK: interpreted the data, supervised the findings of this work, contributed to the writing of the manuscript AT: interpreted the data, supervised the findings of this work, contributed to the writing of the manuscript RD: interpreted the data, supervised the findings of this work ST: interpreted the data, supervised the findings of this work HK: carried out the experiment, analyzed the data ME: conceived and planned the experiments, supervised the findings of this work.

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Data availability
Data available on reasonable request from the authors.

Declarations

Ethics approval and consent to participate
Obtained from University of Health Sciences, Hamidiye Clinical Research Local Ethics Committee (date of approval: 13/02/2020 reference number of approval: 2020.02.13–09). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written consent was obtained from the patients and healthy volunteers of the control group before blood sampling.

Competing interests
The authors declare no competing interests.

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