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Original Research

Whole Blood Viscosity as a Marker of Thrombosis in Cushing's Disease: An Actor or Ineffective Factor

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ABSTRACT

Objective: There is a tendency for thromboembolic events (TE) in Cushing's disease (CD) because of the cortisol excess itself and associated risk factors for TE. Whole blood viscosity (WBV) as a measure of hemorheological features may impact the development of TE. However, limited data are available on the status of these changes in CD. Herein, we aimed to compare WBV between patients with CD and the control group and evaluate the impact of CD treatment on WBV.

Methods: A total of 34 patients with CD without prior TE history and 30 subjects as the control group were enrolled between 2015 and 2020. Demographic, clinical, and laboratory characteristics of the study groups were recorded. WBV was calculated using the de Simone formula.

Results: Among the corticotroph pituitary adenomas of the CD group, 32 of 34 were microadenomas, and 2 were macroadenomas. Postoperative remission was achieved in all patients. However, a recurrence was observed in 10 patients at 5.8±3.2 year follow-up. There was no difference between baseline WBV, at both low and high shear rates, between the CD and control groups ($p>0.05$). Furthermore, the WBVs at both low and high shear rates were also similar before and after treatment in the CD group (16.3±1.8 versus 15.4±1.7, $p=0.2$, for WBV at the high shear rate; 40.5±38.2 versus 25.4±35.2, $p=0.23$, at the low shear rate).

Conclusion: In this small-sized preliminary study, the WBV at both shear rates revealed no difference between the CD and control groups. There was also no impact of CD treatment on WBV at follow-up. However, further large-scale studies are necessary to confirm our study findings.

Keywords: Cushing's disease, hypercoagulability, shear stress, whole blood viscosity

INTRODUCTION

Thromboembolic events (TE) occur 4 times more frequently in Cushing's syndrome (CS) (1). CS is associated with the risk factors of thromboembolism, such as obesity, hypertension, and diabetes. Although increased coagulability and reduced fibrinolysis because of cortisol excess are believed to be the cause of prothrombotic tendency, other pathogenetic mechanisms have not been determined (2).

The thrombosis process is classically triggered by Virchow's 3 factors, including endothelial damage, impaired blood flow, and increased blood clotting tendency. A direct relationship is present among impaired blood flow, blood viscosity, and thrombosis susceptibility (3). High blood viscosity, the inherent resistance of blood in vessels to flow, is the only rheological factor linked with major cardiovascular risk factors (4). Endothelial damage and thrombosis also occur as a result of increased shear stress in hyperviscosity. Therefore, there is a close and dynamic association among these three mechanisms. Blood viscosity can be measured with various techniques. Whole blood viscosity (WBV) can be quickly and accurately calculated using the de Simone formula (5).

However, there is no study regarding the role of blood viscosity and rheology in patients with the CD. Hemorheological alterations should be considered, especially for venous TE, for better knowledge in such a patient group. Therefore, we aimed to evaluate (i) the baseline WBV at both low and high shear rates in the CD and control groups, and (ii) the WBV at both low and high shear rates before (hypercortisolism state) and after (normal cortisol state) CD treatment.

METHODS

Study Participants

Our study has a cross-sectional retrospective design. The study was conducted at Ankara University School of Medicine, Depart-

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ment of Endocrinology, Ankara, Turkey. A total of 34 patients diagnosed with CD and who had a remission after successful transsphenoidal surgery were enrolled between 2015 and 2020. Among the corticotroph pituitary adenomas of the CD group, 32 of 34 were microadenomas, and 2 were macroadenomas. The diagnosis of CD was based on clinical features, loss of diurnal rhythm of cortisol secretion, elevated urinary free cortisol levels, and absence of cortisol suppression after low-dose oral dexamethasone and high-dose oral dexamethasone suppression tests, imaging pituitary adenomas at magnetic resonance imaging, and inferior petrosal sinus sampling if necessary. Remission was determined as the regression of the clinical features and normalization of hypercortisolism in terms of Adrenocorticotropic hormone ACTH, cortisol, and urinary free cortisol levels (morning serum cortisol values 5 mg/dL and UFC 5-55 µg/day within 7 days of selective tumor resection) (6). A total of 30 healthy subjects were randomly selected from the outpatient clinic admissions for the control group.

Patients with hematological disease, including anemia, acute illness, and acute/chronic infectious or inflammatory disease, those who underwent invasive procedures/surgery within 6 months of recruitment, those who had active malignancy, previous venous/arterial TE, history of recurrent abortus, ongoing anticoagulant or antiplatelet therapy, diseases with hypoalbuminemia (chronic renal failure, hepatic insufficiency, and heart failure), lymphatic or venous system-mediated chronic stasis, and cigarette smoking were all excluded.

None of the patients received anticoagulant therapy during the preoperative or perioperative period. All of the coagulation parameters (prothrombin time, activated partial thromboplastin time (aPTT), and thrombin time) were in the normal reference ranges for all patients.

Laboratory Evaluation

Demographic, clinical, and laboratory data were recorded for each participant. Blood samples were obtained from each patient at 8.00 A.M. in an 8-h fasting state to do a complete blood count analysis and biochemistry panel, including serum cortisol and ACTH levels before surgery after surgery. Serum cortisol concentrations were ascertained by immunoenzymatic assays Access cortisol assays, Beckman Coulter, Brea, CA, USA). The intra-assay coefficients of variation (CVs) were less than 5%. Serum ACTH concentration was measured by the electrochemiluminescence immunoassay (Roche Elecsys 2010 analyzer, Roche Elecsys-ACTH, Roche Diagnostics, Indianapolis, IN, USA), and the intra-assay CV was less than 6%. We ascertained the urinary free cortisol by radioimmunoassay (DIAsource Immunoassays S.A., Louvain-la-Neuve, Belgium), and the intra-assay CV was less than 7%. Normal values used in our laboratory were as follows: early morning cortisol: 5–19.4 mg/dL; ACTH: 25–60 pg/mL; and UFC: 5-55 µg/day.

Main Points:

- Although the linkage between hypercoagulability and Cushing's syndrome is very well determined, the missing mechanism of thrombosis as endothelial dysfunction, stasis, and rheological properties of blood coagulation have not been studied yet.
- This is the first study evaluating the hemorheological features of patients with Cushing's disease as a possible cause of thrombosis.
- We did not find an association between whole blood viscosity and hypercortisolemia parameters.

The estimation of WBV (centipoise, cP) was calculated in both high shear rate (HSR = 208 s^{-1}) and low shear rate (LSR= 0.5 s^{-1}) with previously validated formulas. Hematocrit was calculated by an automated analyzer by multiplying the red cell count by the mean cell volume and given in percent (Coulter counter; Beckman Coulter Diagnostics, Brea, CA, USA). Total protein was measured using a Beckman Coulter AU 5800 analyzer with its commercial kits and given in g/L. For HSR (208 s^{-1}), (0.12×HcT) + 0.17 (TP-2.07) and for LSR (0.5 s^{-1}), (1.89×HcT) + 3.76 (TP-78.42) were the formulas used to calculate WBV (7).

The Institutional Ethics Committee approved the study protocol (12/2020, R451), and the study protocol complied with the principles outlined in the Declaration of Helsinki.

Statistical Analysis

The Shapiro–Wilk test was used for the assessment of normality. Numerical variables with normal distribution are represented as mean±standard deviation, and numerical variables with a skewed distribution are represented as median (minimum and maximum). Categorical variables are presented as numbers and percentages. The paired student t-test was used to compare the measurements at two-time points (at the time of diagnosis and after remission) for ACTH, cortisol, WBV-HSR, and WBV-LSR. Since the 24-h urinary cortisol levels were not normally distributed, nonparametric tests were conducted to compare these parameters. The Wilcoxon test was used to compare the change in 24-h urinary cortisol levels between baseline and after treatment. According to the parameter distribution, normal or nonnormal, the correlation coefficients and their significance were calculated using the Spearman or Pearson tests, respectively. All statistical analyses were performed using SPSS statistical software (IBM Corp., released in 2015, IBM SPSS Statistics for Windows, Version 23.0, IBM Corp., Armonk, NY, USA). A two-tailed p-value <0.05 was determined as statistically significant.

RESULTS

Study Population and Laboratory Features

The median age of the CD group was 48±12 years, and the mean age at CD diagnosis was 42±13.5 years. In the CD group, 79.4% (27 of 34) of patients were female. Table 1 shows the demographic, clinical, and laboratory features of study groups both at baseline and after the CD group treatment**.** Postoperative remission was achieved in all patients. However, a recurrence was observed in 10 patients at 5.8±3.2 years follow-up. Pasireotide treatment was initiated and continued in a 39-year-old female patient after a recurrence in the second year of the reoperation. In addition, 5 patients received steroid replacement therapy. The mean gluco-

Table 1. The demographic, clinical, and laboratory features of Cushings' disease and control group

	Before treatment (a)	After treatment (b)	Control group (c)		
	(n:34)		(n:30)	p A–B	p A–C
Age (years)	48 ± 12		45.5±14.7	–	0.75
Age at Diagnosis (years)	42 ± 13,5				
ACTH levels (25–60 pg/mL)	85,9 ±59,6	54,7 ± 42,8		<0.01*	
Serum cortisol levels (3.7 – 19.4 mg/dl)	26,1 ± 13,9	10 (0,19–20)		<0.01*	
24–hour urinary cortisol (5–55 µg/day)	494 (349–4000)	50 (9–1200)		<0.01*	
Hematocrit (%)	40.8±5.9	38.9±5	40±4.2	0.19	0.22
Total protein (mg/dl)	6.8±0.6	6.6±0.5	7.3±4.6	0.4	0.62
WBV at HSR (208/s)	16.3 ± 1.8	15.4 ± 1.7	16.2±1.9	0.2	0.33
WBV at LSR (0.5/s)	40.5 ± 38.2	25.4 ± 35.2	39.2±30.7	0.23	0.54

ACTH: Adrenocorticotroph Hormone WBV: Whole Blood Viscosity HSR: High Shear Rate LSR: Low Shear Rate

corticoid replacement dosage after surgery in these 5 patients was 5 (2.5–5) mg.

WBV at Baseline and After Surgery

At baseline, there was no difference in WBV-HSR and WBV-LSR between CD and control groups (p=0.33 and p=0.54, respectively). Furthermore, WBV-HSR and WBV-LSR were similar before and after treatment (16.3±1.8 versus 15.4±1.7, p=0.2, for WBV-HSR; 40.5±38.2 versus 25.4±35.2, p=0.23, for WBV-LSR) (Table 1).

Correlation analysis revealed that preoperative cortisol levels were significantly correlated with pretreatment levels (r=0.5, p=0.007), pretreatment 2-mg dexamethasone test (r=0.45, p=0.03), pretreatment 24-h urinary cortisol levels (r=0.73, p<0.001), and posttreatment ACTH levels (r=0.37, p=0.04). Moreover, posttreatment cortisol levels were significantly correlated with the pretreatment 2-mg dexamethasone test (r=0.47, p=0.02), pretreatment 24-h urinary cortisol levels (r=0.41, p=0.04), posttreatment ACTH levels (r=0.71, p<0.001), and posttreatment 24-h urinary cortisol levels (r=0.75, p<0.01). However, neither WBV-LSR nor WBV-HSR results were correlated with cortisol levels.

DISCUSSION

Our small-sized preliminary study findings showed that WBV, at both low and high shear rates, in the CD group was similar to that in the control group. In addition, no significant change was observed in WBV at both low and high shear rates before and after CD treatment. To our knowledge, this study was the first to investigate the role of WBV in CD patients.

CS is associated with a hypercoagulable state and increased risk of venous thromboembolism. TE contribute to high mortality rates in CS with pulmonary embolism, ischemic cardiac disease, and stroke (8). The studies show a shortening of the aPTT and elevated levels of factor VIII, factor IX, and von Willebrand factor in CS (9-12). Moreover, an excess of the fast-activating plasminogen activator inhibitor 1 (PAI-1 or SERPINE1) was shown to lead to the impairment in the fibrinolytic system (2). It was hypothesized that the TE might occur because of cortisol-induced upregulation of mRNA transcription of coagulation parameters and the increased activity of fibrinolysis indexes (13).

This study was conducted to investigate the missing piece of the puzzle for TE. As early as the 1920s, it was suggested that localized or general intravasal aggregation of red cells might contribute to thrombus formation (14). Thrombosis is associated with high blood viscosity owing to increased red cell aggregation (15). Blood is a thixotropic and multiphase fluid, and its viscosity is influenced by the flocculation–deflocculation equilibrium of red cells, the interaction between velocity gradient and red cells, and plasma (14).

Blood viscosity is briefly defined as the blood's internal resistance, contributing to endothelial shear stress (16). Both increased shear stress and viscosity induce endothelial damage and inflammation, and tend to thrombosis (3). WBV was investigated for both venous and arterial thrombosis because blood viscosity was suggested as a useful surrogate measure of perfusion for routine use in the general population. It was higher in patients with deep venous thrombosis, obstructive sleep apnea syndrome, retinal vein thrombosis, coronary slow flow phenomenon, systemic lupus erythematosus, aortic sclerosis, and obese patients (3, 4, 17-20). The Edinburgh Artery Study, the most comprehensive prospective study of WBV, showed a cognitive decline and an increased risk of stroke (21). WBV was also associated with insulin resistance, and these data suggest that elevated WBV is associated with insulin resistance (22).

Although considering that insulin resistance is a very well-known condition in CD and WBV might also be increased, we could not find such an increment in WBV in patients with hypercortisolism compared with the healthy control group.

Supraphysiological doses of glucocorticoids lead to accelerated skeletal muscle protein breakdown, stimulation of proteolysis, and protein synthesis inhibition (23). Several studies have revealed lower serum albumin and total protein levels in patients with CS (24, 25). Moreover, cortisol was believed to enhance the formation and increase the proliferation of erythroid cells (26) and to be associated with slightly increased hemoglobin and hematocrit levels (27). As a result of the prediction equation that has been validated for WBV, increased hematocrit and decreased levels of protein could have resulted in similarities between CD before treatment and the control group for WBV. However, the total protein and hematocrit levels were similar between groups in our study.

Our study should be interpreted with some limitations. First, we did not directly measure WBV with a viscometer. We had no data about other hemorheological parameters such as erythrocyte deformability and aggregation, and plasma viscosity. Apart from these, the lack of comparison with a group with CD with thrombosis because of this condition's rarity was another limitation of the study. The optimal cutoff level of WBV at LSR and HSR levels in predicting thrombosis could not be studied because of the limitation, as mentioned above.

CONCLUSION

Our study results revealed that the WBV, at both low and high shear rates, was similar between CD and control groups and in patients with CD either in hypercortisolemic or eucortisolemic state. We propose that more accurate results can be obtained with large-scale prospective studies with viscometer-based analysis.

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