ORIGINAL RESEARCH

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Assessment of Soluble Endothelial Protein C Receptor and Thrombomodulin Levels in Newly Diagnosed Myeloproliferative Neoplasms

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ABSTRACT Objective: Thrombotic complications are considered as significant causes of morbidity and mortality in patients with myeloproliferative neoplasms (MPN). The aim of this prospective study was to assess the soluble EPCR (sEPCR) and thrombomodulin (TM) levels of MPN patients at diagnosis and to evaluate their association with disease characteristics. **Material and Methods:** A total of 41 patients [male/female: 22/19; age: 58(31-87) years] who were diagnosed as MPN and 20 healthy volunteers were included in this study. Serum sEPCR, TM, protein C, protein S, D-dimer and fibrinogen levels were measured at the time of diagnosis. **Results:** Serum sEPCR and TM levels were evaluated at diagnosis in a total of 41 MPN patients and 20 healthy controls. Median sEPCR and TM levels were not different between males and females. Median sEPCR and TM levels were found to be similar in the patient and control groups. JAK2V617F positivity did not represent a significant association with sEPCR and TM levels. Protein C, protein S, D-dimer ve fibrinogen levels were not statistically different between the patient and control groups. **Conclusion:** The small sample size may be an explanation for the similar levels of sEPCR and TM in MPN patients and control group. Despite the efforts in order to understand the underlying mechanism of the hypercoagulable state in MPN, current data is inadequate to highlight the hidden steps of this complicated network. Thus, further studies are warranted to overcome this obstacle.

Keywords: Myeloproliferative disorders; activated protein c receptor; thrombomodulin; thrombosis; blood coagulation

Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are chronic myeloproliferative neoplasms (MPN) characterized by a clonal expansion of abnormal hematopoietic stem cells. The clinical course is marked by mainly thrombohemorrhagic complications including a potential to transform into myelofibrosis and acute leukemia.[1-4]

Thrombotic events, which can be the first manifestation of the disease, may occur at venous, arterial or microcirculatory sites. The probability of major thrombosis varies between 7,6-29,4% and 11,2-38,6% for newly diagnosed ET and PV, respectively.[3,5-7] Although thrombohemorrhagic complications are considered as the most common causes of morbidity and mortality in MPNs, the etiology and pathogenesis of the clotting abnormalities still remain unclear.[3,5-7] Clinical factors including age, previous history of thrombosis, obesity, hypertension and hyperlipidemia as well as elevated blood cell counts (leukocytosis, erythrocytosis and thrombocyto-

sis) may contribute to an increased thrombosis risk. Secretion of procoagulant, proteolytic and inflammatory cytokines, leukocyte derived proteases, hyperviskosity and expression of adhesion molecules may generate a prothrombotic state. Furthermore, patients may present with a hypercoagulable state characterized by an increased concentration of several plasma markers of hemostatic system activation. Several studies have indicated the impact of genetic thrombophilic factors such as Factor V Leiden or prothrombotic mutations and JAK2V617F mutational status and allele burden on thrombotic risk in MPN patients.[1-4,6-11]

The protein C (PC) pathway plays a pivotal role in the regulation of the coagulation cascade through inactivation of factor Va and factor VIIIa, which is mediated by an intereaction between activated PC (APC) and endothelial PC receptor (EPCR). Anticoagulant effect of PC becomes more evident when thrombin/thrombomodulin (TM) complex is stimulated during this process.[12-15]

The aim of this prospective study was to assess the soluble EPCR (sEPCR) and TM levels of MPN patients at diagnosis, based on a comparison with healthy controls and to evaluate their association with disease characteristics.

MATERIAL AND METHODS

A total of 41 patients [male/female: 22/19; age: 58(31-87) years] who were diagnosed as MPN [PV: 9 (22%); ET: 28 (68,3%); PMF: 3 (7,3%); unclassified: 1 (2,4%)] and 20 healthy volunteers were included in this prospective study (Table 1). Diagnosis of MPN was based on World Health Organization 2016 Diagnostic Criteria.[16] Serum sEPCR, TM, PC, protein S (PS), D-dimer and fibrinogen levels were measured at the time of diagnosis. Serum sEPCR and TM levels were measured by Enzyme-Linked Immuno Sorbent Assay (ELISA) using CUSABIO Human Soluble Endothelial Protein C Receptor and Human Thrombomodulin ELISA Kit.

STATISTICAL ANALYSIS

Descriptive statistical analysis was used for patient characteristics. Continuous variables in two groups were compared by Mann–Whitney U test and categorical variables with Chi–squared test. Spearman test was used for correlation analysis. A threshold value of $p<0,05$ was considered as statistically significant. The calculations were performed with SPSS 16.0 (SPSS Inc, Chicago, Illinois).

The study was approved by the Ethical Committee of Gazi Medical School.

RESULTS

Median sEPCR and TM levels were not different between males and females in the patient group ($p>0.05$, $p>0.05$). Median sEPCR level was similar between the patient [43,1(1,9-171,8) ng/ml] and control groups [44,4(14,9-157,1) ng/ml] ($p>0,05$). Furthermore, sEPCR levels were not found to be different among disease subgroups [PV: 42,7(6,2-110,1) ng/ml; ET: 46,7(1,9-171,8) ng/ml; PMF 10,9(2,4-113,9) ng/ml] ($p>0,05$).

Similarly, median TM levels were not different either between the patient [41,2 (3,3-140,3) ng/ml] and control groups [41,9 (19,1-145,4) ng/ml] ($p>0,05$) or among disease subgroups [PV: 42,7(6,9-119,1) ng/ml; ET: 43,9(5,7-140,3) ng/ml; PMF: 26,3(3,3-41,2) ng/ml] ($p>0,05$).

JAK2V617F was demonstrated in a total of 26 (63,4%) patients [PV: 9 (100%); ET: 14(50%); PMF: 3(100%)]. Median levels of sEPCR and TM were not different between JAK2V617F positive and negative patients ($p>0.05$, $p>0.05$). Protein C, PS, D-dimer ve fibrinogen levels were similar between the patient and control groups ($p>0,05$). There was a negative correlation between sEPCR and fibrinogen levels ($p=0,035$; $r=-0.669$). A positive correlation was indicated between JAK2V617F mutation and leukocyte count ($p=0,002$; $r=0,602$) and uric acid level ($p=0,003$; $r=0.611$). Similarly, there was a positive correlation between lactate dehydrogenase and leukocyte count ($p=0,002$; $r=0.424$) and uric acid level ($p=0,028$; $r=0.311$).

DISCUSSION

In this prospective study, serum sEPCR and TM levels were evaluated at diagnosis in a total of F41 MPN patients and 20 healthy controls. Median

TABLE 1: Patient characteristics.

	Patient (n=41)	Control (n=20)	p value
sEPCR (ng/mL) [Median (range)]	43,1 (1,9-171,8)	44,4 (14,9-157,1)	>0,05
PV (n=9)	42,7 (6,2-110,1)		
ET (n=28)	46,7 (1,9-171,8)		
PMF (n=3)	10,9(2,4-113,9)		
TM (ng/mL) [Median (range)]	41,2 (3,3-140,3)	41,2 (19,1-145,4)	>0,05
PV (n=9)	42,7 (6,9-119,1)		
ET (n=28)	43,9 (5,7-140,3)		
PMF (n=3)	26,3(3,3-41,2)		
Hb (g/dL) [Median (range)]	14,7 (7,7-18,9)		
Hct (%) [Median (range)]	44,9 (22,9-59,5)		
WBC (µL) [Median (range)]	9120 (5860-24710)		
Platelet (µL) [Median (range)]	584000 (102800-1369000)		
LDH (U/L) [Median (range)]	261,5 (165-931)		
Uric Acid (mg/dL) [Median (range)]	5,1 (2,6-11,9)		
ALT (U/L) [Median (range)]	17 (4-98)		
Creatinine (mg/dL) [Median (range)]	0,8 (0,5-1,2)		
Protein C (%) [Median (range)]	91(42-129)		
Protein S (%) [Median (range)]	71,5(37-130)		
D-Dimer (ng/mL) [Median (range)]	136(1-824)		
Fibrinogen (mg/dL) [Median (range)]	289,5(212-392)		
Bone Marrow Fibrosis (Grade) [Median (range)]	2(1-3)		
JAK2V617F Mutation (%) [Median (range)]	0,2(0,0014-0,89)		

Abbreviations: ALT: Alanine Transaminase; ET: Essential Thrombocythemia; Hb: Hemoglobin; Hct: Hematocrit; LDH: Lactate Dehydrogenase; PMF: Primary Myelofibrosis; PV: Polycythemia Vera; sEPCR: Soluble Endothelial Protein C Receptor; TM: Thrombomodulin; WBC: White Blood Cell

sEPCR and TM levels were found to be similar in the patient and control groups. JAK2V617F positivity did not represent a significant association with sEPCR and TM levels. Protein C, PS, D-dimer ve fibrinogen levels were not statistically different between the patient and control groups.

Thrombotic complications are considered as significant causes of morbidity and mortality in patients with MPN. Several studies have investigated the underlying mechanism of the tendency to thrombosis in this particular group of patients.[17-20] A potential hypercoagulable state is indicated based on a series of modifications in different steps of the hemostatic network including endothelial cells, certain cytokines, adhesion molecules and coagulation factors. Clonal proliferation of hematopoietic progenitor cells as well as abnormal characteristics of endothelial cells cause an increased production of procoagulant microparticles which may lead to an "APC-resistant" phenotype in MPN patients similar to clotting system activation.[2] Duchemin et al showed that circulating microparticles may represent an acquired "TM-resistance" condition in MPN patients which may be responsible for the hypercoagulable state although a predictive value of TM-resistance for thrombosis was not declared.[3]

In another study by Tripodi et al, endogenous thrombin potential ratios, which were taken as indexes for procoagulant imbalance and reflect a resistance to TM, were higher in MPN patients and directly correlated with platelet counts and inversely with the plasma free PC, PS and antithrombin levels. Additionally, clot formation time on thromboelastometry was shorter and maximal clot firmness was greater in the patient group.[9]

In a study by Arellano-Rodrigo et al, ET patients with thrombosis were indicated to have significantly higher levels of factor V, factor VIII, von Willebrand factor (vWF), sP-selectin and sCD40L when compared to patients without thrombosis. Furthermore, patients with JAK2V617F mutation had significantly lower levels of free PS and higher levels of tissue factor, sP-selectin, SCD40L, vWF and sTM.[21]

In a prospective study of 37 patients with ET and 34 with PV, significant elevations in leukocyte activation markers including CD11b, leukocyte alkaline phosphatese, elastase and myeloperoxidase and endothelial cell markers such as vWF and TM were reported. Polycythemia vera patients had significantly higher levels of prothrombin fragment F1+2, thrombin-antithrombin complexes and D-dimer.[22]

In the present study, median sEPCR and TM levels were not statistically different between the patient and control groups. There are several studies which investigated the potential role of sEPCR and TM levels in MPN. In a study by Ducros et al, 148 patients with hematological malignancies and 101 healthy volunteers were analyzed for sEPCR and D-dimer levels. As a substantial proportion of these patients (67%) had plasma sEPCR levels higher than controls, authors claimed that plasma sEPCR level may be a feasible marker which provides an illuminant insight into thrombotic risk assessment in patients wih hematological malignan cies.[5] Some reports have emphasized a reasonable relationship between sEPCR and TM levels and thrombotic complications. A total of 56 patients with central retinal vein occlusion (CRVO), 26 patients with branch RVO and 78 healthy subjects were enrolled in a study by Gumus et al. Elevated levels of sEPCR were considered to be a risk factor for CRVO in this study.[23] Similarly, Javanmard et al found that low plasma sTM concentration was associated with an increased risk of cerebral venous sinus thrombosis, despite a lack of association with plasma sEPCR levels.[24]

Atalay et al measured sEPCR levels in 25 MPN patients and 29 controls. In contrast with our results, sEPCR levels were found to be significantly higher in ET and PV patients when compared to the control group. Levels of coagulation activation factors including thrombin-antithrombin complex, D-dimer and prothrombin fragments 1+2 were also higher in ET and PV patients. Thus, authors suggested that elevated sEPCR levels may predict a high possibility of a prothrombotic state. However, sEPCR levels were not statistically different between the patients with and without thrombosis.[25]

CONCLUSION

In the presented study, the small sample size may be an explanation for the statistically insignificant similarity of sEPCR and TM levels in MPN patients and controls which is generally in contrast with previous reports. The study might be more informative if prospective follow-up of thrombosis was available. Despite the efforts in order to understand the underlying mechanism of the hypercoagulable state in MPN, current data is inadequate to highlight the hidden steps of this complicated network. Thus, further studies are warranted to overcome this obstacle.

Conflict of Interest

Authors declared no conflict of interest or financial support.

Authorship Contributions

Consultancy, data collection, analysis and evaluation, reference screening, article writing, responsibility for study materials: *Semiha Çalkaya;* ***Creation of hypothesis, consultancy, study design, analysis and evaluation, reference screening, article writing, critical examination:*** *Zeynep Arzu Yegin;* ***Data collection and processing, analysis and evaluation, article writing, source/fund supply, responsibility for study materials:*** *Özlem Gülbahar;* ***Data collection and processing, responsibility for study materials:*** *Mehmet Çingirt;* ***Data Collection:*** *Zübeyde Nur Özkurt;* ***Data Collection:*** *Kadir Acar;* ***Creation of Hypothesis, Source/Fund Supply:*** *Gülsan Türköz Sucak.*

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