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THE ASSOCIATION OF HIGH MOBILITY GROUP BOX-1 PROTEIN WITH DEEP VEIN THROMBOSIS

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ABSTRACT

Aim: We aimed to investigate relation between high mobility group box-1 protein (HMGB1) levels in peripheral blood and deep vein thrombosis (DVT). **Materials and Methods:** For this study, HMGB1 levels were compared between lower extremity acute-subacute DVT patients and healthy volunteer subjects. Fifty-three patients (18 to 65 years of age) presenting to the cardiovascular surgery clinic of Private Gaziantep Medikal Park Hospital between April 2018 and April 2019 and 47 controls were included in the study. Biochemical analyses were conducted by the hospital laboratory using the ELISA method. The SPSS for Windows, version 25.0 software was used for statistical analyses of the study data. **Results:** Increased HMGB1 protein levels were found both in the entire patient group and in patients with proximal and distal DVT versus healthy controls. In the patient group, HMGB1 levels did not significantly differ in relation to age, gender and comorbidities. **Conclusion**: Increased HMGB1 protein levels as detected in patients with acute/subacute deep vein thrombosis compared to the normal population may be due to inflammation caused by the disease itself as well as the effects of HMGB1 on platelet functions to promote thrombus formation.

KEYWORDS: HMGB-1; Deep Venous thrombosis; Venous thromboembolism.

INTRODUCTION

Venous thromboembolism or its most common form, deep vein thrombosis, is a significant cause of morbidity and mortality. Its incidence is roughly 1/1000 in developed countries and 1/100 in the elderly population.[1] Venous thromboembolism originates from the veins in the leg and pelvis in 85% and upper extremity veins in 5-6% of the cases. Since deep vein thrombosis (DVT) occurs in up to 70% of cases with pulmonary thromboembolism (PTE) and more than half of DVT patients develop PTE, these two can be regarded as a single clinicopathologic entity.[2]

The High Mobility Group Box (HMGB) proteins are non-histone nuclear proteins with various functions in the cell.[3] HMGB1 is a 30 kDA protein which is expressed by eukaryotic cells and binds to chromatin.[4] HMGB1 is a 215 amino acid protein that is composed of three domains: two positively charged deoxyribonucleic acid (DNA)-binding domains (box 1 and box 2) and a negatively charged C terminal.[5] HMGB1, HMGB2 and HMGB3 belong to the HMGB protein family. While the expression of HMGB3 and HMGB2 is limited to some cells and occurs only during early stages of life, HMGB1 is widely expressed in almost all cells and continues to be ubiquitously expressed in adulthood.[6] HMGB1 acts as a chromatin-binding factor. HMGB-1 modifies the interaction of a number of transcription factors including p53 and steroidal hormone receptors with DNA by binding non-specifically to the small groove of DNA. It has a key role in DNA repair, transcription, cell differentiation, extracellular signaling and somatic recombination.[7-8] Aside from its nuclear functions, it serves as an extracellular signaling molecule and is passively released by necrotic cells and actively secreted by inflammatory cells.[9] In the extracellular environment, HMGB1 interacts with several receptors such as RAGE (receptor for advanced glycation end product), TLR (Toll-like receptors)-2, TLR-4 and TLR-9. RAGE is the receptor that mediates intracellular actions of extracellular HMGB1.[10]

The aim of the present study was to investigate the relation between peripheral blood HMGB1 levels and venous thrombosis in patients diagnosed with acute or subacute DVT and to provide new insights that could help elucidate the role of HMGB1 protein in the development of DVT.

MATERIALS AND METHODS

Fifty-three patients (18 to 65 years of age) presenting to the cardiovascular surgery clinic of Private Gaziantep Medikal Park Hospital between April 2018 and April 2019 who were newly diagnosed with acute/subacute (<4 weeks) lower extremity deep vein thrombosis and 47 age- matched healthy controls were included in the study. Pregnancy, patients with autoimmune and connective tissue diseases, patients with a history of severe psychosis, patients receiving cancer therapy and women taking oral contraceptives were excluded from the study. The patients were diagnosed using venous Doppler ultrasound by radiologists working at the same hospital, along with physical examination findings. Healthy controls were included in the study folowing brief anamnesis and physical examination. 5 cc venous blood samples were collected from the participants into biochemistry tubes under sterile conditions and sera were separated by centrifugation. Sera were collected into microtubes and serum samples were stored at -80ºC until the time of ELISA testing. Approval for the study was obtained from the Ethics Committee for Clinical Trials of SANKO University (02/2019). All participants were informed about the nature and scope of the study and signed written informed consent forms before enrollment. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki

HMGB1 Measurement

HMGB1 levels were measured by the Biochemistry Laboratory of Medical Park Hospital using the ELISA device available. A commercially available kit (Rel Assay Diagnostics® Mega Tıp Ltd, Turkey) was used for HMGB1 quantification. The double- antibody sandwich enzyme immunoassay technique was used for the analysis. All concentration/absorbance curves and data calculations for HMGB1 testing were obtained using the integrated software of the BioTek ELx808 (Winooski, Vermont, USA) absorbance reader. Regression equation was calculated based on concentration and optical density (OD) values.

Statistical analysis

The SPSS for Windows 25.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Whether the variables followed a normal distribution was checked using both visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). A p value greater than 0.05 at Kolmogorov-Smirnov test indicated a normal data distribution. For data with a normal distribution, the Student's t-test was used to assess the differences between patient and control groups; otherwise, Mann-Whitney U test was employed. For categorical variables, the chi-square test was used to compare the two groups. The statistical power of the study was 0.9996184 calculated using the Power (1-β err prob) formula.

RESULTS

The study sample consisted of a total of 100 participants including 53 patients and 47 controls. Compared to the patient group, control group had a greater mean age (51.75±15.01 versus 56.53± 9.95 years, respectively) but the difference was statistically non-significant (p=0.061). Also, gender distribution was not significantly different between the study groups. However, significantly increased HMGB1 levels were found in the study group than in control group (p<0.001) (Table 1). No correlation was identified between patient age and serum HMGB1 levels (r=-0.038, p=0.787). There were no significant differences between males and females in terms of distal and proximal DVT and comorbidities in the patient group (Table 2). HMGB1 levels did not significantly differ between the study groups in relation to clinical features (Table 3).

Table 1: Demographic and clinical data of the study groups.

	Controls (n=47)	Deep Vein Thrombosis (n=53)	p
Age (years) mean± standard deviation	56.53± 9.95	51.75± 15,01	0.061*
Gender	Male: 31 (34.0%) Female: 16 (66.0%)	Male: 30 (56.6,6%) Female: 23 (43.4%)	0.339**
HMGB1 (pg/mL) Median (min-max)	12.60 (3.27 26.75)	35.98 (2.18- 172.76)	p<0.001***

* Student's t-test. ** Chi-square test, ***Mann-Whitney U test.

Table 2: Comparison of clinical features in the patient group by gender.

	Male (n=30)	Female (n=23)	P
Proximal DVT	29 (58.0%)	21 (42.0%)	0.402
Distal DVT	8 (61.5%)	5 (38.5%)	0.679
CAD	3 (75.0%)	1 (25.0%)	0.440
Immobility	7 (77.8%)	2 (22.2%)	0.160
Previous surgery	8 (72.7%)	3 (27.3%)	0.225
Hypertension	2 (50.0%)	2 (50.0%)	0.782
Diabetes mellitus	8 (57.7%)	2 (9.1%)	0.112

n: Number of subjects, DVT: Deep Vein Thrombosis; CAD: Coronary Artery Disease

Table 3: Comparison of serum HMGB1 levels between study groups in relation to clinical features.

	HMGB1		
	Yes n (%)	**No n (%)**	**p**
Comorbidity	8 (15.1%)	45 (84.9%)	0.823
History of DVT	3 (5.7%)	50 (94.3%)	0.098
DM	10 (18.9%)	43 (81.1%)	0.892
Hypertension	4 (7.5%)	49 (92.5%)	0.114
Hyperlipidemia	2 (3.8%)	51 (96.2%)	0.191
Hypertension	2 (50.0%)	2 (50.0%)	0.782

Among 53 patients, 40 had only proximal DVT (HMGB1 (in pg/mL): median (min-max) 35.32 (2.18-172.76), 10 had both proximal and distal DVT (HMGB1: median (min-max) 30.78 (14.03-52.79) and 3 patients had only distal DVT (HMGB1: median (min-max) 42.15 (37.87-90.10).

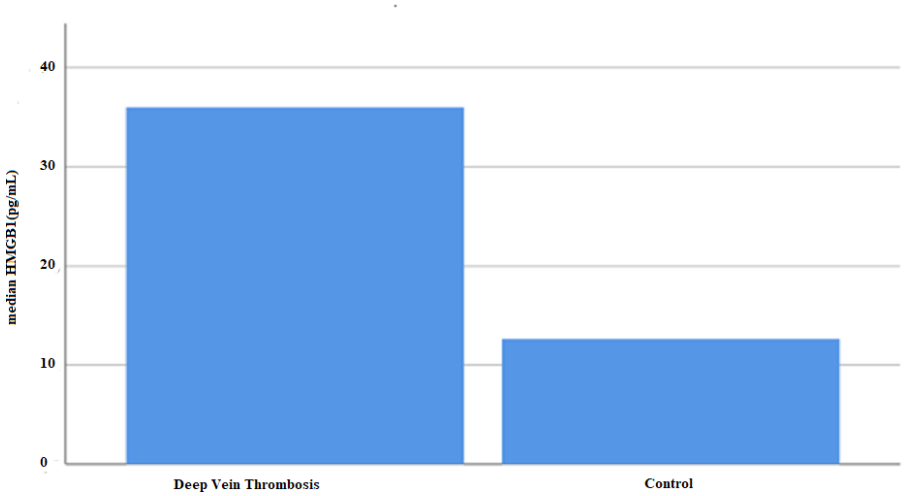

Figure 1: Comparison of serum HMGB1 levels between patients with deep vein thrombosis and healthy controls.

DISCUSSION

Deep vein thrombosis (DVT) is among the most prevalent cardiovascular disorders but its pathophysiology has not been fully understood. Recently, sterile inflammation was shown to promote coagulation during DVT but underlying molecular mechanisms are not clear. HMGB1 has been found to have a critical role in atherosclerosis, myocardial ischemia-reperfusion injury, heart failure, acute coronary syndrome, vasculitis, pulmonary vascular disease, cerebral artery disease and peripheral artery disease.[11-12] A limited number of studies exist in the literature on the association of venous thrombosis with HMGB1. In an experimental study from 2016, Gu et al. demonstrated upregulation of HMGB1 expression in the brain tissues of rats using an animal model of cerebral venous sinus thrombosis. The same authors also reported protective effects of human soluble thrombomodulin against cerebral venous sinus thrombosis through inhibition of HMGB1.[13]

In another study using a mouse model of venous thrombosis induced by flow reduction in the vena cava inferior, Stark et al.[14] identified a marked increase in HMGB1 levels in developing inflammation. Moreover, they showed that HMGB1 has a proinflammatory role and HMGB1 released from platelets induces other inflammatory markers and as a result, they suggested that HMGB1 could be a new target for anti-inflammatory strategies in DVT prophylaxis.[14]

In one study, HMGB1 was shown to promote degeneration and aneurysm of endogenous aortic vessel via TL4 receptor in vascular smooth muscle cells by enhancing release of several inflammatory cytokines (interleukin-6, monocyte chemoattractant factor, matrix metalloproteinases).[15]

The present study could not identify whether increased levels of HMGB1 protein as measured in the patient group are a cause or a consequence of DVT and this is the major limitation of the study. Eventually, DVT is a condition where oxidative damage and inflammation play a central role.[16] However, it may be wrong to interpret higher HMGB1 levels as a direct consequence because as discussed above, the protein itself has an adverse effect on platelet aggregation (13- 14). We believe that there may be several initiators or triggers of the disease but HMGB1 itself contributes to the development of DVT by inducing thrombus formation via its effects on platelets. HMGB1 levels measured by ELISA method can guide clinical diagnosis and the use of molecules that inhibit HMGB1 may be effective in preventing the development of the disease. Further studies are warranted to clearly

establish the link between HMGB1 and DVT.

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