CLINICAL SIGNIFICANCE OF HEMOSTASIS SCREENING TESTS IN MULTIPLE MYELOMA

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ABSTRACT

Introduction*: In multiple myeloma cases, the tendency to both bleeding and thrombosis increases as a result of disruption of the hemostatic balance. We aimed to investigate the hemostasis parameters and their clinical importance, the relationship of hemostasis parameters with other prognostic factors in patients with multiple myeloma.*

Materials and methods*: In our study, the files of 150 MM patients were retrospectively analyzed. Age, gender, hemogram and biochemistry parameters, hemostasis parameters, beta 2 microglobulin, immunoglobulin levels, disease type and stage were recorded. Thirty healthy cases were examined as the control group. Statistical evaluations were made by the SPSS 20 program.*

Results*: The platelet value in female cases was significantly lower than in male cases (p=0.005). A significant association was found between the stage of the disease and the platelet value (p=0.02). A positive correlation was determined between prothrombin time and lactate dehydrogenase value (p=0.01, r=0.196). There was a negative correlation between activated partial thromboplastin time and albumin, thrombocyte levels, and a positive correlation with D Dimer (p=0.02 r=-0.34, p=0.01 r=-0.37, p=0.01 r=0.38, respectively). In the control group, prothrombin time, activated partial thromboplastin time, international normalized ratio, D Dimer values were significantly lower (p=0.002, p=0.48, p=0.007, p<0.001), and fibrinogen and thrombocyte values were significantly higher (p=0.003, p<0.001) compared to the multiple myeloma group. No correlation was found between hemostasis parameters and bleeding, thrombotic events.*

Conclusion*: Hemostasis tests of patients with multiple myeloma are defective. Negative effects of female gender, advanced disease, elevated lactate dehydrogenase, and low albumin values on hemostasis parameters were determined.*

Keywords: *bleeding, thrombosis, multiple myeloma, prothrombin time, activated partial thromboplastin time.*

DOI: 10.19193/0393-6384_2022_1_63

Received *March 15, 2021;* ***Accepted*** *October 20, 2021*

Introduction

Multiple myeloma (MM) is a malignancy that constitutes approximately 10% of hematological malignancies and is characterized by an uncontrolled increase of clonal plasma cells. Bone lesions, renal failure, tendency to infections, clinical and laboratory findings of anemia and hypercalcemia are common in MM cases. In addition, the tendency to both bleeding and thrombotic events increase due to the deterioration of the hemostatic balance in these cases. Thrombocytopenia due to infiltration of the bone marrow with plasma cells or triggered by cytokines thrombocyte dysfunction, heparin-like anticoagulant, monoclonal thrombin inhibitor, amyloidosis, and acquired vwf deficiency are among the causes of bleeding[1,2]. The increased monoclonal protein binds to clotting factors. Therefore, coagulation factors and VWF deficiency occur[3,4]. On the other hand, monoclonal antibodies bind to alpha 2 antiplasmin, causing its inhibition and increased fibrinolysis. Coppola et al. reported that clinically bleeding is less common in MM cases and bleeding is weakly associated with disturbances in hemostasis tests[5].

The risk of deep vein thrombosis (DVT) is 7 times higher in patients with malignancy. The risk of venous thrombosis increases most in hematological cancers after lung and gastrointestinal system cancers. In hematological malignancies, the risk of DVT is increased 28 times[6]. Acute promyelocytic leukemia is the hematological cancer that increases the risk of DVT the most. MM is the second hematological malignancy that increases the risk of DVT[7]. Malignancy-related thrombosis is more common, especially in the first 3 months after diagnosis in MM[8]. The frequency of DVT in MM cases has been reported as 3%-10%[9,10]. Increase in monoclonal immunoglobulin (Ig) increases the tendency to thrombosis by causing hyperviscosity. Increased lupus anticoagulant and antiprothrombin levels contribute to the tendency to endothelial damage and thrombosis. Proinflammatory cytokines such as interleukin-6 and tumor necrosis factor, which play an important role in the pathogenesis of MM, can trigger coagulation pathways[11]. Ayurveda et al. reported that factor VIII and VWF levels increase and protein S level decreases in MM cases[12]. In addition, imide group drugs and corticosteroids used in the treatment of MM increase the tendency to thrombosis. It was reported that arterial and venous thrombosis were associated with an increased risk of mortality in MM cases.

Prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT) are hemostasis screening tests. These tests are inexpensive and can work in almost any hospital. In this study, we aimed to investigate the hemostasis screening tests in our MM cases and the factors affecting these tests.

Material and methods

In this study, the files of 196 patients who were followed up with the diagnosis of MM in the Hematology Department of Atatürk University Medical Faculty Hospital were retrospectively analyzed. 150 cases with no missing data were included in our study. The study followed ethical guidelines consistent with the Declaration of Helsinki. It was approved by the Ethics Committee of the Atatürk University (number: B.30.2.ATA.0.01.00/243) and informed consent form was obtained from all patients. The criteria revised by the International Myeloma Study Group (IMWG) in 2014 were used for the diagnosis of symptomatic MM. The cases were staged according to the International Scoring System (ISS). Age, gender, hemogram parameters, blood urea nitrogen, creatinine, total protein, albumin, globulin, calcium, sedimentation rate, C-reactive protein, beta 2 microglobulin, IgG, IgA, IgM levels, disease stages, MM subtype, fibrinogen, D Dimer, PT, aPTT, INR values were recorded. The relationship between platelet, fibrinogen, D Dimer, PT, INR, aPTT and other parameters was evaluated. In MM cases, PT, aPTT, INR, fibrinogen, D Dimer values that after 2-4 cycles of chemotherapy treatment were recorded and compared with pre-treatment values. Bleeding and thrombotic events of the cases were recorded. The relationship between bleeding and thrombotic events and hemostasis parameters was investigated. In addition, 30 healthy cases were evaluated retrospectively as the control group. Platelet, fibrinogen, D Dimer, PT, INR, aPTT values of the control group were recorded. Myeloma and control groups were compared in terms of these parameters.

Data were evaluated with IBM SPSS 20 program. The Shapiro Wilk test was used to assess whether the data were normally distributed. Data were presented as number, percentage, mean±standard deviation. The correlation between the variables was made with Pearson correlation analysis if the data were in normal distribution, and with Spearman correlation analysis if they were abnormally distributed. Comparison between the two groups was made with the independent T-test if the groups were normally distributed, and with the Mann-Whitney U test if they were abnormally distributed. Three group comparisons were made with the one-way ANOVA test. $P<0.05$ was considered statistically significant.

Results

The mean age of 150 MM cases was 65.83±11.26; 92 (61.3%) cases were male and 58 (38.7%) cases were female. The mean age of the control group was 65.37±14.08. Of the cases, 22 (73.3%) were female and 8 (26.7%) were male. There was no significant difference between the MM and control groups in terms of age and gender distribution. In 150 MM cases, IgG kappa was present in 76 (50.7%), IgG lambda in 32 (21.3%), IgA kappa in 12 (8%), IgA lambda in 6 (4%), kappa in 13 (8.7%), lambda in 11 (7.3%).

The laboratory findings of our cases with MM were shown in Table 1. Hemostasis

parameters except for the platelet count did not differ according to gender in the MM group (Table 2). Thrombocytopenia and thrombocytosis were detected in 18 (12%) and 4 (2.7%) patients, respectively. PT and INR were prolonged in 41 (27.3%) patients. A prolonged aPTT was observed 18 (12%) patients. An elevated plasma fibrinogen was observed in 19 (12.7%) patients while reduced fibrinogen was 6 (4%) patients. In addition, high D Dimer was detected in 21 (14%) of our MM cases. In Table 3, hemostasis parameters of the MM and control groups were compared.

Laboratory Parameters	Mean±Standard Deviation
Leukocytes (μL)	6160±2250
Platelets (μL)	157890±104230
Hemoglobin (g/dL)	10.92±2.25
Sedimentation rate (mm/h)	59.42±36.04
Blood urea nitrogen (mg/dL)	29.8±22.16
Creatine (mg/dL)	1.64±1.7
Total Protein (g/dL)	7.18±1.77
Albumin (g/dL)	3.31±0.71
Globulin (g/dL)	3.9 ±1.86
C reactive protein (mg/L)	46.34±57.41
B2 microglobulin (mg/L)	8.2±7
IgA (g/L)	2.16±3.64
IgG (g/L)	40±25.3
IgM (g/L)	1.1±4.88

Table 1: Laboratory parameters of MM cases.

	Female	Male	p-value
aPTT (secs)	29.51±6.88	28.53±5.29	0.35
PT (secs)	16.27±5.97	15.61±5.07	0.54
Fibrinogen (mg/dL)	424.29±178.28	345.39±107.52	0.08
D Dimer (ng/mL)	1954.57±1788.82	2628.85±2341.22	0.17
Platelet (μL)	138655±105362	187741±95907	0.005
INR	1.2±0.5	1.2±0.4	0.6

Table 2: Hemostasis parameters according to gender in MM cases.

	MM group	Control group	p-value
PT (secs)	17.38±14.75	12.3±1.4	0.002
aPTT(secs)	29.13±6.32	28,3±3.74	0.48
Fibrinogen (mg/dL)	398.93±162.29	251,42±54.19	0.003
INR	1.2±0.3	1.05±0.12	0.007
Platelet (μL)	157890±104230	228266±67638	0.001
D Dimer (ng/mL)	1194.73±130.28	162±27.08	0.006

Table 3: Hemostasis parameters of MM and control groups.
Prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT)

Hemostasis parameters of our MM cases after chemotherapy treatment were as follows; PT: 17.38±14.75 secs, aPTT: 29.13±6.32, fibrinogen: 398.93±162.29 mg/dL, INR: 1.2±0.3, platelet: 157890±104230 μL, D Dimer: 1194.73±130.28 ng/mL. A significant decrease in PT, INR, aPTT, fibrinogen, D Dimer and a significant increase in thrombocyte value were detected after chemotherapy (p=0.001, p=0.005, p=0.007, p=0.009, p=0.03, p=0.008, respectively).

Fortynine (32.7%) of our MM cases were stage 1, 44 (29.3%) were stage 2, 57 (38%) were stage 3. No significant correlation was found between disease stage and hemostasis parameters except platelet count (Table 4).

Disease stage	Platelet count	p-value
Stage 1	199277±76815	
Stage 2	152116±126451	0.02
Stage 3	140438±91123	

Table 4: Platelet count according to Multiple Myeloma stage.

The relationship between hemostasis parameters and other parameters in MM cases was evaluated. A positive correlation was found between PT and LDH (p=0.017, r=0.196). A negative correlation was found between aPTT and albümin, thrombocyte levels, and a positive correlation with D Dimer (p=0.02 r=-0.34, p=0.01 r=-0.37, p=0.01 r=0.38, respectively).

Upper gastrointestinal bleeding was found in 3 (2%) of our MM cases, hematuria in 2 (1.3%), deep vein thrombosis in the lower extremity in 6 (4%), and pulmonary thromboembolism in 4 (2.7%) of them. There was no significant relationship between hemostasis parameters and bleeding and thrombotic events.

Discussion

The screening tests used for the evaluation of hemostasis are PT, aPTT and thrombin time. Although abnormalities are frequently detected in these tests in MM cases, but it reported that abnormal hemostasis tests was not associated with clinically significant bleeding[13,14]. Less than 15% of MM cases have a bleeding events, usually minor[15]. 43 patients with monoclonal gammopathy were examined in a study, and it was reported that 26 patients (60%) had a laboratory hemostasis defect. However, bleeding symptoms were seen in only five (11.62%) cases. In our study, abnormal hemostasis tests, which caused a tendency to bleeding, was detected in 83 (55.3%) cases, but bleeding was detected in only 5 (3.3%) of our cases. This result was consistent with the literature.

Varying rates of thrombocytopenia and thrombocytosis were reported in MM patients. Gupta et al. reported 3 cases diagnosed with MM while being investigated for immune thrombocytopenia[16]. Immune thrombocytopenia was not detected in any of our cases. Gogia et al. compared 29 MM patients with 30 control subjects[17]. They

determined thrombocytopenia in 58.5% of patients and thrombocytosis in 3.4%. Kyle et al. observed thrombocytopenia in 5% and thrombocytosis in 2% of MM patients[18]. In our study, thrombocytopenia was found in 12% of our cases and thrombocytosis in 2.7%. Our thrombocytosis rate is similar to the literature. The difference in thrombocytopenia rates may be due to the difference in the number of cases and the disease stages of the patients included in these studies.

In studies, the most common hemostasis screening test abnormality in MM patients was reported as prolonged PT[19-21]. In some studies, it was found that the PT value was associated with the serum paraprotein level[19,22-23]. Pandey et al. detected that prolonged PT rate was 25% in plasma cell diseases[19]. They also observed a significant prolongation in PT as the MM stage progressed. However, they did not find a significant relationship with PT in terms of age, gender and MM type. In our study, prolonged PT was the most common hemostasis abnormality, which was detected in 27.3% of our cases. This result was consistent with the literature. In our study, no correlation was found between PT value, disease stage and MM subtype. However, we found a positive correlation between the PT and the LDH value.

Huang et al. found a negative correlation between fibrinogen and M protein levels[24]. It reported that IL6 causes an increase in fibrinogen level in MM cases[3]. Gogia et al. found high fibrinogen level in 34.5% of MM cases[17]. Elice et al. reported this rate as 68% (25). In our study, hyperfibrinogenemia was found in 12.7% of the cases. This rate is lower than the literature. This may be due to the difference in the number of patients. We did not find a relationship between fibrinogen level and other prognostic parameters and MM subtype.

In a study, 78 newly diagnosed MM cases were examined and elevated D Dimer was found in 63% of the cases[25]. Elice et al. also reported this rate as 63%[20]. Auwerda et al. examined 134 MM and 124 control patients and they reported that no significant difference in terms of D Dimer between the MM and control groups[12]. But, Huang et al. reported that the D Dimer value was significantly higher in MM cases compared to normal cases[24]. In another study, 29 MM patients were analyzed and elevated D Dimer was found in 75.9% of MM cases[17]). In our study, elevated D Dimer was found in 14% of our cases. In addition, we detected that the D Dimer was significantly higher in the MM group than the control group according to literature.

Imid group drugs used in the treatment of MM cases increase the tendency to thrombosis. In one study, 4446 patients with MM were examined and VTE was detected in 7.4% of the patients at 1-year follow-up[26]. 2837 of these cases were included in the study after lenalidomide treatment was approved for use in MM patients in 2006. While VTE was associated with increased mortality at 6 months in all patients, It was associated with increased mortality at both 6 and 12 months in patients with VTE using lenalidomide[26]. Kristinsson et al. examined 9399 MM patients and determined that the mortality rate increased in MM patients with venous thromboembolism and arterial thrombosis[27].

Gogia et al. found prolonged aPTT (69%) as the most common abnormal hemostasis screening test in MM patients[17]. They found no relationship between aPTTand MM clinical and laboratory parameters and prognostic markers. 101 MM patients were determined and no correlation was found between aPTT and MM stage, M protein type, serum light chain concentration in another study[24]. Teng et al. found that prolonged aPTT was an independent prognostic factor in IgA myeloma[21]. In our cases, prolonged aPTT was found as 12%. The albumin value is a laboratory test used in ISS staging. We found a negative correlation between aPTT and albumin value.

Sokol et al. stated that coagulation parameters can be used as a prognostic marker[28]. They examined 36 MM patients and noted a reduction in the subjects' D Dimer levels with treatment. In our case, a significant decrease in PT, INR, aPTT, fibrinogen, D Dimer and a significant increase in thrombocyte value were detected after chemotherapy.

In conclusion, abnormalities in hemostasis tests, which are associated with bleeding and tendency to thrombosis, were found in MM cases. However, the effect of abnormalities in these tests on bleeding and thrombotic events is not fully known. In our study, no relationship was found between bleeding and thrombotic events and hemostasis parameters. Therefore, prospective studies with a large number of cases should be planned and the relationship between hemostasis parameters and bleeding/ thrombosis should be investigated. Some studies have reported that the risk of mortality increases in myeloma cases with both arterial and venous thrombosis. Therefore, early identification and regular follow-up of patients at risk for thrombosis

is important. We also found that abnormalities in hemostasis tests improved after chemotherapy treatment. Therefore, prospective studies with a large number of cases should be planned for the use of hemostasis parameters as a prognostic biomarker.

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