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Original Research Article

Correlation of prothrombin time and activated partial thromboplastin time with serum immunoglobulin and M-band in newly diagnosed multiple myeloma patients

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ABSTRACT

Background: Multiple myeloma is the second most frequent malignancy which constitute 13% of hematologic cancers. Thrombotic and hemorrhagic complications have been frequently observed in multiple myeloma patients.
Methods: The study was conducted in the department of pathology, Government medical college Srinagar. A total of fifty (50) patients were recruited for the study. The patients were advised coagulation profile and complete myeloma profile.
Results: Our findings indicate that prolonged PT is associated with high serum IgG levels. A mild to moderate correlation was seen with kappa-free light chains and an inverse correlation was seen between PT and lmbda-free light chains.
Conclusions: Screening of multiple myeloma for hemostatic abnormalities at the diagnosis should improve prognosis in such cases.

Keywords: APTT, M-band, Myeloma, PT

INTRODUCTION

Multiple myeloma is a hematological malignant tumor caused by the malignant clonal proliferation of terminal B lymphocytes, namely plasma cells. There is an abnormal increase of monoclonal immunoglobulin or light chain which is the main serological manifestation.[1,2] Bone marrow is the site of origin of nearly all plasma cell myelomas and in most cases, there is disseminated bone marrow involvement, other organs may be secondarily involved. The clinical spectrum spans from asymptomatic to highly aggressive disease. Diagnosis is based on a combination of clinical, morphological, immunological and radiological features.[3] It is usually seen in the elderly, but in recent years, it has been seen in younger people also. Because the onset of the disease is hidden and the clinical manifestations are diverse, it is usually misdiagnosed at the first instance.[4] It has been seen that abnormal coagulation is the main cause of death in patients with Multiple myeloma, which seriously affected the patients' health and quality of life.[5] Although, the pathological basis of hemorrhage in patients was thought to be vascular endothelial cell injury and dysfunction but, presently, it is believed that the mechanism of abnormal blood coagulation in multiple myeloma patients may be that M protein can specifically inhibit the activity of various blood coagulation factors, which leads to the inhibition of blood coagulation function.[6,7] The tumor can invade the blood vessel wall and promote the release of pro-coagulant factors from the blood vessel wall. Moreover, tumor cells metastasize and invade tissues and organs, resulting in tissue damage and endothelial cell

damage. After endothelial cell injury, a large number of tissue factors can be released to activate the body's coagulation system, which then leads to coagulation-fibrinolysis dysfunction in these patients.[8,9] D-dimer (D-D), fibrinogen (FIB), prothrombin time (PT), activated partial thromboplastin time (aPTT) are all important indexes reflecting the body's coagulation function.[10]

The study was conducted to assess the correlation of prothrombin time (PT) and activated partial thromboplastin time (aPTT) with serum immunoglobulin and M-band in newly diagnosed multiple myeloma patients.

METHODS

This cross-sectional observational analytic study was conducted in the department of pathology, Government Medical College, Srinagar over a period of one and half years starting from 1st April 2021 to 30th September 2022. During the first 15 months data was collected and during the next 3 months data entry, analysis, and write-up were carried out. A detailed history was taken from each patient with special reference to the presence of bone pain, manifestations of anemia, any history of infection and symptoms of renal disease such as flank pain, oliguria, and altered sensorium due to uremia. A thorough clinical examination was done after obtaining written informed consent from all patients for their inclusion in the study.

Exclusion criteria

All treated cases of multiple myeloma were excluded.

Sample size

A total of fifty (50) patients were recruited for the study.

Sample plan

The selected multiple myeloma patients were informed about the objectives of the study. Proper written informed consent was taken from the selected patients who agreed to participate in the study in English/ Urdu language. Then the relevant information regarding their socio-demographic variables, and other desired variables as per the preformed questionnaire was obtained. The EDTA sample was run within 2 hours of collection on an automated hematology analyzer (Sysmex XN-1000), based on the electrical impedance method to obtain complete blood counts.

Examination of stained peripheral blood film

Peripheral smears were prepared from freshly drawn venous blood and stained by Leishman stain. A note was made on changes in red cells, leucocytes, and platelets. A differential leucocyte count was done.

Tests of coagulation

Both prothrombin time (PT) and activated partial thromboplastin time (aPTT) were performed in fully automated coagulation analyser (Sysmex CS-2400) based on clot detection by scattered light detection method, after running the internal controls, before processing the sample.

Multiple myeloma comprehensive profile, serum immunoglobulin profile including serum electrophoresis, immunofixation and free light chain assay was done.

Operational definitions

The laboratory tests which were done in the department of pathology, GMC Srinagar, during this study were as per the following reference range and the specification of the analyzer (XN-1000)/ machine:

Table 1: Multiple myeloma comprehensive profile.

Multiple myeloma comprehensive profile			
Test name	**Unit**	**Reference range**	**Method**
Serum albumin	gm/dl	3.50-5.20	BCG
Serum creatinine	mg/dl	0.51-0.91	Compensated Jaffe's reaction, IDMS traceable
Serum urea	mg/dl	17.0-43.0	Urease UV
Serum calcium	mg/dl	8.80-10.60	Arsenazo III
Serum beta 2 microglobulin	mg/dl	609.0-2366.0	CLIA
Serum immunoglobulin profile			
Serum immunoglobulin IgG	mg/dl	700.0-1600.0	Immunoturbidimetry
Serum immunoglobulin IgM	mg/dl	40.0-230.0	
Serum immunoglobulin IgA	mg/dl	70.0-400.0	
Free light chains			
Kappa, free light chain	mg/dl	3.30-19.40	Nephelometry
Lambda, free light chain	mg/dl	5.71-26.30	
Kappa/lambda ratio	mg/dl	0.26-1.65	

Ethical consideration

Ethical clearance was obtained for conducting the study from the ethical committee of Government Medical College Srinagar. Written informed consent was obtained from the participating stakeholders at every level.

Data analysis

Data was compiled using a Microsoft 2016 Excel spreadsheet and analyzed by IBM SPSS V.23. Descriptive statistics were computed to describe the socio-demographic characteristics of participants and to summarize the distribution of each of the dependent (outcome) and independent variables

RESULTS

In this study, a total of 50 suspected multiple myeloma (MM) patients were recruited over a stipulated period. Among these patients 31 (62%) were males and 19 (38%) were females. The mean age of the patients was 63.78±10.61 years with a minimum age of 45 years and maximum age of 90 years.

Table 2: Demographic characteristics of patients.

Age	Frequency	Percentage
40-49	6	12
50-59	6	12
60-69	25	50
70-79	8	16
80-89	4	8
90-99	1	2
Total	50	100

Table 3: Distribution of organ impairment (CRAB) in multiple myeloma patients.

Clinical features (CRAB)	Frequency	Percent
Anaemia	48	96.0
Hypercalcemia	27	54.0
Renal Failure	11	22.0
Bone Diseases	16	32.0

The CBC of the study participants revealed that the mean hemoglobulin was 8.80±1.83 gm/dl with a minimum Hb of 6.20 gm/dl and a maximum 13.60 gm/dl as shown in Figure 3. The mean TLC was 6261±2412 per mm^3 with a maximum of 12300 per mm3 and minimum of 1240 per mm^3 and the mean platelet count was found to be 169440±124635 per mm^3 with a maximum of 890000 per mm3 and a minimum of 19000 per mm^3 (Table 4).

The coagulation profile of the patients showed that the mean PT was 16.0±3.3 seconds with a minimum of 10.6 Sec. and a maximum of 31.5 Sec. The mean aPTT was 36.7±9.9 seconds with a minimum of 24.7 seconds and a maximum of 68.1 seconds. The PT was prolonged in 66% of the participants and aPTT was prolonged in 12% of the patients (Table 5).

Table 4: Complete blood count in multiple myeloma patients.

Complete blood count		Frequency	Percent
Hb level	Anaemic	47	94.0
	Normal	3	6.0
	Total	50	100.0
Total leucocyte count	Leucopenia	9	18.0
	Normal	40	80.0
	Leucocytosis	1	2.0
	Total	50	100.0
Platelet counts	Low	17	34.0
	Normal	32	64.0
	High	1	2.0
	Total	50	100.0

Table 5: Coagulation profile in MM patients.

Coagulation Profile			
	Levels	**Frequency**	**Percent**
PT	Normal	17	34.0
	Prolonged	33	66.0
	Total	50	100.0
aPTT	Prolonged	6	12.0
	Normal	44	88.0
	Total	50	100

Table 6: Pattern of plasma cell infiltration on BMB in MM patients.

Bone marrow biopsy (pattern)	Frequency	Percent
Diffuse	30	60.0
Interstitial	16	32.0
Mixed	4	8.0
Total	50	100.0

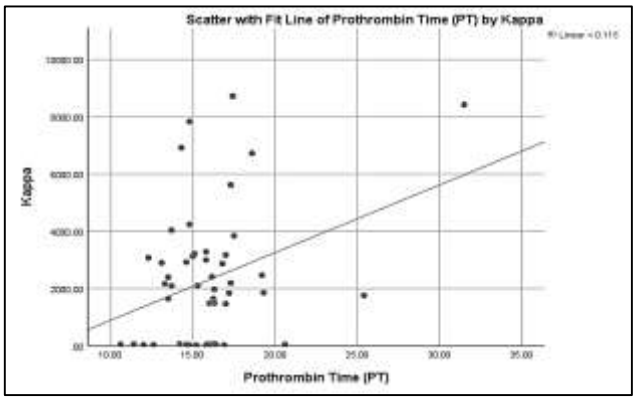

Figure 1: Scatter plot of PT by kappa free light chain in MM patients.

Bone marrow aspiration of the patients showed that the mean percentage of plasma cells was 41.56±23.41% with

a minimum of 11% and a maximum of 97% as shown in Figures 3 and 4.

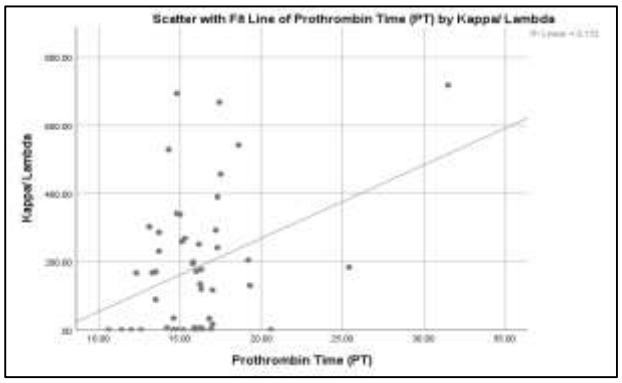

Figure 2: Scatter plot of PT with kappa/lambda free light chain ratio in MM patients.

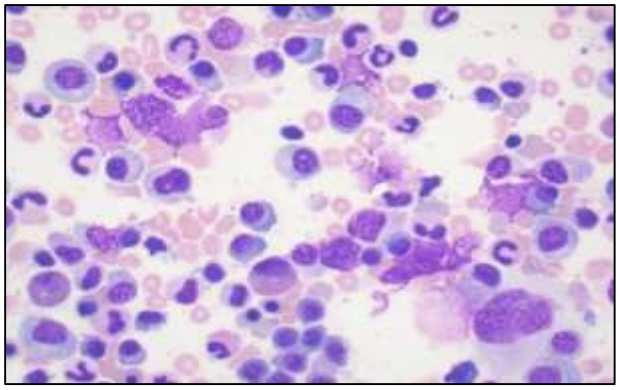

Figure 3: Bone marrow aspirate showing plasmacytsosis (100x).

Pearson correlation test was done between prothrombin time and serum immunoglobulin, kappa and lambda free light chains after plotting the scatter plot to check for outliers, direction and homoscedasticity as shown in Figures 1 and 2. It was noted that there was no correlation between PT and serum IgG, IgA, and IgM, but mild to moderate correlation was seen with Kappa-free light chains with a correlation coefficient of 0.33 with a significant p value <0.05. An inverse correlation was seen between PT and lambda free light chains with a correlation coefficient of -0.24 with a significant p value <0.05. Again, mild to moderate correlation was seen with kappa/lambda free light chain ratio with a correlation coefficient of 0.36 with a significant p value <0.05.

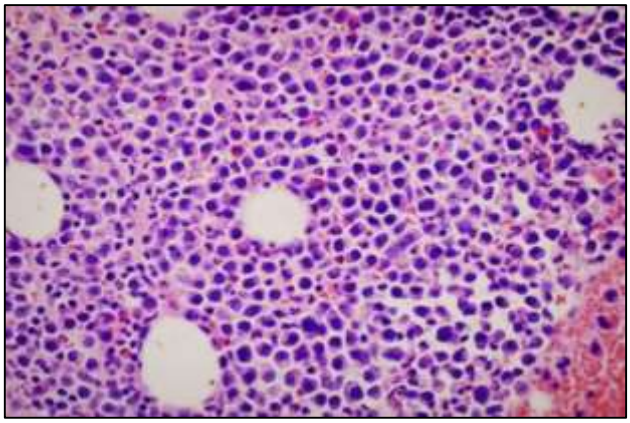

Figure 4: Bone marrow biopsy with diffuse pattern of infiltartion by plasma cells (40x).

Table 7: Shows correlation of PT and M-band in MM patients.

Pearson correlation		M-band (gm/dl)
PT	**Correlation coefficient**	0.109
	P value	0.451
	(N)	50

Table 8: Ccorrelation of aPPT and serum immunoglobulin, kappa and lambda free light chains and M-band in MM patients.

Pearson correlation		Serum IgG	Serum IgA	Serum IgM	Kappa	Lambda	Kappa/lambda	M-band (gm/dl)
aPPT	Correlation coefficient	0.015	-0.022	-0.065	0.175	-0.142	0.110	0.067
	P value	0.919	0.882	0.652	0.224	0.327	0.446	0.642
	(N)	50	50	50	50	50	50	50

Table 9: Association of prolonged prothrombin time (PT) with high serum IgG levels.

Prothrombin time (PT)	Serum immunoglobulin IgG			Total	Statistical test
	Low	Normal	High		
Normal	0 ()0.0%	3 (17.6%)	14 (82.4%)	17 (100.0%)	Fisher's exact test P value <0.04
Prolonged	2 (6.1%)	0 (0.0%)	31 (93.9%)	33 (100.0%)	
Total	2 (4.0%)	3 (6.0%)	45 (90.0%)	50 (100.0%)	

Table 10: Association of prolonged prothrombin time (PT) with M-band corresponding to IgG in MM patients.

Prothrombin time (PT)	M-band (serum immunoglobulin)		Total	Statistical test
	IgG	IgA		
Normal	15 (31.3%)	2 (100.0%)	17 (34.0%)	Fisher's exact test P value <0.05
Prolonged	33 (68.8%)	0 (0.0%)	33 (66.0%)	
Total	48 (100.0%)	2 (100.0%)	50 (100.0%)	

A correlation test was done between PT and M-band after plotting the scatter plot but again no correlation was found. aPTT and serum immunoglobulin, kappa and lambda free light chains and M-band were again subjected to Pearson correlation, but as shown in Table 7 there was no statistically significant correlation.

There was an association noticed during analysis between prothrombin time (PT) with serum immunoglobulin IgG as shown in Table 8. Prolonged PT was associated with high serum IgG levels in 93.9% of the cases with statistically significant p value <0.04.

A similar association was found with PT and M-band (serum immunoglobulin IgG and IgA) as shown in Table 10. Prolonged PT was associated with M-band corresponding to IgG levels in 68.8% of the cases with a significant statistical difference (p value <0.05).

DISCUSSION

In multiple myeloma, the pathophysiology of coagulopathy is multi-factorial.[11] The pathogenesis of the observed clotting abnormalities in these patients is probably complex.[12,13] Elevated M-protein levels are correlated with abnormal values in the TT, PT, and aPTT. The high blood concentration of M-protein with its impact on the fibrin polymerization process may be the cause of prolongation of not only PT, aPTT but also TT.[14] The correlation if any between PT and aPTT with serum immunoglobulin and M-band in newly diagnosed patients of multiple myeloma will help us to access disease severity, treatment and prognosis in these cases.

In our study 50 cases were recruited. The coagulation profile of the patients showed that the mean PT was 16.0±3.3 seconds and the mean aPPT was 36.7±9.9 seconds. The PT was prolonged in 66% of the participants and aPTT was prolonged in 12% of the patients and the reported frequency of prolonged PT in patients with myeloma was highly variable. Teng et al observed prolonged PT in only 4.5% of patients. Although prolonged PT alone has no impact on survival, in a study conducted on 252 patients of MM and other plasma cell dyscrasias, an isolated prolonged PT was the most frequent abnormal coagulation test seen in 25% of patients. There was no correlation between prothrombin time and serum immunoglobulin, kappa, and lambda-free light chains. It was noted that there was no correlation between PT and serum IgG, IgA and IgM but a mild to moderate correlation was seen with kappa-free light chains with a correlation coefficient of 0.33 with a significant p value <0.05. An inverse correlation was seen between PT and lambda free light chains with a correlation coefficient of -0.24 with a significant p value <0.05. Again, mild to moderate correlation was seen with kappa/lambda free light chains with a correlation coefficient of 0.36 with a significant p value <0.05. As seen in this study, Pandey et al reported prolonged PT in 44% of patients.[15] Panday et al reported that multiple myeloma was more likely to have prolonged PT than patients with other plasma cell neoplasms and monoclonal protein level was significantly higher in patients with isolated prolonged PT and correlated with PT.[15]

A small sample size in our study was taken due to limited allotted time which may have affected the results of the study. However, it didn't critically affect our findings and we still obtained estimates even with this sample size. There were missing data on some patients and biases of judgment on bleeding diathesis. However, we tried to reduce this bias by excluding the patients with known bleeding diathesis.

CONCLUSION

Our findings indicate that prolonged PT is associated with high serum IgG levels, so can cautiously be used as a predictor for the disease, as the sample size was small. But at the same time, this study has opened the window for other researchers to take this study on large scale for validation of the findings. A mild to moderate correlation was seen with kappa-freelight chains and an inverse correlation was seen between PT and lambda-free light chains. Again, a mild to moderate correlation was seen with kappa/lambda free light chain ratio. These findings can further be explored as limited research in this field has been done so far and MM being one of the commonest plasma cell dyscrasia, makes it more demanding.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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