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CORRELATION OF BONE MARROW PLASMACYTOSIS WITH ABNORMAL BLEEDING PATTERN AND CONGO RED STAINING

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ABSTRACT:

Background: The plasma cell is round or oval in shape, the cytoplasm is abundant and intensely basophilic, usually deep blue. The nucleus is small in relation to the cell size, round or oval, eccentrically placed, and contains dense masses of chromatin arranged in pyramidal blocks against the nuclear membrane, giving the characteristic "cartwheel" appearance. Plasma cell dyscrasias form a heterogeneous group of diseases characterized by expansion of the number of monoclonal bone marrow plasma cells that produce monoclonal immunoglobulins.

Material and Methods: A thorough clinical evaluation of the patients with bone marrow plasmacytosis coming to SS Hospital, BHU from October 2017 to May 2019 was done, including history taking, physical examination and relevant investigations. Patients not giving consent for participation in the study were excluded.

Results: The mean prothrombin time among 42 patients was 15.3 seconds. Prothrombin time was prolonged in 17 patients (40.5%). The mean APTT among 42 patients was 32.1 seconds. APTT was prolonged in 19 patients (45.2%). Isolated prolongation of PT was observed in 3 patients (7.1%) and isolated prolongation of APTT was observed in 3 patients (7.1%). Platelet aggregation studies were performed in 33 patients. Platelet aggregation with adrenaline was abnormal in 11 patients (33.3%) and aggregation with ADP was abnormal in 18 patients (54.5%).

Conclusion: Generalised weakness and easy fatiguability was the commonest presenting complaint and bone pain was relatively less common than in other studies. Bone lesions were also relatively less common in our study as compared with other studies. Active bleeding was observed in 3 patients with 2 of them having epistaxis and one with per rectal bleed. Coagulation tests were abnormal in many cases of plasma cell dyscrasias but most patients were asymptomatic. Abdominal /gluteal fat pad aspiration and Congo red staining showed faint positivity in only one patient.

Keywords: Bone marrow, Plasmacytosis, Congo red staining, Electrophoresis, PT and APTT

Introduction:

Plasma cell dyscrasias form a heterogeneous group of diseases characterized by expansion of the number of monoclonal bone marrow plasma cells that produce monoclonal immunoglobulins.[1] The presence of a monoclonal protein is indicative of an underlying clonal plasma cell or B cell disorder. These disorders encompass a spectrum of disease entities ranging from clinically benign monoclonal gammopathy of undetermined significance (MGUS) to clinically significant diseases such as multiple myeloma and Waldenström macroglobulinemia.[2]

The plasma cell is round or oval in shape, the cytoplasm is abundant and intensely basophilic, usually deep blue. The nucleus is small in relation to the cell size, round or oval, eccentrically placed, and contains dense masses of chromatin arranged in pyramidal blocks against the nuclear membrane, giving the characteristic "cartwheel" appearance.[3]

The name plasma cell was first used by Waldeyer in 1875. His description, however, included several types of cells; and in 1881 Unna redefined the cells as he observed them in a case of lupus, emphasizing the characteristic basophilia of the cytoplasm

(granuloplasm). In 1895, Marschalko emphasized that the appearance of the nucleus with its characteristic arrangement of angular chromatin blocks and its eccentric position within the cell are to be used as stringent criteria for the identification of plasma cells. The modern period of plasma cell study originated in 1937, with the clinical observations of Bing and Plum, who noted the close association of hyperglobulinemia and the presence of plasma cells. Subsequent studies in hyperimmunized rabbits were carried out by Bjornboe and Gormsen, who demonstrated that antibody production correlated with massive plasma cell proliferation in the spleen. In his doctoral thesis, Fagraeus left little doubt about the importance of plasma cells in antibody formation. Indisputable evidence in favour of antibody production by plasma cells was provided by Coons, who introduced the powerful technique of immunofluorescence to immunology. Plasma cells containing antibody were detected in the red pulp of the spleen, the medullary cords of the lymph nodes and the focal granulomata of immunized animals.[4]

Plasmacytosis is defined as presence of plasma cells in circulating blood or presence of unusually large proportions of plasma cells in tissues or exudates. Bone marrow plasmacytosis can be seen in association with a variety of conditions. Reactive plasmacytosis can be seen in chronic infections like visceral leishmaniasis, HIV, tuberculosis, infectious mononucleosis, autoimmune diseases like SLE and rheumatoid arthritis, hypersensitivity reactions, megaloblastic anaemia, haemolytic anaemia, diabetes mellitus, cirrhosis, malignancies, lymphoproliferative disorders and Castleman's disease.[5]

MATERIAL AND METHODS

A thorough clinical evaluation of the patients with bone marrow plasmacytosis coming to SS Hospital, BHU from October 2017 to May 2019 was done, including history taking, physical examination and relevant investigations. Patients not giving consent for participation in the study were excluded.

Sample collection

Blood sample was collected in EDTA vial for complete blood counts by Beckmann Coulter Haematology autoanalyzer, in serum / plain vial for liver function tests, renal function tests, serum calcium by BS 800 analyser manufactured by Mindray.

Collection of samples for peripheral blood smears

- Under strict asepsis, left ring finger was pierced with a 22-gauge needle and first drop of blood was wiped off with clean cotton.
- Subsequent free-flowing drops of blood were collected on several clean glass-slides.
- Smears of adequate size were prepared using another clean glass-slide as a spreader and air-dried and stained with Leishman stain.

Collection of bone marrow aspirate

- The patient and his/her attendants) were described the procedures briefly and written consents were obtained.
- Patient was made to lie down in prone position or lateral decubitus position on the bed.
- Cloths were removed over the area of lower back and waist region and required area was exposed.
- Bilateral posterior superior iliac spines were palpated and gentle thumb pressure was applied to mark the area.
- After proper hand-wash and wearing sterile pair of gloves, sensitivity test for lignocaine was done. For this, 0.5-1 ml of 2% lignocaine was injected intradermally over ventral aspect of upper part of forearm. After that patient was observed for 5-10 minutes for any symptoms of hypersensitivity like redness, itching, dryness of mouth, palpitation, breathlessness etc.
- Then patient's exposed area (over lower back) was cleaned thoroughly and sequentially with spirit-betadine-spirit.
- Under proper aseptic precautions, local skin and subperiosteal infiltration was done with 2% lignocaine over and around the areas of bilateral posterior superior iliac spines.
- A sterile bone marrow aspiration needle (Salah's needle) of appropriate size was introduced through one of the anaesthetized areas by semi-rotatory movements and cortex was penetrated to reach the marrow space.
- About 0.5 ml of aspirate was obtained by creating negative pressure in a 20 ml syringe and smears were prepared and air-dried and stained with Leishman stain.
- In case of a 'dry tap', or if no particles (‘fragments’) obtained, the bone marrow aspirate was repeated at a slightly different angle or at another site.
- After obtaining adequate amount of bone marrow aspirate, bone marrow aspiration needle was taken out slowly with semi-rotatory movements and the site was pressed with gauge piece till haemostasis was achieved.
- After that bandage was applied and patients were instructed to seek medical advice for any complication like bleeding etc.

RESULTS:

After thorough search of patients suspected of having clinical features related to or indicative of plasma cell related abnormalities, 51 patients were included in the study, 43 patients were newly diagnosed cases of multiple myeloma, 1 was follow up case of multiple myeloma, 1 was a case of MGUS, 4 were cases of megaloblastic anaemia, 1 was a case of kala azar and 1 was a case of amyloidosis associated nephropathy.

Age distribution of multiple myeloma patients

Table 1: Age distribution of Multiple myeloma patients

Total Number of Patients n=43		
Age (Years)	Patient Number	Percentage %
0-30	2	4.7
30-40	3	7.0
40-50	6	14.0
50-60	14	32.6
60-70	14	32.6
70-80	3	7.0
80-90	1	2.3
90-100	0	0
Mean age (Yrs)	57.79	
Standard Error	1.84	
Median Age	60	
Standard Deviation	12.12	
Minimum age	25	
Maximum age	88	

Among 43 newly diagnosed multiple myeloma patients, the mean age was 57.8 years and median age was 60 years. The ages of the patients ranged from 25 years to 88 years with 2 patients (4.7%) less than 30 years of age and 4 patients (9.4%) more than 70 years of age, including 1 patient (2.3%) more than 80 years of age. 3 of the patients (7.0%) were between 30 and 40 years of age, 6 patients (14.0%) were between 40 and 50 years of age, 14 patients (32.6%) were between 50 and 60 years of age and 14 patients (32.6%) were between 60 to 70 years of age.

Table 2: Haemoglobin levels in multiple myeloma patients

Total Number of Patients n=43		
Haemoglobin (g/dl)	Patient Number	Percentage
Less than 7	27	62.8
7 to 10	11	25.6
More than 10	5	11.6
Mean Haemoglobin (g/dl)	6.7	
Standard Deviation	2.4	
Minimum	2.6	
Maximum	12.8	

Among 43 patients included in the study, the mean haemoglobin level was 6.7 g/dl. The minimum haemoglobin level at the time of presentation was 2.6 g/dl. 27 patients (62.8%) had haemoglobin less than 7 g/dl, 1 patient (25.6%) had haemoglobin levels between 7 to 10 g/dl and 5 patients (11.6%) had haemoglobin of more than 10 g/dl.

Table 3: Plasma cells in bone marrow of multiple myeloma patients

Plasma cells in bone marrow %	Patient Number
Less than 10	4
10 to 30	17
30 to 60	15
More than 60	6
Mean	34.1%
Median	30.5%
Minimum	6%
Maximum	82%

Plasma cells in bone marrow of multiple myeloma patients

Out of 43 patients of newly diagnosed multiple myeloma, the mean and median plasma cell percentage in bone marrow were 34.1 percent and 30.5 percent respectively. The maximum plasma cell concentration was 82% and minimum plasma cell percentage was 6%. 4 patients (9 .3 %) had 10% or less plasma cells in the examined bone marrows. 6 patients (13.9%) had more than 60% plasma cells, 17 patients (39.5%) had 10 to 30% plasma cells and 15 patients (34.9%) had 30 to 60% plasma cells in their bone marrow, while 1 bone marrow was unsatisfactory for any comment as it had no bone marrow elements and only blood. Plasmablastic morphology was seen in 4.7% patients.

Among 4 patients with less than or equal to 10% bone marrow plasma cells, one patient had 7% bone marrow plasma cells with lytic bone lesions and serum creatinine of 2.8 mg/dl. One patient had 6% bone marrow plasma cells with serum creatinine of 2.1 mg/dl, serum calcium level of 12 mg/di and haemoglobin of 4.5 gm/dl. One patient had 6% plasma cells with bone lesions and haemoglobin of 3.7gm/dl. One of the patients had 10% plasma cells with haemoglobin of 5.4g/dl and serum creatinine of 2.1 mg/dl.

Table 4: Serum electrophoresis in multiple myeloma patients

Total Number of Patients n=43		
	Patient number	**Percentage**
No M peak	**10**	**23.3**
M peak in gamma region	**24**	**55.8**
M peak in beta region	**4**	**9.3**
M peak between beta and gamma region	**4**	**9.3**
Biclonal gammopathy	**1**	**2.3**

M peak on serum electrophoresis was seen in 33 patients (76.7%) out of 43. 24 patients (55.8%) had M peak in the gamma region, 4 patients (9.3%) had an M peak in beta region and 4 patients (9.3%) had an M peak in between beta and gamma regions. Two M peaks were present in 1 patient (2.3%) (biclonal gammopathy). No M peak was observed in 10 patients (23.3%).

Coagulation tests in multiple myeloma patients

The mean prothrombin time among 42 patients was 15.3 seconds. Prothrombin time was prolonged in 17 patients (40.5%). The mean APTT among 42 patients was 32.1 seconds. APTT was prolonged in 19 patients (45.2%). Isolated prolongation of PT was observed in 3 patients (7.1%) and isolated prolongation of APTT was observed in 3 patients (7.1%). Platelet aggregation studies were performed in 33 patients. Platelet aggregation with adrenaline was abnormal in 1 patient (33.3%) and aggregation with ADP was abnormal in 18 patients (54.5%).

On Congo red staining of abdominal/gluteal fat pad aspirate, faint positivity was observed in 1 of the smears and rest others were negative.

One of the patients was a case of MGUS, he was 40 years old at the time of diagnosis, with B positive blood group presented with complains of generalized weakness and bone pain, had pallor on examination, normocytic normochromic anaemia, normal TIC and platelet count, 8% plasma cells in bone marrow, M peak in gamma region and serum creatinine of 1.6 mg/dl, normal LFT, normal coagulation profile, normal serum calcium levels and no bone lesions.

One patient was a case of kala azar, 49-year male with O negative blood group, presented with chief complains of fever and pain in left upper abdomen. Examination findings showed pallor and hepatosplenomegaly with pancytopenia, reversal of albumin to globulin ratio, normal serum creatinine, calcium and coagulation profile, polyclonal increase in gamma globulins on serum electrophoresis and bone marrow having LD bodies and 8 to 10 percent plasma cells.

One patient was a case of amyloid nephropathy on follow up, now presenting with edema, sudden onset breathlessness and fever. Examination revealed pallor and pedal edema. There was raise serum creatinine, anemia, mild leucoytosis and nephrotic range proteinuria.

Rest four patients were cases of megaloblastic anemia with chief complains of generalized weakness and easy fatiguability, pallor on examination with cytopenias involving one or more lineages and plasma cells ranging from 6% to 8%.

Correlation was done between serum total protein levels and bone marrow plasma cell percentage with serum calcium levels, rouleaux formation, PT, APTT, platelet aggregation studies and Congo red staining. However, no correlation was found between serum total protein levels and bone marrow plasma cell percentage with serum calcium levels, rouleaux formation, PT, APTT, platelet aggregation studies and Congo red staining ($p > 0.01$ in all).

DISCUSSION:

Bone pain was the most common complain, present in around 60-90% of patients according to ICMR data, and anaemia and fatigue was present in about 70% of the patients. According to P Kaur et al, the most frequent complaints were bone pain was in 50% patients and generalized weakness and fatiguability in 46.4%. As per Kyle et al, bone pain was most common complaint, present in 58% of patients at the time of diagnosis.[6,7,8]

In our study, pallor was the most common examination finding, in accordance with WHO. Pallor was present in 38 patients (88.3%), out of 43 patients of multiple myeloma. 1 patient (2.3%) had cervical lymphadenopathy (probably unrelated to multiple myeloma. None of the 43 patients had hepatomegaly, splenomegaly, Icterus or family history of multiple myeloma.

According to the data of Mayo clinic, the liver was palpable in 4% of the patients, and spleen was palpable in 1%. Lymphadenopathy was present ni 1% of patients.[7,9]

In our study, 16.3% patients had leucopenia, compared with 7.2% and 20% in studies of P Kaur et al and Kyle et al respectively. In our study, 20.9% patients had leucocytosis, compared with 35.7% and 8% of patients in studies of P Kaur et al and Kyle et al respectively.[7,8]

Thrombocytopenia was present in 10 patients (23.2%) and thrombocytosis was present in 1 patient (2.3%) in our study. According to study by P Kaur et al, 25% patients had thrombocytopenia. According to Kyle et al, thrombocytopenia was present initially in 5% of patients and thrombocytosis was present in 2% of patients.[7,8]

In our study, plasma cells were present in peripheral blood in 3 patients, including 80% plasma cells in one patient with plasma cell leukaemia. According to data from Kyle et al, 3% patients had plasma cells in peripheral blood including 1 patient of plasma cell leukaemia. According to study by P Kaur et al, 2 patients out of 28 showed plasma cells in peripheral blood including 1 patient of plasma cell leukaemia. According to WHO, plasma cells were found in peripheral blood smears in about 15% of cases.[7,8,9]

In our study, the mean and median plasma cell percentage in bone marrow was 34.1% and 30.5% respectively. The maximum plasma cell concentration was 82% and minimum plasma cell percentage was 6%. 4 patients (9.3%) had 10% or less plasma cells in the examined bone marrows. 6 patients (13.9%) had more than 60% plasma cells. According to data from Mayo clinic, the median value of plasma cells in bone marrow was 50%, 4% patients had less than 10% plasma cells in bone marrow. As per WHO, there are < 10% plasma cells in the bone marrow aspirate smears in 5% cases. Patients with <10% plasma cells in bone marrow were diagnosed multiple myeloma by presence of other myeloma defining events.

The mean serum creatinine level was 3.97 mg/dl in our study. It was more than or equal to 2 mg/dl in 26 patients (60.5%). According to Kyle et al, the median serum creatinine level was 1.2 mg/dl. 19% patients had serum Creatinine levels of 2 mg/dl or more. Serum Creatinine with levels above 2 mg/di was found in 20% patients according to WHO and ICMR

data and 77.3% and 15 to 20% patients according to studies by P Kaur et al and Prakash et al respectively. According to Perkins et al, asymptomatic prolongation of PT and APTT were observed in 59% and 18% of multiple myeloma patients. According to Kyle et al, prolongation of PT was observed in 37% of multiple myeloma cases. According to a study by Meera Sikka et al, the most frequent abnormal haemostatic parameter was prolonged APTT. PT and APTT were prolonged in 48.3% and 68.9% patients respectively. [7,10,11]

Bleeding was observed in 3 patients. In 1 patient with epistaxis, PT, APTT and platelet aggregation studies were al deranged. The patients presenting with per rectal bleeding had prolonged PT and APTT both but normal platelet aggregation studies. In another patient with epistaxis PT and APTT were normal. Thus, patients of multiple myeloma with abnormal laboratory parameters of bleeding may not have overt bleeding manifestations.

Correlation was done between serum total protein levels and bone marrow plasma cell percentage with serum calcium levels, rouleaux formation, PI, APTT, platelet aggregation studies and Congo red staining. However, no correlation was found between serum total protein levels and bone marrow plasma cell percentage with serum calcium levels, rouleaux formation, PT, APTT, platelet aggregation studies and Congo red staining (p value was >0.01 level in all). Huang et al.[12] observed correlation between serum M protein and prolonged Prothrombin time. However, no such correlation was observed by M Sikka et al.

There were many limitations in our study. We could not demonstrate clonality of plasma cells, could not carry out molecular and cytogenetic studies and follow up of the patients. We could not determine the heavy chain and light chain isotypes of the monoclonal protein. Most of these limitations were present as the study was undertaken in resource limited settings and most of our patients are of low socioeconomic status who cannot afford all such investigations.

CONCLUSION:

Therefore, to conclude, median age at the time of first diagnosis of multiple myeloma patients is younger in India than in western countries. Generalised weakness and easy fatiguability was the commonest presenting complaint and bone pain was relatively less common than in other studies.

Bone lesions were also relatively less common in our study as compared with other studies. Active bleeding was observed in 3 patients with 2 of them having epistaxis and one with per rectal bleed. Coagulation tests were abnormal in many cases of plasma cell dyscrasias but most patients were asymptomatic. Abdominal /gluteal fat pad aspiration and Congo red staining showed faint positivity in only one patient. This may be due to scant material obtained by aspiration. No significant correlation was found between serum total protein levels and bone marrow plasma cell percentage with serum calcium level, rouleaux formation, PT, APTT, platelet aggregation studies and Congo red staining.

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