Original Article

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Clinicopathological Profile of Patients with Multiple Myeloma: An Ambispective Study

Pathology Section

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ABSTRACT

Introduction: Multiple Myeloma (MM) is a clonal expansion of B lineage plasma cells in bone marrow. MM accounts for 10-15% of haematological malignancies and 1% of all malignant diseases. Though, the aetiology of the disease is largely unknown, various factors have been found to increase the incidence of MM. The present comprehensive research work on MM in the Malwa region of central India is an attempt to establish on clinicodemographic, haematological, biochemical and radiological parameters and finally to stage them according to Durie Salmon Staging (DSS) system.

Aim: To study clinicopathological profile of patients with MM.

Materials and Methods: The present ambispective study was conducted on total 70 cases of MM reported in the Department of Pathology at Sri Aurobindo Medical College and Post Graduate Institute, Indore, Madhya Pradesh, India, from July 2012 to June 2020. The study design included retrospective studies from July 2012 to December 2018 (six years and five months) and prospective studies from November 2017 to April 2019 (one year and five months). The demographic data of patients' including history and clinical findings were recorded. Complete Blood Count (CBC) was done in all cases. Biochemical investigations were also done. Urine samples of all patients were screened for the presence of Bence Jones protein. Biochemical parameters and staging of MM patients was done. Statistical analysis prepared in excels spreadsheet and quantitative data were presented as proportions.

Results: The study included a total of 70 cases of MM diagnosed on peripheral smear and/or bone marrow examination. The male:female ratio was 2.1:1, with a male preponderance. The results of the present study, reported 48 (68.57%) patients were male and 22 (31.43%) patients were females. In the present investigation, the authors diagnosed serum Lactate Dehydrogenase (LDH) levels were estimated in 65, out of 70 patients. Out of which, 25 (35.71%) patients had levels <240 U/L, while 23 (32.85%) and 17 (24.28%) patients had levels between 241-480 U/L and >480 U/L, respectively.

Conclusion: The present study, underlines the association between clinical presentation, laboratory parameters and radiological findings in establishing the diagnosis of MM. The present study emphasises not only the role of a bone marrow aspiration and biopsy in establishing a diagnosis of MM, but also, assessing the plasma cell burden in the marrow.

Keywords: Anaemia, Interleukin-6, Serum creatinine, Tumour necrosis factor-alpha, Vascular endothelial growth factor

INTRODUCTION

The MM is a clonal expansion of B lineage plasma cells in bone marrow associated with production of monoclonal Immunoglobulins (Ig) fragments and their increased concentration in serum and/or urine [1]. The term "Multiple Myeloma" was coined by Rustizky V in 1873 [2]. It is included in the category of mature B-cell neoplasms in 2008, World Health Organisation (WHO) classification of haematopoietic and lymphoid neoplasms [3].

Multiple myeloma accounts for 10-15% of haematological malignancies and 1% of all malignant diseases. It is seen in all races with a higher incidence in African Americans in comparison to Asian. The majority of people with MM are middle-aged or older people [1]. When compared to Western countries, the median age of the Indian population is around 55-62 years [4]. The incidence of MM increases with age and the prevalence of this disease is higher in the male population [1]. Though the aetiology of the disease is largely unknown, various factors like environmental, occupational, radiation exposure, benzene exposure, metal industries, pre-existing medical conditions and lastly genetic factors have been found to increase the incidence of MM [1].

The clinical manifestations arise as a result of bone marrow infiltration by clonal plasma cells and secretion of M proteins. These are characterised by a clinical pentad: anaemia, M protein in the serum/urine or both, bone pain and abnormal bone radiographs, hypercalcaemia and renal insufficiency/failure [1]. Bone pain is the commonest presenting symptom with most of the patients having punched out lytic lesions, osteoporosis, osteopenia or fractures on radiological examination [3].

Majority of patients on peripheral smear show normocytic normochromic anaemia and rouleaux formation. Sometimes, leucoerythroblastic blood picture or plasma cell leukaemia is also seen [5]. Bone marrow examination along with clinical, laboratory and radiological parameter is essential for establishing the diagnosis of MM. Plasma cell morphology, percentage and pattern of infiltration of plasma cells in marrow have significant correlation with clinical stage and survival. Higher percentage of plasma cell fraction is a reliable predictor of relapse in treated MM patients and it also helps to evaluate morphological remission and minimal residual disease in MM patients [6]. Most of the data on MM are from western countries. Due to paucity of data in medical literature, especially from Malwa region, the present study was conducted to evaluate clinical and laboratory parameters in cases of MM to establish local facts. Therefore, aim of the present study was to evaluate clinicopathological profile of patients of MM.

MATERIALS AND METHODS

The present ambispective study was conducted on total 70 cases of MM reported in central clinical laboratory of Department of Pathology, at Sri Aurobindo Medical College and Post Graduate Institute, Indore, Madhya Pradesh, India, from July 2012 to

June 2020. Prior to start of the study, the Institutional Ethics Committee (IEC) approval (Research protocol number ECR/748/Inst/MP/2015/RR-22) was taken. All the cases were of MM diagnosed on peripheral smear and/or bone marrow examination. The study included retrospective studies from July 2012 to December 2018 (six years five months) and a prospective study from November 2017 to April 2019 (one year five months).

Sample size calculation: In the present investigation, 6-7 cases of MM were diagnosed per year on peripheral smears and/or bone marrow aspirates in the Department of Pathology. Hence, the study was feasible for a sample size of 50 cases in a duration of eight years. During the present study, the authors found extra 20 cases. Therefore, overall 70 cases of MM selected for the present study on the basis of inclusion criteria.

Inclusion and Exclusion criteria: In present study, all cases of MM diagnosed on peripheral smear and/or bone marrow examination were included. Moreover, clinically suspected cases of MM but not proved on peripheral smears and/or bone marrow examination and subjects on treatment for MM were excluded from the present study.

Study Procedure

A demographic, clinical, haematological, radiological, biochemical data were collected from the bone marrow record registers from the Department of Pathology and Minimal Residual Disease (MRD) files for the retrospective cases whereas for the prospective cases all the patients' details, history and clinical findings were recorded as per the proforma in all cases of MM.

The CBC was done in all cases by Haematology Beckman coulter LH 750. Peripheral blood and bone marrow smears were stained by Geimsa stain, while Haematoxylin and Eosin (H&E) staining were performed on received bone marrow biopsies. CBC, Erythrocyte Sedimentation Rate (ESR) findings were correlated with those of peripheral smear and bone marrow aspirates. Stained peripheral blood and bone marrow smears were also examined for rouleaux formation and background staining.

Biochemical investigations i.e., serum levels of calcium, creatinine, lactate dehydrogenase and protein were done in all cases by VITROS 5,1 FS system (Ortho-Clinical Diagnostics-J&J Company) in the central clinical laboratory of the Institute. Samples of serum and protein were outsourced to referral laboratory for electrophoresis testing and reports obtained were interpreted. The reports were given in the terms of serum β_2 microglobulin levels, serum lactate dehydrogenase, serum calcium, serum creatinine level and serum albumin level with a normal range of 0.70-3 µg/mL, 125-240 IU/L, 9-10.2 mg/dL, 0.72-1.4 mg/dL and 3.5-5.2 mg/dL, respectively. Investigation of MM is based on the selection criteria revealed in 2003 by International Myeloma Working Group, as well as, adapted by WHO in 2008 [7].

Urine samples of all patients were screened for the presence of Bence Jones protein by the heat test. The disease was diagnosed on the basis of haematological, clinical, and radiological and biochemical parameters and staging of MM patients was done according to the diagnostic criteria laid by Durie BG and Salmon SE [8]. Broad morphological classification of MM:

Plasma Cells (PC) were further classified into two broad groups: (1) the plasmacytic group, with non nucleolated PCs; and (2) the plasmablastic cell group, with nucleolated PCs [9].

STATISTICAL ANALYSIS

A structural proforma was used to collect the data. Data was entered in a Microsoft Excel spreadsheet. Quantitative information was presented as proportions and also presented in percentage. A mean and standard deviation were used to express quantitative data.

RESULTS

The study included a total of 70 cases of MM diagnosed on the basis of peripheral smear and/or bone marrow examination. These patients were also studied for clinical presentations, biochemical and radiological investigations. The presenting age of the patients ranged from 36 to 82 years with an average age of 58.26 years. Out of the 70 patients, majority i.e., 21 (30%) patients were aged between 51 and 60 years followed by 20 (28.57%) patients between 61 and 70 years. Only 3 (4.29%) patients were less than 40 years of age. The male:female ratio was 2.1:1, with a male preponderance. The results of the present study, reported 48 (68.57%) patients were males and 22 (31.43%) patients were females.

Low backache 67 (95.71%) patients were the most common clinical features observed in MM patients. Generalised weakness and pallor were present in 32 (45.71%) and in 22 (31.43%) patients, respectively. A total of 24 (34.28%) patients presented with symptoms of renal failure (anuria and/ oedema). Other symptoms like weight loss, infection (urinary tract infection and diarrhoea), pain abdomen, breathlessness (due to pneumonia and Chronic Obstructive Pulmonary Disease (COPD) and neurological manifestations (paraplegia, paraparaesis and neurogenic bladder) were present in 10 (14.29%), 8 (11.43%), 5 (7.14%), 4 (7.14%) and 6 (8.57%) patients, respectively [Table/Fig-1].

Age range (in years)	No. of participants (n=70)	Percentage (%)
31-40	4	5.71
41-50	16	22.86
51-60	21	30
61-70	20	28.57
71-80	6	8.57
81-90	3	4.29
Gender		
Male	22	31.43
Female	48	68.57
Clinical features		
Low backache	67	95.71
Generalised weakness	32	45.71
Pallor	22	31.43
Weight loss	10	14.29
Renal Insufficiency	24	34.28
Infection	8	11.43
Pain in abdomen	5	7.14
Breathlessness	4	5.71
Neurological manifestations	6	8.57

[Table/Fig-1]: Demographic data and clinical features.

Haemoglobin values ranged from 4.8 gm% to 13.4 gm% with a mean of 9.06 gm%. Haemoglobin was <8.5 gm% in 25 (35.71%) patients, whereas 45 (64.29%) had haemoglobin value >8.5 gm% [8].

Peripheral Blood Smear (Rouleaux Formation)

Anaemia (<10 gm%) on peripheral smear was observed in 45 out of 70 patients (64.29%) [Table/Fig-2]. Rouleaux formation was observed on the peripheral smear of 41 (58.57%) patients, of which 32 (78.04%) patients had plasmacytic type myeloma and 9 (21.95%) patients had plasmablastic type myeloma. Plasma cells were found in the peripheral blood smear of 4 (5.71%) cases. Out of which, 2 (2.85%) cases were of plasmacytic type and 2 (2.85%)

plasmablastic type. Leukemoid reaction was found in the peripheral blood smear of 1 (1.43%) case and 1 case (1.43%) presented with plasma cell leukaemia (29% plasma cells on PS).

Initial investigation	Absent	Present	N
Osteolytic lesion, osteoporosis, fracture {X-rays, Computed Tomography (CT) scan}	5	65	70
Urine Bence Jones proteins	54	6	60
M band (serum electrophoresis)	1	69	70
Rouleaux formation (Peripheral smear)	29	41	70

[Table/Fig-2]: Distribution of cases of MM according to initial investigations.

As shown in [Table/Fig-3], serum LDH levels were estimated in 65 out of 70 patients. Out of which 25 (35.71%) patients had levels <240 U/L, while 23 (32.85%) and 17 (24.28%) patients had levels between 241-480 U/L and >480 U/L, respectively; whereas the serum β_2 microglobulin levels were available in only 30 (42.85%) patients. Renal insufficiency (i.e., S.creatinine ≥2 mg/dL) was seen in 41 (58.57%) patients. Whereas, the majority of the patients 54 (77.14%) present with serum albumin levels below 3 gm/dL.

Variable	Level	No. of patients	Percentage (%)
Serum β_2 microglobulin (µg/mL)	Positive (≥3 µg/mL)	25	35.71
	Negative (<3 µg/mL)	5	7.14
	NA	40	57.14
Serum lactate dehydrogenase (IU/L)	<240	25	35.71
	241-480	23	32.85
	>480	17	24.28
	NA	5	7.14
Serum calcium levels (mg/dL)	<10.2	33	47.14
	10.3-10.9	10	14.28
	≥11	27	38.57
Serum creatinine levels (mg/dL)	≤1.4	21	30
	1.5-1.9	8	11.42
	≥2	41	58.57
Serum albumin levels (gm/dL)	<3	54	77.14
	>3	16	22.85

[Table/Fig-3]: Blood investigations.

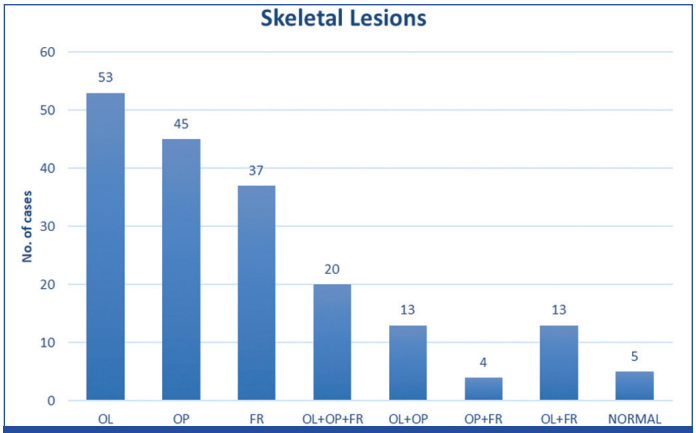
Radiological Findings

Radiological findings were available in all 70 patients. The skeletal lesions were present in 65 (92.86%) patients as osteolysis, osteoporosis and fractures; either singly or in various combinations. The commonest radiological findings were osteolytic lesions present in 53 (75.71%) patients followed by osteoporosis found in 45 (64.29%) patients. Fractures were found in 37 (52.86%) patients and combination of osteolysis and fractures were seen in 13 (18.57%) patients. Osteoporosis was found in 45 (64.29%) patients, and combination of osteoporosis and osteolysis in 13 (18.57%) patients, of osteoporosis and fracture in 4 (5.71%) patients and combination of all three in 20 (28.57%) patients. Normal radiographs were observed in 5 (7.14%) patients [Table/Fig-4].

Site of osteolytic lesions: The osteolytic lesions were present in 53 (75.71%) patients which ranged from single to multiple and involved the skull, pelvis, vertebrae, long bones, like the femur and humerus, and flat bones like the scapula, ribs, clavicle and mandible. In 42 (60%) cases, Skull was the most common site to be involved.

Sites of Fractures

Presented data with fracture of single or multiple site like fracture of the pelvis and long bones was seen in only 1 (2.70%) patient, no patient was seen of clavicle and compression fractures of vertebrae in 35 (94.59%) patients [Table/Fig-5].



[Table/Fig-4]: Graphical representation of skeletal lesions in cases of MM.
OP: Osteoporosis; OL: Osteolytic lesion; FR: Fracture

Site	Frequency	Percentage (%)
Cervical and thoracic vertebrae	5	13.51
Thoracic and lumbar vertebrae	30	81.08
Long bones	1	2.70
Clavicle	0	0
Pelvis	1	2.70

[Table/Fig-5]: Distribution of fracture sites in cases of MM (n=37).

Bone Marrow Aspiration and Biopsy

Bone marrow aspirate was performed in all the 70 patients; however, it was reported as haemodiluted in three patients. Hence, the [Table/Fig-6] shows percentage of PCs in bone marrow aspiration and biopsy in 67 patients. The percentage of plasma cells ranged from 10-90% with a mean of 35.90%.

Percentage of PCs in aspirate	Percentage of PCs in biopsy			
	<20%	20-50%	>50%	Total
<20%	5	16	0	21
20-50%	0	23	10	33
>50%	0	0	13	13
Total	5	39	23	67

[Table/Fig-6]: Percentage of PC infiltrate in bone marrow aspirate and biopsy in cases of MM.

Bone Marrow Biopsy (BMB)

The BMBs of all 67 (95.71%) patients were reviewed for assessment of percentage of plasma cells and their infiltration pattern. A total of 39 patients had the plasma range between 20-50%. Whereas, 23 patients showed >50% of plasma cells and five patients showed <20% of plasma cells. Majority of patients revealed presence of plasma cells (on bone marrow biopsy) to be in the range of 20-50%, 42 (60%) and the most common pattern of infiltration on biopsy was the interstitial, 30 (42.86%) one followed by diffused pattern, 19 (27.14%), while the least common was nodular pattern, 4 (5.71%).

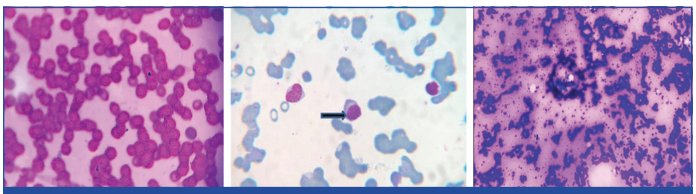
As shown in [Table/Fig-7], {C=Hypercalcaemia, R=Renal failure, A=Anaemia, B=Bone pain (CRAB)} features were studied in all 70 patients. Twelve (17.14%) cases showed the presence of all the four features $C^+R^+A^+B^+$ i.e., hypercalcaemia (S.calcium ≥11 mg/dL), renal insufficiency (S.creatinine ≥2 mg/dL), anaemia (Hb <10 gm%) and bony lesions. Six (8.57%), 1 (1.43%), 2 (2.86%) and 11 (15.71%) cases had only three features i.e., $C^+R^+A^+$, $C^+A^+B^+$, $C^+R^+B^+$ and $R^+A^+B^+$, respectively. Two (2.86%) C^+A^+, 7 (10%) R^+B^+, 9 (12.86%) A^+B^+, 2 (2.86%) C^+ B^+ and 3 (4.29%) R^+A^+ patients presented with only two features from the CRAB criteria while 1 (1.43%) C^+, 1 (1.43%) A^+ and 9 (12.86%) B^+ patients presented with only one feature. Four cases (5.71%) of smouldering MM were also observed.

CRAB features	Frequency (n)	Percentage (%)
C, R, A, B	12	17.14
C, R, A	6	8.57
C, A, B	1	1.43
C, R, B	2	2.86
R, A, B	11	15.71
C, A	2	2.86
R, B	7	10
A, B	9	12.86
C, B	2	2.86
R, A	3	4.28
C	1	1.43
R	0	0
A	1	1.43
B	9	12.86
SMM	4	5.71

[Table/Fig-7]: Showing the CRAB features in cases of MM.
C: Hypercalcaemia; R: Renal failure; A: Anaemia; B: Bone pain; SMM: Smoldering multiple myeloma

The hypercalcaemia was observed in 27 (38.57%) patients, features of renal insufficiency were seen in 41 (58.57%) cases, anaemia was noted in 45 (64.28%) cases and patients that presented with bony lesions were 53 (75.71%). Majority of patients 53 (75.71%) presented with stage III of which 19 (27.14%) presented with stage IIIA and 34 (48.57%) presented with stage IIIB. Seven (10%) patients presented in stage IIA and 7 (10%) in stage IIB. Three (4.29%) patients presented in stage IA.

The results can be further explained in photomicrographic form of peripheral Smear Rouleaux Formation (fields Stain 400X) [Table/Fig-8], plasma cell on peripheral Blood Smear (Geimsa Stain 1000X) [Table/Fig-9], and Bone Marrow Aspiration (BMA) showing Bluish Hue and rouleax formation (Geimsa stain, scanner view) [Table/Fig-10]. These results are indicative of the characteristic rouleaux formation, increased levels of plasma cells and increase in monoclonal immunoglobins (cause of the bluish hue in the MM patients).

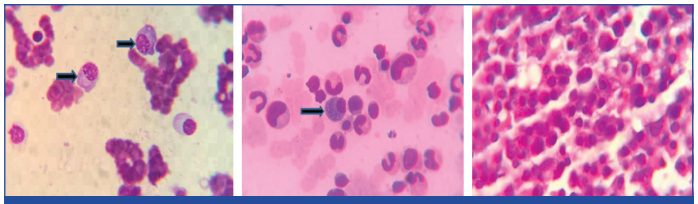


[Table/Fig-8]: Photomicrograph of peripheral smear Rouleaux formation (fields stain 400X).
[Table/Fig-9]: Photomicrograph of showing plasma cell on peripheral blood smear (Geimsa stain 1000X).
[Table/Fig-10]: Photomicrograph of Bone Marrow Aspiration (BMA) shows bluish hue and Rouleax formation (Geimsa stain, scanner view). (Images from left to right)

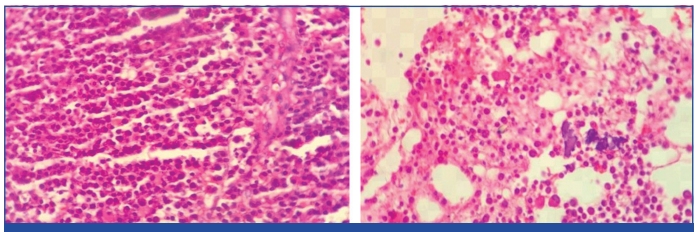
Furthermore, images of flame cells and Russell body cells [Table/Fig-11a,b] along with mature and immature plasma cells infiltration in BMB [Table/Fig-12] are cited in the current literature to further validate the findings. Diffuse pattern and interstitial pattern of infiltration of plasma cells infiltrating bone marrow were also observed, which can be visualised under [Table/Fig-13a,b]. Similarly, [Table/Fig-14a,b] showing plasma cells with binucleated forms and plasma cells with multinucleate forms were also quoted in the text.

Prognostic Variables

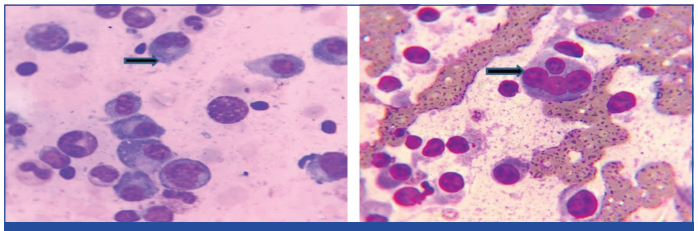
In the plasmablastic group, total of 18 patients were involved among which, most of the patients were more than 50 years of age, 14 (77.78%); 11 (61.11%) of which were having serum creatinine levels of more than or equal to 2 mg/dL and 15 (83.33%) of them having albumin levels of less than 3 g/dL.



[Table/Fig-11a]: Photomicrograph showing flame cells and Russell body cells on BMA (Geimsa stain, 1000X).
[Table/Fig-11b]: Photomicrograph showing flame cells and Russell body cells on BMA (Geimsa stain, 1000X).
[Table/Fig-12]: Photomicrograph showing both mature and immature plasma cells in filteration in BMB (H&E, 1000X). (Images from left to right)



[Table/Fig-13a]: Photomicrograph of showing diffuse pattern and interstitial pattern of infiltration of plasma cells infiltrating bone marrow biopsy (H&E, 400X).
[Table/Fig-13b]: Showing the diffuse pattern and interstitial pattern of infiltration of plasma cells infiltrating bone marrow biopsy on pertivular area ofthe slide (H&E, under 400X). (Images from left to right)



[Table/Fig-14a]: Photomicrograph of BMA showing plasma cells with binucleated forms (Geimsa stain, 1000X).
[Table/Fig-14b]: Photomicrograph of BMA showing plasma cells with multinucleated forms (Geimsa stain, 1000X). (Images from left to right)

DISCUSSION

The present study comprised of 70 cases of MM, diagnosed on CBC and peripheral smear and further assessed by bone marrow examination and biochemical tests over duration of eight years. The present study is an attempt to focus on various causes, clinical presentation and various haematological, radiological and biochemical parameters.

The present study showed renal failure as the third most common presenting feature of MM which is in concordance with the studies by Subramanian R et al., and Kaushik R et al., [10,11]. The latter study has also reported equal incidence of both fever and renal failure amounting to the third most common presenting feature, whereas Vahini G et al., have reported generalised weakness and pallor while Basharat S et al., have reported fatigue as the third most common presenting symptom of MM [12,13].

On peripheral smear findings, in the index study, anaemia (<10 gm%) was observed in majority i.e., 64.29% patients. These findings were in concordance with the findings of Sultan S et al., (63.9%) [14]. Rouleaux formation was seen in 41 (58.57%) patients in the present study. This finding was consistent with the study of Vahini G et al., [12] (50%) and closer to the study of Kaushik R et al., [11] (70%) while Subramanian R et al., [10] and Kaur P et al., [15] have reported rouleaux formation on peripheral smear exam in (91%) and (82%) cases, respectively.

One of the most important diagnostic criteria of CRAB symptoms is hypercalcaemia. However, it was seen only in 26 (37.14%) patients. These findings were in concordance with the studies of Kaushik R et al., Basharat S et al., Kaur P et al., Diwan AG et al., and Chowdhury MRK et al., where hypercalcaemia was reported in 11.76%, 17.5%, 42.8%, 5% and 9.38% patients, respectively

[11,13,15-17]. In the index study, diagnosis of MM was made in those with normal calcium levels (8.4-10.2 mg/dL) in 25 cases (35.71%) and below normal levels (<8.4 mg/dL) in 9 patients (12.86%). Low calcium levels might be due to renal impairment. This could also be due to varying food habits, or due to different environmental factors. However, 3 (4.29%) patients had low calcium levels without renal impairment. This suggests that hypercalcaemia is not always a part of MM and hence, a high index of suspicion is necessary to diagnose MM. Hypocalcaemia in MM secondary to vitamin D deficiency has been reported previously [18]. Vitamin D deficiency could be another factor contributing to hypocalcaemia other than renal failure in myeloma patients.

Renal function impairment is a ubiquitous phenomenon in MM. The main cause of renal failure is cast nephropathy due to light chain excretion and glomerular deposition of Ig. Other major causes include myeloma kidney, hypercalcaemia and dehydration. In the current study, raised serum creatinine i.e., ≥2 mg/dL was found in 41 (58.57%) patients, which is similar to the findings in the study of Kaur P et al., (2014) (77.3%) [15].

In the present study, hypoalbuminemia (serum albumin <3 g/dL) was seen in 77.14% patients with A:G ratio reversal in 92.86 patients. While Chowdhury MRK et al., and Kyle RA et al., reported hypoalbuminemia in 15% and 43.75% patients, respectively [17,19]. Serum albumin level was found to be a significant prognostic factor for assessing disease severity in symptomatic MM in a previous study. The present study shows a higher percentage of cases with hypoalbuminemia because of late stage of presentation and enrolment of a limited number of patients. Serum β_2-microglobulin is one of the most important prognostic factors though raised β_2-microglobulin level is a poor prognostic sign. In present study, 83.33% patients had their serum β_2-microglobulin ≥3 µg/mL.

The M band is detectable in 97% of myeloma patients. They may have either intact Ig or fragment or a free light chain on serum/urine protein electrophoresis, whereas cases with non detectable monoclonal proteins are referred to as non secretors (1-3%) [1]. Serum LDH levels were estimated in 63 out of 70 patients. More than half (58.73%) of MM patients included in the index study presented with elevated serum concentrations of LDH (>280 IU/L). These findings were in agreement with the studies of Jurczyszyn A et al., where (49%) patients reported raised serum LDH levels while the above observations were slightly higher than Shin J et al.,; wherein LDH levels above normal were seen in (37%) cases [20,21]. In the early stages of MM, lactate dehydrogenase levels are rarely raised; nevertheless, as the prognosis of disease, LDH levels rise to levels higher than at diagnosis [22]. Patients with high LDH levels have a shorter median overall survival than those with normal LDH levels.

The level of tumour mass can be assessed by LDH levels hence their increase during the course of the disease may refer to the increased levels of tumour, relapse or the existence of extra plasmacytomas [23]. Radiological findings were available in all 70 patients in whom osteolytic lesions was the most common skeletal lesion 75.71% followed by osteoporosis, which was seen in 64.29% cases. In the present study, osteoporosis was reported in a higher percentage of patients i.e., in (64.29%) while Diwan AG et al., have reported in (40%) [16]. The presence of pathological fracture in the present study was seen in 52.86% patients, which was in agreement with the study by Sharma A et al., (57.4%) [24].

Skull was the most common site of osteolytic lesion (79.25%) which was comparable to Kaur P et al., [15] while the most common site of pathological fracture was thoracic and lumbar vertebrae (81.09%), which were in agreement with the study by Jayashankar E and Roshinipaul T [25]. Bone marrow examination is the cornerstone of the diagnosis of MM along with other laboratory and clinical parameters. Bone marrow examination is essential not only for demonstration of plasmacytosis (as a major criterion for diagnosis of MM), but it also provides valuable information regarding plasma cell morphology and infiltration pattern, which can help in determining the prognosis of the disease [10].

In the index study, the median plasma cell percentage on the BM biopsy (67%) was higher than that on BM aspirate (48%). Also, the number of cases >50% plasma cells on the BM biopsy were significantly higher than the BM aspiration. It was also observed that 58% of patients had plasma cell infiltrate in the range of 20-50% on the biopsy which was closer to but slightly lower on the aspirate (52%). Thus, BM biopsy PC percentage is a better indicator of tumour load than BM aspirate. Similar findings were observed in a study by Pich A et al., where PC percentage on BM biopsy (50%) was higher than on BM aspiration (38%) [26]. Romanowsky stained BM aspiration is subject to multiple preanalytical and analytical variables. Even though the BMA provides excellent morphological details, the number of PCs estimated may be less than the actual burden, as MM is often a focal process [27]. Adhesion markers such as CD56 are expressed by PCs due to which they tend to bind to each other as well as to the stroma. This phenomenon explains their tendency to aggregate on the trephine biopsy. As a result of the strong adhesion, they may be under represented on the BMA smears [28]. Due the lipophilic nature they remain trapped in the bone marrow spicules; even when they are aspirated, therefore, the differential counts are performed in the cell trails. Since PCs distribution in the marrow spicules is variable, hence, there is interobserver variability. In approximately 9% of cases of MM, the bone marrow may be fibrotic and, in such cases, PCs are underestimated on BMA smears as a result of haemodilution [29]. Hence, both BM smears and sections should be evaluated. In the present study, mature myeloma (65.67%) was the most common variant and intermediate myeloma (7.46%) was the least common variant. Similarly, the study by Sharma A et al., also showed mature myeloma (50%) as the most common variant and intermediate myeloma (2%) the least [24]. The first histological classification and staging criteria published by Bartl R et al., was based upon morphological features on BM aspiration and biopsy [30]. They classified plasma cells into two categories: plasmacytic- with predominantly non nucleolated PCs and plasmablastic- with predominantly nucleolated PCs. In the present study, myeloma was further classified into two broad categories, depending on the location of the nucleus, presence of nucleoli and nuclear chromatin. These criteria of morphological classification of plasma cells are similar to criteria used by Bartl R and Frisch B [30]. Out of 67 cases, nearly 73.13% had plasmacytic morphology and 26.87% had plasmablastic morphology. This finding was in agreement with the study by Sharma A et al., where 52% cases had plasmacytic morphology while 48% showed plasmablastic morphology [24]. In MM patients, various patterns of infiltration of bone marrow can be observed namely, interstitial, focal/nodular, diffuse, mixed and paratrabecular. In the present study, majority of cases had interstitial involvement (42.86%) followed by diffuse (27.29%), mixed (24.29%) and nodular (5.71%). Similarly, interstitial infiltration was also the predominant pattern of infiltration in the study by Sharma A et al., (46%) [24].

In other words, evidence or absence of end organ damage or the so-called CRAB criteria helps in reaching a differential diagnosis amongst active symptomatic MM, Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smouldering Multiple Myeloma (SMM) [31]. It is vital to distinguish these two things when

it comes to classification, diagnosis and therapy. The prognosis of MM is influence by CRAB factors. In the present study, CRAB features were studied in all 70 patients. Results were in agreement, with the study by Talamo G et al., who studied 170 consecutive cases of MM and found among patients with symptomatic MM, 74% presented with CRAB features [32].

The current staging system being followed is the Revised International Staging System (RISS), however, in developing countries like India, many hospitals continue to follow the Decision Support System (DSS), mainly because its parameters are easily available and affordable. International Staging System (ISS) though easier to follow because of limited parameters, requires $\beta_2$microglobulin levels while RISS in addition to $\beta_2$microglobulin levels also requires cytogenetic study by iFISH which is done in very few centres and is also expensive. Yet, even in the DSS system, compliance to all the criteria like immunofixation and quantification of monoclonal proteins in urine and serum are difficult due to financial constraints and lack of availability. Thus, the current study followed the DSS system and staged patients based on haemoglobin, serum creatinine, serum calcium, serum electrophoresis and skeletal survey by plain radiography. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). Majority of the patients presented very late in the disease and this could be the cause for most of the present study participants belonging to Durie Salmon stage III. However, this was in concordance with the studies by Sharma A et al., (56.8%) [24].

Similar to the present study, the study by Greipp PR et al., found higher age of presentation (median age=65) and lower serum albumin levels (median=3.3 gm/dL) in patients with plasmablastic morphology than in non plasmablastic (median age=3.6 gm/dL) and (median=63), respectively [33]. Thus, we can say that PB cases tend to have more aggressive and advanced disease manifested by more frequent hypercalcaemia, renal insufficiency, anaemia and a lower albumin level.

Median survival can be reduced by several months by the presence of a single adverse prognostic factor. An important question is, which single factor or whether a combination of factors provides the best prognostic information for patient care. A risk-based staging system that uses a combination of independent prognostic factors provides superior prognostic information than any prognostic factor alone [34]. An international prognostic index for myeloma, that uses a combination of well-validated and readily available yet simple factors, is being developed by a panel of top myeloma researchers from around the world.

Limitation(s)

The present study was, both a retrospective and prospective study and its findings cannot be generalised. Immunohistochemistry was not available in any of the cases. However, immune fixation was available in 30 cases only. Lastly, there was a lack of follow-up data to see the disease progression and median survival. Despite the above limitations; the present study was a regional study, which provided essential informative data for stratification in present set-up.

CONCLUSION(S)

The present study establishes a correlation between clinical, laboratory and radiological findings of MM. The clinical outcomes include low backache and lytic lesions on skeletal survey. Bone marrow aspirate and biopsy revealed predominant presence of plasmacytic morphology, interstitial pattern of infiltration in stage III patients and low cellularity in SMM.

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