"Multiple Myeloma: A clinicopathologic and cytogenetic analysis of 45 patients in a tertiary care centre."

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ABSTRACT

Aims & Objectives:Clinico-pathological analysis of all cases diagnosed as MM. To find genetic abnormalities by cytogenetics & fluorescence in situ hybridization(FISH) in this heterogenous group.
Material and Method: All the cases of MM diagnosed between January 2012 to July 2014 were included in the study. The clinical details including presentation, electrophoresis, renal parameters were taken from clinical records. The bone marrow slides were retrieved and reviewed. Conventional cytogenetics and FISH analysis was performed in 14 and 8 patients respectively.
Results: The study included 45 patients in the age range of 40-82 years with male predominance (M:F=1.5:1). Most of the patients (21) presented with complaints of back pain and weakness. Renal dysfunction was seen in 29 patients with mean creatinine of 2.5mg/dl. Immunoelectrophoresis revealed M band in 19 cases. The bone marrow plasma cell ranged from 15% to 90%. The commonest morphology was that of Marshalko type whereas plasmablastic type was rare. The cytogenetic abnormalities detected were aneuploidy, trisomy 1 and 9, 13q deletion.
Conclusion: This study reflects our experience of 45 patients of MM with respect to clinical features, bone marrow morphology and cytogenetic analysis.
Key Words: Multiple myeloma, bone marrow, cytogenetics

Introduction

The term myeloma was coined by Rustizky in 1873.Multiple myeloma (plasma cell myeloma, myelomatosis, or Kahler disease) is a rare form of adult hematological malignancy accounting for 13% of all hematologic malignancies.Multiple myeloma (MM) is a paraproteinemiacharacterized by neoplastic/clonal proliferationof plasma cells (B-cells) in the bone marrow.

The spectrum of diseases ranges from monoclonal gammopathy of unknown significance (MGUS) to plasma cell leukaemia. The neoplastic cells produce monoclonal immunoglobulin (M protein) and free light chain proteins designated as kappa or lambda. Depending on the type of immunoglobulin produced, multiple myeloma (MM) can be classified into IgA MM, IgD MM, IgE MM, IgM MM, IgG MM, light chain (L.C) MM, and non-secretory MM [1].
It generally affects elderely between ages of 60-70 years with slight male prepondrance.The primary cause of multiple myeloma is idiopathic. Genetic predisposition, radiation , exposure to industrial and agricultural toxins may be implicated in some of the cases.
The clinical symptoms that are suspected for a plasma cell disorder include back pain, weakness or fatigue, osteopaenia, osteolytic lesions, spontaneous fractures and recurrent infections [2]

Hypercalcemia, renal impairment, anemia, and lytic bony lesions(CRAB symptoms)are currently the clinical diagnostic criteria for myeloma [2,3]Morphology of the plasma cells, beta-2 microglobulin levels, end organ damage and the stage of the disease are the prognostic parameters.

MM is associated with complex cytogenetic and molecular abnormalities which can be of prognostic significance. The detection of cytogenetic abnormalities in multiple myeloma is important for risk stratification and the new treatment strategies. Risk stratification with Cytogenetics and FISH studies have helped classify patients into high risk, intermediate risk and good risk categories which are helpful in guiding therapy and in predicting resistance to drugs in theses patients. [4]

Detection of chromosomal abnormalities in MM by conventional cytogenetics and specific target arrangements by Fluorescence in situ hybridization (FISH) should be included in the initial diagnostic workup for patients suspected of MM.

Table 1.WHO 2016: Mayo Stratification of Myeloma and Risk-Adapted Therapy. Adapted from Chesi M and BergsagelPL [4]

Standard risk (60%)	Intermediate risk (20%)	High risk (20%)
t(11;14)	t(4;14)	Del 17p
t(6;14)	Del 13	t(14;16)
Hyperdiploid	Hypodiploid	t(14;20)
All others		GEP high-risk signature
OS: 8-10 years	OS: 4-5 years	OS: 3 years

GEP, gene expression profiling; OS, overall survival

Although MM is not curable, treatment options like autologous hematopoietic stem cell transplantation and advances in chemotherapy have improved the quality-of-life and increased survival.[2]

In our study,clinico-pathological analysis of all cases diagnosed as MM was done. We tried to look into genetic abnormalities by cytogenetics & fluorescence in situ hybridization(FISH) in this heterogenous group which can be of prognostic significance.

Materials and Methods:

Retrospective analysis of 45 cases of MM diagnosed at Dept.of Pathology was done.

Clinical parameters included weakness due to reduced hemoglobin, backache, lytic lesions of bones
Lab findings include biochemical and hematological parameters.
Biochemical parameters include total serum proteins, serum albumin levels, A/G ratio, blood urea, serum creatinine,serum calcium.
The peripheral smears, BMA/BMBx slides were reviewed. Hematological parameters include Hemoglobin levels (<10 g/dl or >10 g/dl), Rouleaux formation on peripheral smear, Erythrocyte Sedimentation Rate ESR(raised).
Bone marrow aspiration and bone marrow biopsy are done in all the cases. Percentage of plasma cells in the aspirate was estimated by a 500-cell count (10-30% or>30%).
Trephine biopsy was decalcified in 1% acetic acid. Trephine biopsy was referred to as adequate if the trephine had at least 3 marrow spaces below the sub-cortical space and the aspirate had a goodnumber of particles .Bone marrow biopsy slides were reviewed for the pattern of involvement(nodular, interstitial,mixed and diffuse) and plasma cell morphology.
Bone marrow aspirates processed for cytogenetic workup by IL6 based Karyotyping (14 cases) ,iFISH/mFISH analysis (8 cases) and Zeiss microscope with Metasystem Software. Karyotype were analyzed on Ikaros Software. Interphase and metaphase FISH studies performed with 13q14.3, t(4;14)/FGFR3 and P53 on Isis .

Results (n=45) :

Age of the patients ranged from 30years to 80years.Majority of the patients are between 30-50 years of age with median age of 48 years.The disease is more common in males than in females with M:F of 2:1.Total number of males are thirty and females are fifteen.(Bar diagram B-1)

Most common clinical features are backache and bony pains due to osteolyticlesions.Other features include generalised weakness and joint pains.(Bar diagram B-2)

Hemoglobin levels of less than 10gm% are seen in 62% of the cases.Peripheral smear examination revealed rouleaux formation in 28 cases.ESR is elevated (≥25mm in 1st hour)in 29 cases.75% of the cases showed plasma cells in the range of 10-30% on bone marrow aspiration.11 cases shoed plasm cells >30%

Results of hematological parameters are summarised in Table 2

Bone marrowbiosy(BMB)was done in all the cases. Mixed pattern- Interstitial & Nodular were seen in42% cases.Nodular pattern as seen in 20 %cases and Interstitial pattern as seen in 24% cases.Diffuse type with packed marrow was seen in 6 cases

BMB (pattern of involvement) issummarised in Table 3

In the present study, albumin levels were low in 7 cases , Low A/G ratio was seen in 33 cases. Hypercalcemia is seen in 8 cases (33%), hyperuricemia in 32 cases.Increased serum creatinine levels were seen in 28 cases

Biochemical parameters are summarised in bar diagram B-3

Serum protein electrophoresis was done in 30 cases. 25 patients in the study had the Thick M band and the other 5 cases had thin M band on serum protein electrophoresis. Out of 30 positive cases, M-spike was localized in the gamma region (γ) in 25 cases, beta region (β) 3 cases, and α2 region in 2 cases. (Table-4)

Beta 2 microglobulin is <3.5 in 18 cases, 3.5-5.5 in 12 cases, > 5.5 in 15 cases.(bar diagram B-4)

Conventional cytogenetics performed among 14 cases, showed, diploidy, trisomies, near haploidy/haplodiploidy, In our study chromosome 13 is commonly involved. FISH study performed among 8 cases, they showed 13q 14.3 deletion in 3, normal in 5.

Cytogenetics and FISH Results are summarised in Table 5.

Discussion:

Multiple myeloma is a disease of elderely.In our study, age range is from 40-82 years with a mean age of 48 years whereas Dash [5],Kapoor[6], Phekoo[7]and Hussain[8]reported mean age of 64, 65, 73 and 61 years, respectively. Majority of the patients are between 30-50 years of age.

M;F ratio is 2;1 with a male predominance.Similar findings were observed by Dash[5],Kapoor[6], and Phekoo[7] Female predominance as observed by Hussain [8]

Most common clinical features are backache and bony pains due to osteolytic lesions. The malignant cells cause inhibition of osteoblasts and stimulation of osteoclasts leading to the appearance of lytic bone lesions. Hypercalcemia is due to the increase in osteoclastic activity. Anemia was found in 62% cases with hemoglobin of less than 10gm% as compared to other studies [7,8,9,10]. The anemia was mostly normocytic normochromic.Underlying pathophysiology is anaemia of chronic disease,relative erythropoietin deficiency due to renal impairment and myelosuppression due to chemotheraphy.Replacement of normal hematopoietic precusors by the clonal plasma cells is also the reason for cytopenias.

Peripheral smear examination revealed rouleaux formation in 28 cases(62%) in contrast to Diwanetal[11]where it was observed in only 35% of cases

Erythrocyte sedimentation rate (ESR) is elevated (≥25mm in 1st hour)in 64% cases as compared to Hussian etal[8] whereas Diwan[11] reported 100% cases with elevated ESR. Immunoglobulins secreted by plasma cells cause increase in blood viscosity which leads to increased ESR

In normal individuals, plasma cells constitute 1% in the bone marrow but in multiple myeloma, clonal plasma cells in bone marrow increases up to 80% depending on severity [11].
In our study,75% of the cases showed plasma cells in the range of 10-30% on bone marrow aspiration. The mean bone marrow plasma cells were 66%, and >10% in 70% of cases in a study by Hussianetal 8 as against the 31% and >10% in 92% cases reported by Dash [5].
The infiltration pattern of myeloma is classified as one of three patterns – nodular, interstitial, or diffuse, and these patterns are often mixed[12]
In the nodular pattern, myeloma cells displace normal hematopoietic cells and fat cells, and form a clear nodular lesion in the bone marrow. The distribution of nodular lesions is unequal in the bone marrow.
In the interstitial pattern, myeloma cells form a small cluster and infiltrate in between normal hematopoietic cells. Identification of a myeloma cells by immunohistochemical staining is necessary sometimes in these cases.
In the diffuse pattern, a large number of myeloma cells diffusely infiltrate the bone marrow, and the expansive areas are replaced by myeloma cells. The diffuse pattern progresses from a localized nodule to an interstitial pattern. In this pattern,normal hematopoietic cells are markedly reduced. Bone marrowbiosywas done in all the cases. Mixed pattern- Interstitial and nodular were seen in42% cases.Nodular pattern as seen in 20 %cases and Interstitial pattern as seen in 24% cases. Diffuse type with packed marrow was seen in six cases.
Serum albumin is a significant prognostic factor that reflects the severity of disease progression.Serum albumin is reduced due to cytokine impairment of albumin synthesis and excess degradation. Other causes include such as malnutrition, renal dysfunction, and hepatic impairment[13–15].
In the present study, albumin levels were low in 7 cases 15% in contrast to Hussain et al [8] where it as seen in 55% of cases. This is similar to previous studies that observed low albumin levels in MM patients [11,13].
A low AGR could be the consequence of sustained protein loss, such as that associated with proteinuria. Proteinuria results in undernutrition and inflammation. Low A/G ratio was seen in 33 cases. It is associated with high risk for liver and hematologic malignancies which includes multiple myeloma[14].
Renal function impairment is a common phenomenon in multiple myeloma. Various causes include cast nephropathy , amyloidosis, light chain or heavy chain deposition disease, plasma cell infiltration and interstitial nephritis In this present study, renal failure was associated with hypercalcemia in 8 cases(33%), hyperuricemia in 32 cases. Increased serum creatinine levels were seen in 28 cases.
Serum protein electrophoresis was done in 30 cases. All the patients had M spike similar to Diwan et al.[11]. Twenty five patients in the study had the Thick M band and the other five cases had thin M band on serum protein electrophoresis Out of 30 positive cases, M-spike was localized in the gamma region (γ) in 25 cases, beta
region (β) 3 cases, and α2 region in 2 cases.
Chopra et al. reported that 84.8% of the cases had an M band in the gamma (γ) region and that 15.2% cases had an M spike in the β- region [15].Tripathy reported M spike in the γ- region in 87.5% cases and 12.5% cases in the β- region [16] Like Kyle et al. [17]study two spikes (biclonal) were not found in any patients. In a study by Sunitha etal,[18] out of 16 M band positive cases, there was an M spike in the gamma region in 14 (87.5%) cases and 2 cases(12.5%) had an M

band in the beta region. Col.G S Chopra etal[15] reported that 84.8% of the cases had an M band in the gamma (γ) region and that 15.2% cases had an M spike in the beta (β) globin region . Beta 2 microglobulin is <3.5 in 18 cases,3.5-5.5 in 12 cases,≥ 5.5 in 15 cases.
β2M has been confirmed as ahighly significant prognostic factor in each study in which it has been examined; it reflects tumor burden and renal impairment
MM have highly complex karyotype in the majority of patients[19]. The frequency of genomic abnormalities was reported to be around 50-90% in literature[1,4,5,11-14]The identification of high-risk and low-risk cytogenetic groups is important for predicting response or resistance to therapy. The cytogenetic findings are included inthe consensus statement of the European Myeloma Network and International Myeloma Working Group[20-27].
Cytogenetics was done in fourteen cases. Ten cases showed diploidy and hyperploidy, two cases shoed near haploidy with 13q deletion. Cytogenetic results revealed hyperdiploid clone characterized by a distinct pattern of chromosome gains, and hypodiploid clone often accompanied by –13/13q deletion similar to Kuehl WM,Retal[28]
Survival studies have shown that hypodiploidy and missing or partial deletion 13, and abnormalities of 11q and 22q have been significantly associated with worse prognosis. FISH was done for 8 cases which showed 13q 14.3 deletion in three cases, normal in five cases. Comparision of chromosomal abnormalities and FISH results with a similar study by Perumaletal[29].is summarized in Table-6

	N=30 (Perumal et al.)	**N=14 (Present study)**
Chromosomal abnormalities	7/30	12/14
FISH	N=14/30	N=8
monosomy 13	13/30	3*/8
t(4;14)	4/30	0/2
P53 deletion	0/30	1*/2
normal	16/30	5/8

Conclusion:

Multiple myeloma is a clonal plasma cell proliferation and should be suspected in elderely presenting ith bone pains. Serum protein electrophoresis plays an important rule in detection.BMA is used to know the percentage of plasma cells and for cytogenetics/FISH.Initial results clearly highlight characteristic genetic abnormalities in MM. These chromosomal abnormalities helped in prognostication similar to other studies. This analysis require further correlation with patient survival.

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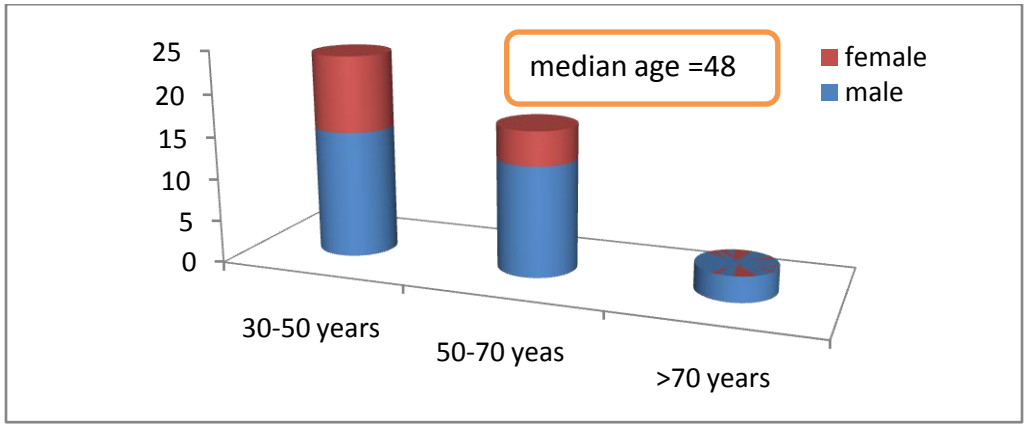
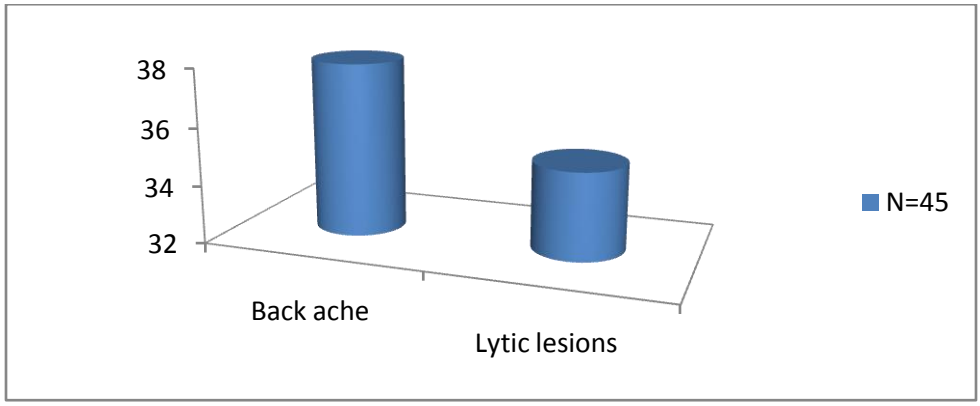
TABLES AND CHARTS



B-1:Age and Sex incidence



B-2:Clinical features

Table 2:Haematological parameters

	Total(n=45)	%
Hb levels <10 g/dl	28	62
>10 g/dl	17	38
Rouleaux formation	28	62
ESR(raised)	29	64
Plasma cells in BMA		
10-30%	34	75
>30%	11	25

Table 3: Bone Marrow biopsy

BMB(pattern of involvement)	**Total (n=45)**
Mixed-Interstitial and Nodular	19 (42%)
Nodular pattern	9(20%)
Interstitial pattern	11(24%)
Diffuse Packed marrow	6(14%)

B-3:BIOCHEMICAL PARAMETERS

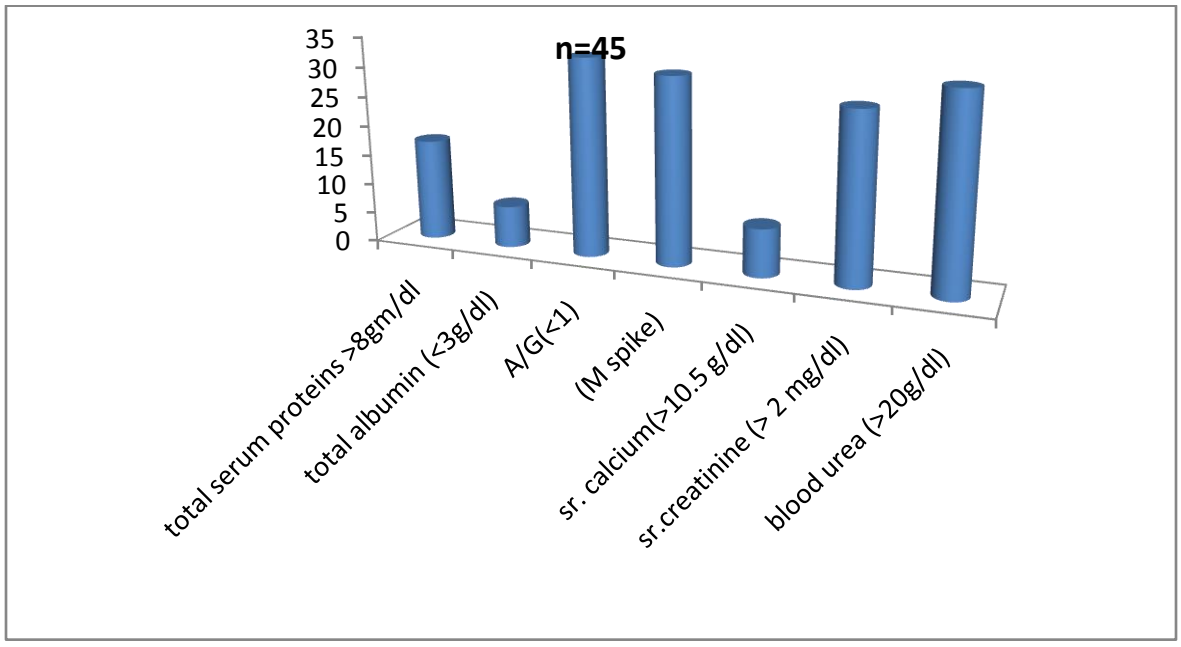


Table 4 : Serum Electrophoresis

	THICK /dense M spike (25 out of	**Thin M spike (5 out of 30)**

	30)	
M spike in gamma region	**21**	**4**
M spike in beta globulin region	**2**	**1**
M spike in between alpha 2 and beta globulin	**2**	

B-4: Beta 2 microglobulin

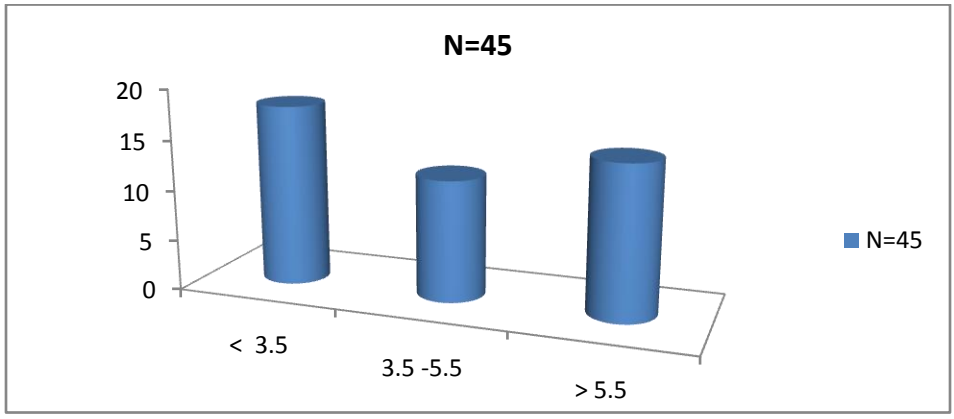


Table 5: Cytogenetics and FISH Results

Cytogenetics	**n=14**	**FISH**	**n=8**
47XY+9	1	Not done	2
47XY+9	2	13q14.3(negative)	1
46,XY[12]/66-69XXY[4]/81-90XXYY[2]	1	13q14.3(deletion)	