



# Cytogenetic Alterations and Correlation with Age and Gender in Patients of Multiple Myeloma: A Study from a Tertiary Care Center in Eastern India

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## Abstract



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## Keywords

- ▶ myeloma
- ▶ FISH
- ▶ cytogenetic aberrations
- ▶ age
- ▶ gender

**Background** Multiple myeloma is a cytogenetically heterogeneous, evolving, and incurable disease. Differences in prevalence of myeloma already exist in Indian subcontinent as compared with Western world countries. This study attempts to investigate differences in incidence of cytogenetic abnormalities (CA) in Eastern Indian patients and study differences in incidence with respect to age and gender.

**Materials and Methods** Interphase fluorescence in situ hybridization (FISH) was applied on purified plasma cells of 280 newly diagnosed myeloma cases using specific probes.

**Statistical Analysis** Data was analyzed using SPSS software version 25.

**Results** Note that 51.07% patients were FISH positive. Del13q was the most common CA. Significant association of del 13q with t(4;14), del 17p, and gain of 1q was seen. The frequencies of FISH positive and negative groups differed in the different age groups; higher number of cases in 41 to 50 years group in FISH positive group ( $p < 0.05$ ) and lower number of cases in FISH positive group in 61 to 70 years ( $p < 0.05$ ) as compared with FISH negative group. Del 17p had higher number of cases in age group 41 to 50 years and 51 to 60 years as compared with other age groups. Incidence of t(11;14) was in 5th to 7th decade while del 13q and t(4;14) had the widest range of age at presentation. Gender disparities were seen in high-risk cytogenetics like del 17p and 1q gain.

**Conclusion** The differences in incidence rate of CAs per se in myeloma cases diagnosed in Indian subcontinent and the differences in incidence with respect to age and gender warrant further multicentric studies.

## Introduction

Multiple myeloma (MM) is a malignancy caused by clonal proliferation of plasma cells. The genomic landscape of the

neoplasm is diverse with heterogeneity at the intracлонаl level.<sup>1</sup> Racial, ethnic, and geographical disparities occur in majority of cancers worldwide. Differences in prevalence of myeloma already exist in Indian subcontinent as compared

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with Western world countries. The current study attempts to study the cytogenetic abnormalities (CAs) in the Eastern part of India and investigate the differences in age and gender characteristics of patients, if any.

## Materials and Methods

All the patients newly diagnosed with MM between year 2014 and 2018 were included in the study. Ethical clearance was taken from the Ethics Committee and informed consent from patients was obtained for the same. The data was collected regarding clinical presentation, pretreatment laboratory parameters, and bone marrow plasma cells. The criteria for the diagnoses were based on the International Myeloma Working Group (IMWG) definition. The cytogenetics was studied by interphase fluorescence in situ hybridization (iFISH). Bone marrow samples were collected at the time of initial diagnostic evaluation and submitted for FISH for detection of CAs, del 13q, del 17p, t(4;14), t(11;14), t(6;14), t(14;16), and t(14;20). Mononuclear cells from bone marrow aspirate were enriched by Ficoll-Hypaque gradient centrifugation. Plasma cells were purified using CD138-coated magnetic beads. Enriched plasma cells were identified by fluorescein isothiocyanate-conjugated anti-human kappa lambda light chain staining, and the purity was 95% (range 70–99%). iFISH was performed on plasma cells using locus-specific probes, LSI 13 (D13S319), LSI 13q34 (control), LSI 17(p13.1) (TP53)/CEP 17(D17Z1), LSI break apart dual color 5' 3'IgH, dual fusion translocation probes *CCND1XT/IgH*, *FGFR3/IgH*, *MAF/IgH* (Vysis Abbott Molecular, Delkenheim, Germany), and *MYC/IgH* (Cancer Genetics, Milan, Italy). A separate set of 100 newly diagnosed patients were studied for 1q gain by iFISH (locus-specific deoxyribonucleic acid probes 1q21/1p36 and 1q25/1p36). The cutoff threshold used was 5% for all the probes.<sup>2</sup> For statistical analysis data were entered into a Microsoft Excel spreadsheet and then analyzed by SPSS (version 25.0; SPSS Inc., Chicago, Illinois, United States). Data was summarized as count and percentages for categorical variables and continuous variables were summarized as medians. Comparison of frequencies was done using proportion test. An  $\alpha$  level of 5% has been taken, that is, if any  $p$ -value is less than 0.05, it has been considered as significant.

## Results

A total of 350 patients attended the myeloma clinic in the study period out of which 70 patients had to be excluded due to inadequate data. A total of 143 (51.07%) patients were found to have the targeted CAs. Note that 57.3% (82/143) patients had single CA while 42.65% (61/143) patients had more than one CA. The individual frequencies of each FISH group are mentioned in **Table 1**. The overall frequencies were del 13q (74/280, 26.4%), del 17p (40/280, 14.3%), t(4;14) (62/280, 22.2%), t(14;16) (6/280, 2.14%), t(14;20) (2/280, 0.7%), and t(11;14) (9/280, 3.2%). Del 13q was the most common CA occurring in 54 out of 61 FISH positive cases

**Table 1** Distribution of cytogenetic abnormalities (CAs) in the study

Cytogenetic abnormalities (CAs) detected based on FISH results			
Cytogenetic abnormalities detected	Frequency, n (%)		
	No. of patients (n)	% (out of total 280 MM patients)	% (out of 143 FISH positive patients)
Isolated CA (n = 82)			
t(4;14)	33	11.8	23.1
del 17p	19	6.8	13.3
t(14;16)	6	2.14	4.2
t(14;20)	2	0.7	1.4
del 13q	20	7.14	13.9
t(11;14)	2	0.7	1.4
More than one CA (n = 61)			
del 13q, t(4;14)	22	7.8	15.4
del 13q, del 17p	13	4.6	9.1
del 13q, 17p, del 14q	8	2.8	5.6
del 13q, t(11;14)	11	3.9	7.7
t(4;14), t(11;14)	7	2.5	4.9
Overall frequency			
del 13q	74	26.4	51.74
del 17p	40	14.3	27.9
t(4;14)	62	22.2	43.45
t(14;16)	6	2.14	4.2
t(14;20)	2	0.7	1.4
t(11;14)	20	7.14	14

Abbreviations: FISH, fluorescence in situ hybridization; MM, Multiple myeloma.

(88%) followed by t(4;14) (in 29/61 cases, 47.5%), 17p (21/61 cases, 34.4%), and t(11;14) (in 20/61 cases, 32.7%).

The median age of presentation was 57 years of age (range 38–74). A total of 177 patients were male and 103 patients were female. The median age was 54 years in the FISH positive group and 59 years in the FISH negative group. The distribution of patients in various age groups is mentioned in **Table 2**. There were no patients in the age group < 30 years. The four cases that presented in age group 31 to 40 years showed significant association with the FISH positive group ( $p < 0.05$ ). In the age group 41 to 50 years, 71% patients (27/38) belonged to the FISH positive group as compared with 29% in the FISH negative group and this was statistically significant ( $p = 0.0003$ ). In the age group 51 to 60 years, both FISH positive and FISH negative groups had highest number of patients and there was no statistically significant difference across the two groups. In the age group 61 to 70 years, the FISH negative group had significantly

**Table 2** Age and gender characteristics of the total MM cases and in the two groups (based on FISH)

Parameters studied	Overall results	FISH positive group	FISH negative group	p-Value
Total number of patients (n, %)	280	143 (51.07)	137 (48.9)	0.60
Median age at presentation (in years)	57	54	59	0.32
Distribution of patients in age groups	N (%)	N (%)	N (%)	
< 30 y	0 (0)	0 (0)	0 (0)	–
31–40 y	4 (1.4)	4 (100)	0 (0)	0.008
41–50 y	38 (13.6)	27 (71)	11 (29)	0.0003
51–60 y	130 (46.4)	70 (53.8)	60 (46.2)	0.22
61–70 y	88 (31.4)	34 (38.6)	54 (61.4)	0.0026
> 70 y	20 (7.1)	8 (40)	12 (60)	0.21
Gender distribution among the patients				
Male (n, %)	177 (63.2)	86 (48.6)	91 (51.4)	0.59
Female (n, %)	103 (36.8)	57 (55.4)	46 (44.6)	0.12
Male:Female ratio	1.7:1	1.5:1	1.97:1	

Abbreviations: FISH, fluorescence in situ hybridization; MM, Multiple myeloma.

higher number of cases (61.4%) as compared with the FISH positive group (38.6%). The 17p deleted cases had younger age of presentation. 13q deleted cases (in isolated group as well as in group with > 1 CA) had the widest range of age at presentation. t(11;14) associated cases had age of presentation in the 6th decade (►Table 3).

The male:female (M:F) ratios in the two groups were 1.5:1 and 1.97:1, respectively (►Table 2). Note that 94.73% of total

17p (isolated) deleted patients lied in the age group 41 to 60 years with almost equal prevalence in male and female patients (►Table 3).

In the set analyzed for 1q gain, 1q gain was seen in 36% of cases. Isolated 1q was seen in only 8.3% cases (3/36). In the remaining 33 cases (91.7%), it was present in association with t(4;14) (16/36, 44.4%), del 17p (14/36, 38.9%), t(14;16) (2/36, 5.6%), and del 13q (23/36, 63.8%). The distribution is shown

**Table 3** Comparison of age and gender distribution in individual FISH positive groups

	del 13q n = 20	t(4;14) n = 33	del 17p n = 19	t(14;16) n = 6	del 13q t(4;14) n = 22	del 13q, del 17p n = 13	del 13q, del 17p, del 14q n = 8	del 13q, t(11;14) n = 11	t(4;14), t(11;14) n = 7
Median age at presentation (y)	56	57	50	62	55	51	51	65	59
Age (range, y)	47–75	44–69	42–55	55–70	38–68	37–64	41–68	54–74	55–67
No of patients in age groups (n, %)									
< 30 y (n = 0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
31–40 y (n = 4)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	2 (50)	0 (0)	0 (0)	0 (0)
41–50 y (n = 27)	3 (11.1)	6 (22.2)	10 (37)	0 (0)	2 (7.4)	5 (18.5)	1 (3.7)	0 (0)	0 (0)
51–60 y (n = 70)	9 (12.8)	17 (24.3)	8 (11.4)	3 (4.3)	14 (20)	4 (5.7)	5 (7.1)	5 (7.1)	4 (5.7)
61–70 y (n = 34)	5 (14.7)	7 (20.6)	1 (2.9)	3 (8.8)	4 (11.7)	2 (5.8)	2 (5.8)	4 (11.7)	3 (8.8)
> 70 y (n = 8)	3 (37.5)	3 (37.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)
Gender distribution									
Male (n)	13	17	10	4	13	9	5	7	4
Female (n)	7	16	9	2	9	4	3	4	3

Abbreviation: FISH, fluorescence in situ hybridization.

**Table 4** Analysis of chromosome 1q gain in 100 MM patients

Frequency of 1q gain cases (n, %)	36 (36)
Median age at presentation (y)	61
Frequency distribution/association with other CAs (n, %, total n = 36)	
Isolated	3 (8.3)
In combination with other CAs, n (%)	33 (91.7)
With del 17p	5 (13.9)
With del 17p, del 13q	9 (25)
With t(4;14)	4 (12.1)
With t(4;14), del 13q	11 (30.6)
With t(14;16)	1 (3.03)
With t(14;20)	0 (0)
With del 13 q	3 (8.3)
With t(11;14)	0
Age distribution (n, %, total n = 36)	
< 30 y	0 (0)
31–40 y	0 (0)
41–50 y	3 (8.3)
51–60 y	15 (41.7)
61–70 y	16 (44.4)
> 70 y	2 (5.6)
Gender distribution	
Male (n)	19
Female (n)	17
Male:Female ratio	1.11

Abbreviations: CA, cytogenetic abnormality; MM, Multiple myeloma.

in **Table 4**. The association with high-risk CAs was seen in 83.3% (30/36 cases) as compared with non-high risk CA (8.3%) and was found to be statistically significant ( $p < 0.0001$ ). The maximum number of cases belonged to 51 to 70 years of age group. The predilection for both males and females was found to be almost equal.

## Discussion

With the advent of cutting edge genomic methodologies, the knowledge about genetic profile of MM has expanded further. However, none of the alterations newly discovered have significantly changed the prognostic scoring system. The minimal FISH panel recommended for genetic testing by IMWG and of particular importance in resource-limited settings are t(4;14)(p16;q32), t(14;16)(q32;q23), and del (17p13).<sup>3</sup> The comprehensive panel recommended by the same group further includes chromosome 1 abnormalities, del 13q, t(11;14), and ploidy category.<sup>3</sup> In the current place of study, the minimal panel along with del 13q and chromosome 1 abnormalities (added recently) are routinely targeted for risk categorization and optimization of therapy keeping

in mind the financial constraints of the patients. Conventional cytogenetics (CG) is not done due to low yield of results owing to the low proliferative capacity of the neoplastic plasma cells.

In the present study, CAs were detected in 51.07% of cases. Previous studies from other countries have reported variable frequencies of CAs ranging from 21.2 to 87%.<sup>4–9</sup> A review of frequencies reported from Indian centers revealed that most of the studies were reported from Western and Southern India. The various centers observed frequencies ranging from 33.3 to 74.9%.<sup>10–13</sup> These studies differed in their results as the range of CAs selected for testing was highly variable and so was the method used for cytogenetic study (FISH only/FISH and conventional CG/CG only). The studies that included ploidy level along with immunoglobulin heavy-chain (IgH) translocations reported higher incidence rates of CAs. Apart from that, another probable explanation to variability in detection rates could be tumor heterogeneity, stage at which the patient was being studied, the technique used for cytogenetic testing, interpretation of data, duration, and sample size of the study.

In the present study, the cases had isolated CA as well as more than one CAs. Del 13q was the most common CA in this study. The tumor suppressor RB1 gene is lost in these deletions. In the present study, 26.4% (74/280) of the total cases showed del 13q. Previous studies from India have also reported lower frequency rates ranging from 25 to 34%.<sup>10,13,14</sup> Monosomy 13 imparts unfavorable prognosis in myeloma, probably due to its frequent association with other high-risk CAs, especially t(4;14). In the current study, two-thirds cases of del 13q were seen in association with other CAs; with t(4;14) (22/143), with del 17p (13/143), with del 17p and del 14q (8/143), and with t(11;14) (11/143). This study observed its association with two high-risk CAs (t(4;14) and del 17p). Acquisition of the deletion during evolution of the disease (from monoclonal gammopathy of undetermined significance phase to smoldering phase to overt myeloma) could be a probable explanation for its association with high-risk CAs, especially primary genetic events. Del 13q is commonly seen in association with t(4;14), the association reported being as high as 80 to 90%. In the current study, it was seen in only one-third of cases with almost an equal number of cases being in association with del 17p. The incidence rate of del 17p and del 13q occurring together has not been reported before. t(4;14) was the next most common CA in the present study. t(4;14) is postulated to be a primary genetic event responsible for plasma cell immortalization.<sup>1</sup> The overall frequency (22.2%) observed in this study was higher than that reported by other studies from Western India (10–15%).<sup>13,14</sup>

Deletion 17p is known to acquire during the course of progression of the disease and is more common to occur in relapsed/refractory setting. Lakshman et al<sup>15</sup> reported acquisition of del 17p at a median of 35.6 months of diagnosis after a median of two lines of therapy. In the present study, it was seen in 14.3% (40/280) of cases in concordance with previous studies.<sup>5,6,12,16</sup> The other CA commonly seen in association with del 17q was del 13q in this study. The

association of del 17p with del 13q was significantly high, 52% (21/40). The incidence rate of two mutations coexisting together is less well described in previous studies.

The CAs that were the least common were t(14;20), t(14;16), and surprisingly t(11;14). The incidence rates of t(14;16) and t(14;20) were in alignment with the reported literature.<sup>17</sup> The incidence rate of t(11;14) was very low as compared with the reported frequency of 20% from western studies.<sup>9</sup> However, other Indian studies have also reported lower incidence rates of t(11;14). Kadam Amare et al<sup>14</sup> reported a low percentage of 5% out of 475 cases, while Dhiman et al<sup>12</sup> did not find any case of t(11;14) out of 93 cases.

In the present study, the age and gender distribution were also studied. The median age at presentation reported for myeloma is 66 to 70 years. In the review of 1,024 cases, Kyle et al<sup>18</sup> observed 37% patients being younger than 65 years. On the contrary, in the present study, 61% of the MM patients were younger than 60 years and only 38% patients belonged to the > 60-year age group. Occurrence of the disease in third decade is also rare. In this study, 4 (1.4%) cases were found in the third decade of life. On comparing the age distribution in FISH positive and negative groups, it was observed that the largest number of patients in both the groups belonged to the age group 51 to 60 years. It was also observed that the incidence of FISH positive cases was significantly higher than the FISH negative cases in the age group 41 to 50 years. And significantly lower number of cases was in the age group 61 to 70 years. Among the individual CAs, the incidence rate of t(4;14) decreased with age and in the 5th, 6th, and 7th decade, respectively, were in decreasing order of 51, 21, and 9%. In a recent study, Cardona-Benavides et al<sup>17</sup> reported similar observation.

Del 17p is reported to have incidence rate increase with age.<sup>15</sup> In the present study, on the contrary, 52% of cases were in the younger age group (41–50 year group) followed by 42 and 5.2%, respectively, in the subsequent higher age groups. Of all the t(11;14) cases, incidence in the 6th and 7th decade combined together was higher than that in the 5th decade as opposed to the rest of the CAs (55% vs. 45%). t(14;16) and t(14;20), though comprised only < 3% of all cases, presented in older age group only.

Overall male preponderance was seen in the present study. Among the FISH positive groups, however, almost 1:1 ratio was seen in isolated del 17p, isolated t(4;14), and combined t(4;14) + t(11;14) groups. An almost equal incidence suggests equal susceptibility in females harboring these mutations. Similar findings have been noted in studies by Boyd et al<sup>19</sup> and Fonseca et al.<sup>20</sup> Boyd et al found higher incidence of IgH translocations in females. In the study by Fonseca et al, t(4;14), t(14;16), and del 17 p had higher or almost equal number of affected patients. In the current study, the group having t(4;14), del 17p, and the ones having t(11;14) along with t(4;14) had almost equal incidence in females as males.

In the current study, 1q gain was seen in 36% of cases. Review of literature reveals its prevalence between 20 and 50% of newly diagnosed MM cases.<sup>21,22</sup> Gain of 1q showed

significant association with high-risk CAs (90%) and equal incidence in females. Association with del 13q was seen in 64.8% cases. This association with del 13q and high-risk cytogenetics has also been seen in previous studies and supported the basis for its adverse prognosis.<sup>23,24</sup>

## Conclusion

This study shows a decade younger age of presentation with significantly higher number of FISH positive cases, that is, in the 4th decade of life. Del 13q was the most common abnormality along with its significant association with other CAs, especially high-risk cytogenetics (del 17p, t(4;14), and chr 1q gain) providing the basis for its adverse prognostic behavior. Low incidence of t(11;14) and del 13q could be due to ethnic differences and diversity. Higher number of female cases in del 17p and t(4;14), 1q gain, and t(11;14) with t(4;14) groups as compared with other CAs, equalizing the M:F ratio hints toward the occurrence for high-risk cytogenetics in female patients. Occurrence of del 17p at the time of diagnosis in a younger age group warrants further study to find if there occurs clinical heterogeneity in 17p deleted newly diagnosed myelomas as compared with the relapsed/refractory myeloma acquiring del 17p at the time of relapse. Larger multicentric studies are needed to authenticate the same.

## Authors' Contributions

K.J., D.M.: Concepts design, definition of intellectual content, literature search, data analysis, manuscript preparation, and manuscript editing. S.S., D.M.: Literature search, data analysis, manuscript preparation, and manuscript editing. M.B., D.M.: Concepts design, definition of intellectual content, manuscript preparation, and manuscript editing.

## Conflict of Interest

None declared.

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