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Research Article

Cytogenetics and FISH Studies in Multiple Myeloma – A Retrospective Study from Western India

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Abstract:
Multiple myeloma is characterized by a complex pattern of extensive genomic aberrations involving many chromosomes and it constitutes about 1% of all malignancies. We have performed conventional cytogenetic (CC) and interphase FISH on 58 cases of MM. Results showed that from 58 cases, only 08 cases had abnormal karyotype by conventional cytogenetic. On the other hand, interphase FISH study with 58 MM patients revealed 08 patients with normal results while 50 patients showed complex genetic aberrations. It included deletions of 13q14 (48.3%), 17p13 (13.8%), 11q13 (27.6%) along with translocation of IgH involving t(4;14)(51.7%) and t(14;16)(1,7%). We conclude that interphase FISH study should be performed in conjunction with conventional cytogenetic for prognostic significance in MM.

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Introduction

Multiple myeloma (MM) is a clonal B-cell malignancy disorder in which malignant plasma cells accumulate in the bone marrow and produce an immunoglobulin, usually monoclonal IgG and IgA. Multiple myeloma comprises 10% of all haematological malignancies and 1% of all neoplastic diseases. The annual incidence of MM is ~30/1000,000 patients. The disease is usually incurable [1]. The Indian incidences of 6000 new cases/year. The male/ female ratio is 1.4:1 and mean 5 year survival rate of 33% [2].

Among various prognostic factors in MM, cytogenetic abnormality (CA) detected by conventional cytogenetic (CC) and FISH studies are major clinical outcome [3]. MM reveals numerical and structural chromosomal abnormalities. It is different from most other haematological malignancies, which are typical less complex and resembles more complexity of solid tumours [4]. The understanding of biology of multiple myeloma has evolved rapidly with introduction of molecular cytogenetic and major advances have been made in the last few decades, this has changed myeloma from incurable to curable disease in number of patients [5, 6].

Molecular studies have demonstrated that primary translocations occur in the early stage of MM, followed by large number of secondary translocation during tumour progression. It is believed that the secondary genomic aberrations are responsible for a more proliferative aberrations phenotype in advanced stage of MM. The structural aberrations such as del(13q), del(17p), del(11q), translocation involving immunoglobulin heavy chain locus (IgH) generally associated with an unfavourable prognosis [7, 8]. Survival studies with MM have revealed hypodiploidy and missing or partial deletion 13q, abnormalities with 11q and 17p have been significantly associated with poor to worse prognosis. IgH (14q32) translocations are also considered as primary genetic events, but some variants may likely act as progression events [1].

In the present study an attempt was made to investigate the frequency of structural and numerical chromosomal aberrations in MM patients from Western India. Total 58 cases of MM were analysed using conventional cytogenetic and interphase FISH study.

Materials and Methods

a) Patients

Fifty eight patients clinically diagnosed with multiple myeloma were studied. The retrospective period of recruitment was form May 2009 to Dec. 2014. The patients included 35 males and 23 females between ages of 41 to 82 years (Median age was 64 years). The consent forms had been signed by the patients and study had been approved by the local Medical college's ethical committee.

b) Cytogenetic study

Bone-marrow samples were cultured for 48 hours to 5 days in Marrow Max medium without mitogen and with 10 ug/ml colcimid solution. Then chromosomal slides were prepared according to standard

procedures. Standard GTG banding was performed [9] on the metaphases obtained. Depending upon availability, 20–25 metaphases cells per sample were analysed. The karyotype description followed ISCN, 2009 [10] recommendations.

Patients were considered hyperdiploidy if there had been 48-72 chromosomes and non-hyperdiploidy if number is less than 46 chromosomes.

C) FISH

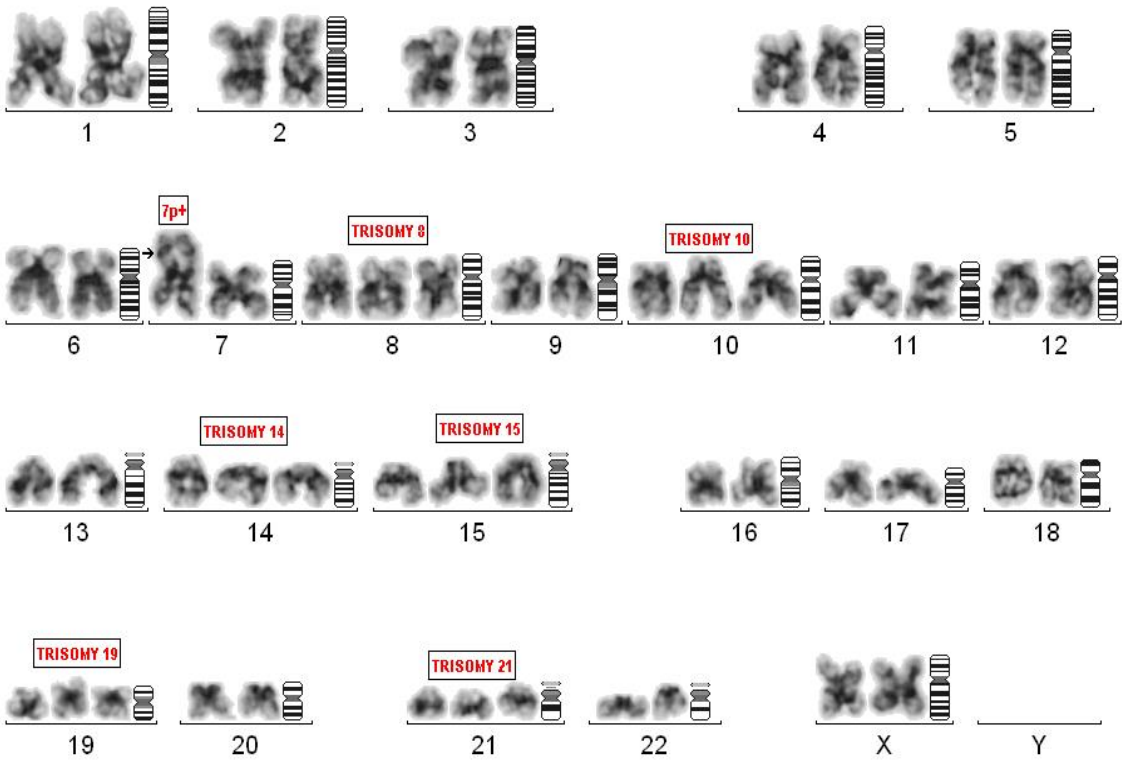
Interphase FISH was performed using specific DNA probes (Kreatech, Netherlands). A multiple myeloma FISH Panel comprising probes for Del 11q23.3 (LSI – ATM probe), Del 13q14.3 (LSI – D13S319 DNA probe), t (11;14) (IgH – 14q32) break apart probe and for del 17p13.1, p53 probes were used. Total two hundred nuclei were enumerated for each FISH Panel probe and average scores was tabulated.

Table 1 Comparison of Cytogenetic and Interphase FISH results of Multiple myeloma patients

No	Patients Sex/Number	Averages Yrs.	Cytogenetic Abnormality (%)	FISH				
				IgH(%)				
				13q14(%)	17p13(%)	11q13(%)	t(4;14)	t(14;16)
	M/35	41 - 84		18(51.4)	07(20)	11(31.4)	18(51.4)	01(2.9)
1			14.3					
	F/23	42 - 70		10(43.5)	01(4.3)	05(21.7)	12(52.2)	-----
2			13.0					
	58	41 - 84		28(48.3)	08(13.8)	16(27.6)	30(51.7)	01(1.7)
Total			13.8					

Results

We have evaluated a total of 58 cases with MM which included 35 males and 23 females having median age 64 years (range 41 – 84 years). Among the 58 patients, cytogenetic study was done in all 58 patients, of which in male 5/35 had abnormal karyotyped while in female 3/23 had abnormal karyotyped. On the contrary, in FISH out of 58 patients, 8(13.8%) had normal results, while 50 had at least one genetic aberration. Both conventional cytogenetic and FISH results are given in **Table 1**. With regard to cytogenetic study a hyperdiploid karyotype with 55 chromosomes is presented in Fig. 1.



52,XX,der(7)add(7p),+8,+10,+14,+15,+19,+21[05] / 46,XX[15]

Fig. 1 G-banded hyperdiploid karyotype of MM patient with numerical and structural changes

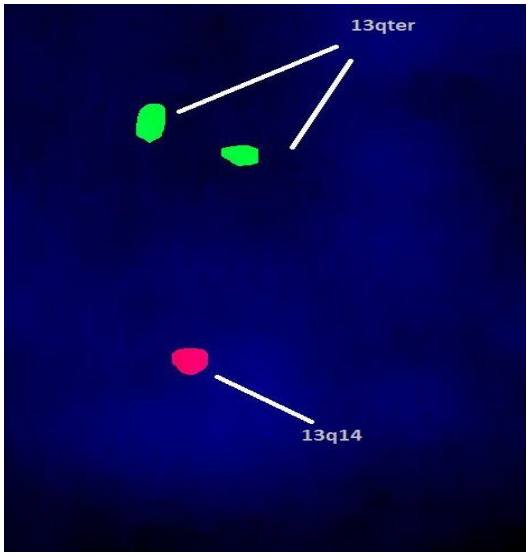


Fig.2 Interphase FISH showing Del (13q14)

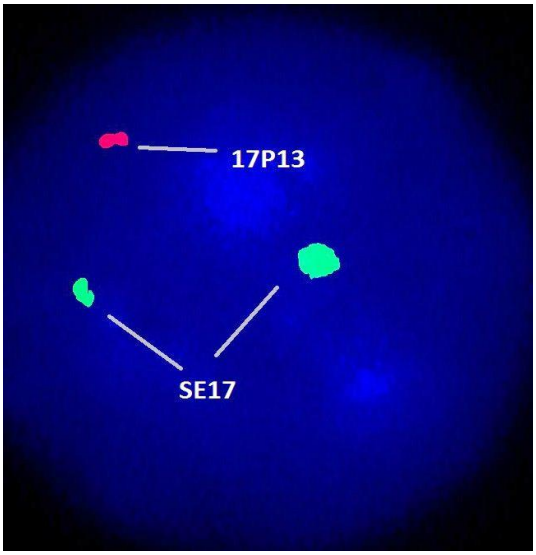


Fig.3 Interphase FISH showing Del 17p13

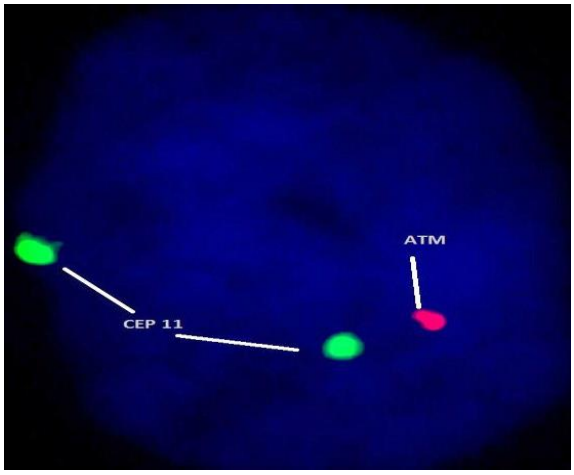


Fig. 4 Interphase FISH showing del (11q13)

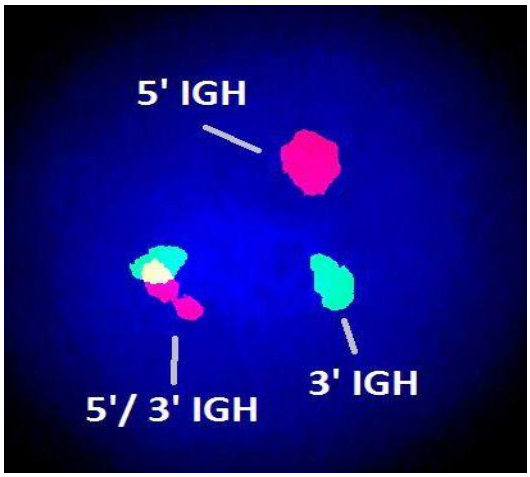


Fig. 5 Interphase FISH showing IgH translocation

FISH results revealed that chromosome 13 was having most frequent changes. Out of 58 patients, 28 patients have 13q14 (48.2%) abnormality (Fig. 2). This includes 51.4% male and 43.3% female. In addition absence of p53 at 17p13 was detected in 8/58(13.8%) patients (07 males and 01 female)(Fig. 3). Similarly 11q13 abnormality was observed in 16/58 (27.6%) involving 11 males and 05 females (Fig. 4). IgH (14q32) aberrations were noted in 31/58 (53.4%) patients. Of which t(4;14) was present in 30 patients (18 males and 12 females), on the other hand, only 01 translocation that is t(14;16) was present in male (Fig. 5). Hyperdiploidy was observed in 14/58 (24.1%) patients by FISH where 08 had 13q14, 03 had 17p13 and 03 with 11q13.

Discussion

Multiple myeloma(MM) has an incidence rate of 102,000 and death rate72,000 per year worldwide. The incidence varies by ethnicity with highest rates observed in African-Americans followed by people of industrialized nations [11]. Cytogenetic analysis of MM has been limited by low proliferative activity of plasma cells in cultures. Despite that, chromosome analysis provides a wide array of chromosome aberrations in proliferating plasma cells from patients with MM.

In the present study using conventional cytogenetic (CC), 13.8% abnormal karyotypes were found. In contrast, interphase FISH studies showed around 80% suggesting that clonal chromosomal abnormalities are frequent in MM. In our study, total hyperdiploidy was observed in 14 patients. The chromosomes gain were +8, +10, +14 +15,+19 and +21.

FISH results showed that most frequent abnormality was deletion of 13q14(48.3%), followed by del 11q13(27.6%) and del 17p13(13.8%) in MM. In majority of del(13q) precedes t(4;14) in the patients with either diploidy or non-hyperdiploidy. With regard to reports from other Asian Countries; a Japanese study revealed a combined figure of 28.6% of t(4;14) and t(11;14) and 36% incidence of del(13q) [12]. Similarly, a South Korean study by Bang et al.,(2006) [13] documented incidences as 37% IGH and 48% of del(13q). Our study found similar results of del(13q) and IgH translocation corroborating with other reports [14, 15].

It has been well documented that del(17p) is considered to be worst prognosis due to loss of TP53 tumour suppressor gene. In our study the rate of deletion(17p) was 13.8% as reported earlier [13]. Very few studies have been carried out on del (11q). Our results showed del(11q) as 27%, which was higher

than earlier reported by Mohamed et al., (2007) as 4% [8]. Normally 11q13 is not routinely checked along MM FISH panel but it is certainly helpful in deciding prognostic outcome.

In summary, this study focuses on MM cases with abnormal karyotype using conventional cytogenetic and interphase FISH techniques. Results revealed that percentage of abnormality especially for del 13q14 and IgH are similar to the study conducted in Asian countries, but del 11q13 is comparatively higher in our study in comparison to other studies reported in the literature [16]. The combined study of CC and interphase FISH support specific chromosomal aberrations which were of major prognostic relevance in MM and comparing data with International Scoring System (ISS) could readily predict prognosis.

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