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Chromosomal Abnormalities in Multiple Myeloma: An Observational Study from South India

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Abstract

Recurrent cytogenetic abnormalities are noted throughout the course of multiple myeloma (MM) from the premalignant stage of monoclonal gammopathy of undetermined significance to end-stage disease. The prospective, observational study evaluated the frequency of structural and numerical chromosomal abnormalities in a cohort of 118 patients diagnosed with MM from south India using conventional cytogenetics. Chromosomal analysis was carried for both fine needle and bone marrow samples and the karyotypes were interpreted as per the International System for Human Cytogenetic Nomenclature. The study identified 6 hyperdiploidy, 2 hypodiploidy and 3 pseudoploidy. The most common numerical abnormalities noted were gain of chromosomes 3, 5, 6 ,7, 11, 15, 16, 18, 19 and 21, and loss of 10, 12, 14, 17 and 22. The study validated the role of CC in conducting primary screening of MM, especially in the resource-poor settings and in remote areas with limited diagnostic facilities

Keywords: Chromosomes; Cytogenetics; Karyotype; Multiple Myeloma.

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Introduction

Multiple myeloma (MM), a cytogenetically heterogenous plasma cell disease, is marked by the presence of several frequent cytogenetic abnormalities throughout the disease course [1]. In terms of disease prognosis, cytogenetic alterations has been identified as an important risk factor and abnormal karyotypes are noted in 30-50% of the patients, especially in the advanced stages of the disease [2]. According to the 2018 systematic analysis published in JAMA Oncology, the mean incidence of MM increased by 126% worldwide and mortality by 94%. The study has underscored that the lack of diagnostic facilities is adding to the increased incidence rate of the malignancy in countries with lower sociodemographic index [3].

Based on the chromosome numbers in the tumor clone, the malignancy can be broadly classified as hyperdiploid MM (≥47 and <75 chromosome) and non-hyperdiploid MM. Non-hyperdiploid MM is further divided into 3 subgroups: hypodiploid (≤44 chromosomes), pseudodiploid (45–46 chromosomes) and near tetraploid (>75 chromosomes). The hyperdiploid clone marked by a distinct pattern of chromosome gain (+3, +5, +7, +9, +11, +19, +21) is associated with better survival, whereas deletions of 1p, 12p, 16q and 17p may have poor outcome or disease progression [4].

Apart from these major chromosomal abnormalities breakpoints at the loci of tumor suppresser

gene, proto-oncogenes or immunoglobulin-related gene especially involving 1p13, 11q13, 6q21, 7p11.2, 14q13, 17p11, and 19p13.3 regions have also been noted in rare cases of MM. [5]

The present study investigated the frequency of structural and numerical chromosomal abnormalities in a cohort of patients with MM from south India. It also explored the feasibility of using conventional cytogenetics (CC) as a primary screening technique for multiple myeloma.

Materials and Methods

The prospective, non-interventional, observational study involved 118 patients newly diagnosed with MM at a super specialty center in south India. The subjects were enrolled between January 2006 and September 2010. The diagnosis was concluded on the basis of the International Myeloma Working Group criteria [6,7]. Informed consents were obtained from all patients prior to the study.

Bone marrow and fine needle aspirations (2 cases) were carried out for all the enrolled subjects. Bone marrow samples were collected according to the standard procedures followed for hematology and cytogenetic investigations. The aspirates were cultured as direct, 24- and 48-hour cultures, without mitogens, in RPMI-1640 medium supplemented with 15% fetal bovine serum at 37^0C. After incubation, the cells were exposed to colcemid (0.10µg/ml) for 30 minutes, followed by hypotonic treatment (0.075 M KCl) for 20 minutes. The cells were subsequently fixed with Carnoy's fixative (methanol-glacial acetic acid, 3:1) and kept in refrigerator overnight. On the following day, chromosomal analysis was performed on the air dried bone marrow samples using the standard G-banding technique (Seabright 1973). [8] Metaphases of good morphology were captured and analyzed by Image Analysis System. The karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature (2005). [9]

With reference to the modal number, hyperdiploidy and hypodiploidy have been used to describe cells with 47–57 chromosomes and 35–45 chromosomes respectively. The corresponding terms near triploidy, near tetraploidy, and pseudodiploidy have been used to define chromosomes 58–80, 81–103 and 46 with numerical and/or structural aberrations [9].

Results

The recruited subjects included 74 males and 44 females between the age range of 31 to 80 years. Conventional cytogenetic analysis of bone marrow (BMA) and fine needle aspirate (FNA, 2 cases) cultures revealed successful karyotype in 77 (87.5%) and complex abnormal karyotypes in 11 patients (12.5%). The numbers of patients noted with hyperdiploidy, hypodiploidy and pseudoploidy were 6, 2 and 3 respectively. The most common numerical abnormalities noted were gain of chromosomes 3, 5, 6, 7, 11, 15, 16, 18, 19 and 21, and loss of 10, 12, 14, 17 and 22. The break points (X) (q13), 3 (q12), 3 (p12), 6 (q23), 9 (q22), & 11 (q13) were involved in deletion and (1) (q21), 19 (p13) & 8 (q24) in addition. The characteristic translocations noted were t (1;6) (q23;q11), t (1;9) (p12;q34), t (11;14) (q13;q32) and t (11;16) (q13;q22). Abnormalities noted during chromosome analyses of the bone marrow and peripheral blood cells are briefed in Table 1 and Figure 1.

Table 1: Abnormalities noted during chromosome analysis of the bone marrow and peripheral blood

Age/ Sex	No. of patients	Samples used	Karyotype
50/M	1	BMA	51, XY, t(1;?)(q21; ?) x2, del (3) (q12), +5, +5, +7, der (9), t (1; 9)(q34; q12), -12 ,der (16), t (11; 16) (q13; q22), -19, +21, -22,+mar/ 55XY, add (1) (q21) x2, dic t (1;9) (p12; q34), del (3) (p12), +5, +5, +7, -14, -17, add (19) (p13),+21, -22, +6mar
17/M	1	FNA	46XY, add (19) (q13)
42/F	1	BMA	46XX, t (2;3) (p23;p27)
68/F	1	BMA	55, X, del (X) (q13), +3, +5, +5, +5, i (8)(q10), add (9) (p24), +11, +11, del (11) (q13),-14, +15, add (16) (q24), -17, +18, +19, +21, +mar/ 46, XX
54/M	1	BMA	46XX, t (11; 14) (q13; q32)
61/M	1	BMA	54XY, +5, del (6) (q21) x2, +6, +7, +7, add (8) (q24) x2, -10, +15,+15, derdic (16), t (1; 16) (p12; p13) x2, +16, +19, +19
48- 50 /M	4	BMA	47XY, +mar/ 46, XY
55/M	1	BMA	78, XY, +X, t (1; 6) (q23; q24), +2, +3, +3, +6, +6, +7, +8, +8, +9, +9, +10, +10, +11, +12, +14, +15, +15, +16, +16, +17, +17, +18, +18, +19, +19, +19, +21,+ 21, +4mar

Cells

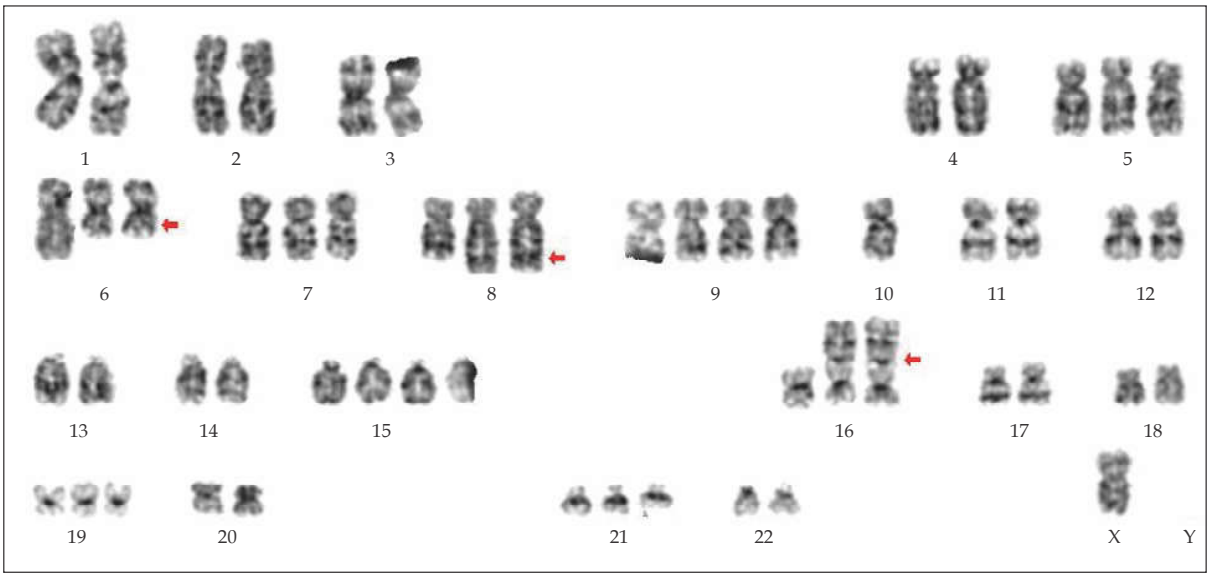

Fig. 1: Karyotyping results of multiple myeloma patients

Discussion

The present study reports CC as an important tool in elucidating the complex and diverse genetic abnormalities associated with MM. The cytogenetic findings helped in identifying two distinct groups of MM: hyperdiploid associated with better prognosis (6 patients) and non-hyperdiploid with poor survival (5 patients). It also helped in establishing the presence of prognostic chromosomal markers such as t (1; 6), t (1; 16), t (11; 14), t (11; 16), and 16(q) abnormalities.

Traditional approaches such as cytogenetic analysis, molecular genetic studies, and fluorescence in situ hybridization (FISH) provide crucial diagnostic and prognostic information in patients with MM. CC plays a paramount role in identifying the chromosomal abnormalities that demarcates patients with good prognosis from poor in relation to therapeutic response. It also holds the advantage of conducting whole genome analysis in a single experiment, whereas FISH targets only specific genes and is expensive when large panel probes are necessary [2].

In concurrence with the current findings, a 2016 single-center study conducted in Korea has highlighted the need of including CC as a part of initial diagnostic work-up in patients suspected with MM. The study considered cytogenetic results obtained from 222 patients with newly diagnosed MM. Among the abnormalities detected, hyperdiploidy with structural aberrations was the predominant finding (44%), followed by hypodiploidy with structural aberrations (28%) [2].

A more recent cytogenetic study conducted by Royal et al. in Indian population has added a few more numerical, structural and clonal abnormalities to the previously reported literature evidence on MM. The researchers noted the existence of a combination of ploidies, i.e., clones of hyperdiploidy, hypodiploidy, hypotetraploidy, and hypertetraploidy, in addition to the commonly reported monosomies. The study also documented the presence of other monosomies such as − 2, −6, −9, −10, −20, −21, and two cases with −Y, and one with −X [5].

Although, cytogenetic analysis provides more valuable information on prognosis, the low proliferation activity of terminally differentiated plasma cells, especially in the early disease stages, is one of the major limiting factors of this technique. In addition, interpretation of the result may be challenging, if the aberrations are cryptic and the chromosomal morphologies obtained through karyotyping are of poor quality [2]. The complementary molecular cytogenetic techniques such as FISH may be required in such cases. The major limitations of the current study are reduced sample size and not introducing FISH data to compare with conventional karyotyping.

A retrospective study from western India has concluded on the necessity of conducting interphase

FISH study along with CC for detecting specific chromosomal aberrations with major prognostic significance in MM. The researchers carried out CC and interphase FISH on 58 subjects and the CC could identify only abnormal karyotype in 8 cases. Whereas, the FISH identified 50 patients with complex genetic aberrations and 8 with normal karyotypes [10].

Conclusion

The present study corroborates the role of CC in conducting initial screening and the primary diagnosis of MM. Owing to the cost-effectiveness; it is highly beneficial for patients belonging to the resource-poor settings and in remote areas with limited access to newer diagnostic facilities.

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