

## ANALYSIS OF COMPLEX CHROMOSOMAL ABNORMALITIES IN A CASE OF MULTIPLE MYELOMA USING SPECTRAL KARYOTYPING

PERUMAL GOVINDASAMY<sup>1</sup>, POOJA S KULSHRESHTHA<sup>2</sup>, PRABU PANDURANGAN<sup>3</sup>, ANIL TARIGOPULA<sup>1</sup>, JAYARAMA S KADANDALE<sup>2</sup>, CHANDRA R SAMUEL<sup>4\*</sup>

<sup>1</sup>Department of Centralised Molecular Diagnostics, Apollo Hospitals, Chennai – 600 006, Tamil Nadu, India. <sup>2</sup>Centre for Human Genetics, Biotech Park, Electronic City Phase I, Bengaluru – 560 100, Karnataka, India. <sup>3</sup>Department of Hematology, Apollo Hospitals, Chennai – 600 006, Tamil Nadu, India. <sup>4</sup>Department of Genetics, Dr. ALMPGIBMS, University of Madras, Taramani, Chennai – 600 113, Tamil Nadu, India. Email: nchandrarsamuel@gmail.com

Received: 05 May 2018, Revised and Accepted: 05 June 2018

### ABSTRACT

**Objective:** It was proposed to determine the chromosomal abnormalities in a 49-year-old male patient with multiple myeloma (MM) employing both conventional and advanced molecular cytogenetic techniques.

**Methods:** GTG-banding and spectral karyotyping (SKY) on fixed metaphases obtained from LPS-stimulated bone marrow cells and interphase fluorescence *in situ* hybridization (iFISH) on unsorted marrow cells were carried out to identify genetic markers of prognostic significance.

**Results:** The abnormal chromosomes observed through conventional cytogenetics could be resolved with SKY technique. The translocation t(4;14)(p16;q32) indicating FGFR3/IGH fusion and deletion of 13q14.3 was noticed using iFISH. The genetic abnormalities confirmed a poor prognostic outcome in the patient who died within 6 months of diagnosis.

**Conclusion:** This report emphasizes the need for multicolor FISH techniques besides iFISH to resolve complex abnormalities and to identify cryptic aberrations of importance in risk stratification of MM patients.

**Keywords:** Chromosomal abnormalities, Interphase FISH, Multiple myeloma, Spectral karyotyping.

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### INTRODUCTION

Multiple myeloma (MM) is a clinically and genetically heterogeneous hematopoietic neoplasm of plasma cells characterized by their accumulation in the bone marrow, production of excessive monoclonal immunoglobulin (M or myeloma protein) in serum and urine, and osteolytic bone lesions. These features lead to pathological bone fragility, nephropathy, immunodeficiency, and hematopoietic disorders [1]. Genetic abnormalities are almost always present in MM patients although an abnormal karyotype can be detected using conventional Giemsa-banding techniques in only 25–35% of them [2]. The low proliferation index of plasma cells and the poor morphology of chromosomes pose additional problems [3]. Analysis with fluorescence *in situ* hybridization on interphase nuclei (iFISH) using chromosome-specific alpha satellite DNA probes confirmed hyperdiploidy, locus-specific probes demonstrated microdeletions, and dual-fusion probes identified cryptic chromosomal translocations involving the immunoglobulin heavy chain (IGH) gene in 14q32.3 such as t(4;14) [4,5]. Guidelines to perform iFISH on plasma cells selected based on morphology, immunophenotyping, or sorting are available [3,6,7].

Non-random chromosome abnormalities of prognostic significance detected by iFISH help to stratify patients into two main groups - (1) the hyperdiploid MM having trisomies of odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 and (2) the non-hyperdiploid MM including translocations involving the IGH gene such as t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16)(q32;q23) in a majority of the cases. Monosomy 13/del(13q), del(17p), and deletion of 1p or amplification of 1q are commonly associated with aggressive disease [5]. With the advent of advanced molecular cytogenetic techniques such as multicolor fluorescence *in situ* hybridization (M-FISH) and spectral karyotyping (SKY) which allowed a simultaneous unequivocal identification of all

the 24 chromosomes, it was possible to unambiguously characterize complex rearrangements and identify the chromosomal origin of marker chromosomes [2,8-10]. However, cytogenetic data employing these methodologies on MM are scarce because of the low mitotic activity of plasma cells, heterogeneity within the clonal population, the technical pitfalls during interpretation of results, and their restricted availability [2,11]. This report describes the application of SKY in the characterization of complex chromosomal abnormalities detected in a newly diagnosed MM patient.

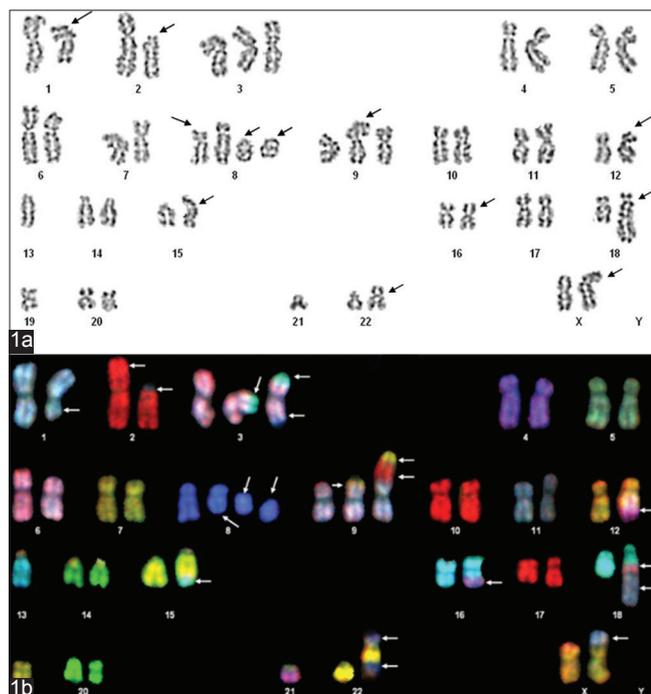
### CASE REPORT

A 49-year-old north Indian man was evaluated at the Department of Hematology, Apollo Hospitals, Chennai-6, in November 2012 for a 2-month history of loss of appetite and severe pain in the back and legs. He was involved in agricultural pesticide business. His medical reports indicated hypercalcemia, systemic hypertension, and azotemia. He was a non-smoker and a teetotaler with no family history of cancer. Trephine biopsy section showed trabeculae of bone with intervening hypercellular marrow particles. There was a diffuse infiltration of atypical plasma cells and mitosis was increased. Immunofixation electrophoresis showed monotypic expression of the immunoglobulin A heavy and lambda light chains. Laboratory investigations revealed platelet count to be 40,000 cells/cu mm, and peripheral smear study showed intense rouleaux formation. Bone marrow cytology was particulate with blood-diluted cell trail and intense plasmacytosis (80% plasma cells). Biochemical investigations showed serum creatinine 1.1 mg/dL, calcium 15.6 mg/dL, serum albumin/globulin reversal, and  $\beta_2$ -microglobulin 5.6mg/L. These findings were consistent with MM. He was started on dexamethasone and bortezomib along with supportive measures. Follow-up studies revealed that the patient had expired within 6 months.

This study was approved by the institutional ethics committee. Detailed case history and bone marrow sample were collected after written informed consent was obtained from the patient. Chromosomal preparations were obtained from unsorted, lipopolysaccharide (Sigma-Aldrich)-stimulated bone marrow cells and were GTG-banded following standard protocols. About 25 metaphases were karyotyped using applied spectral imaging (ASI) systems karyotyping software (Band View version 6.0). Chromosomal anomalies were designated using standard nomenclature. SKY was performed using the same fixed chromosome preparations employing 24-color combinatorically labeled SKYPaint® Probes (ASI Inc., CA) according to manufacturer's instructions. The images were captured using an epifluorescence microscope equipped with a CCD camera. A total of 30 metaphases were analyzed and the breakpoints were determined by comparison with corresponding DAPI-banded chromosomes. The iFISH procedure was carried out on unsorted bone marrow cells fixed on slides employing seven commercially available probes for MM following manufacturer's specifications. About 300 interphase nuclei were scored for the presence of signals for each probe. Images were captured under respective filters in Olympus BX-51 microscope (Olympus, Tokyo, Japan) and analysis was done with the ASI systems software (FISH View version 6.0).

Analysis of GTG-banded metaphases showed a hyperdiploid karyotype with several abnormal chromosomes (Fig. 1a). SKY revealed these derivative chromosomes to result from complex translocations besides cryptic rearrangements in 24 of 30 metaphases (Fig. 1b).

The translocation  $t(4;14)(p16;q32)$  was inferred from iFISH using IGH break apart and fibroblast growth factor receptor 3 (FGFR3)/IGH dual-fusion probes (Fig. 2a-d). The probe LSI D13S319 (13q14.3) showed monoallelic deletion of 13q14.3 locus while the CEP9 probe identified trisomy 9 (Fig. 2e-f).

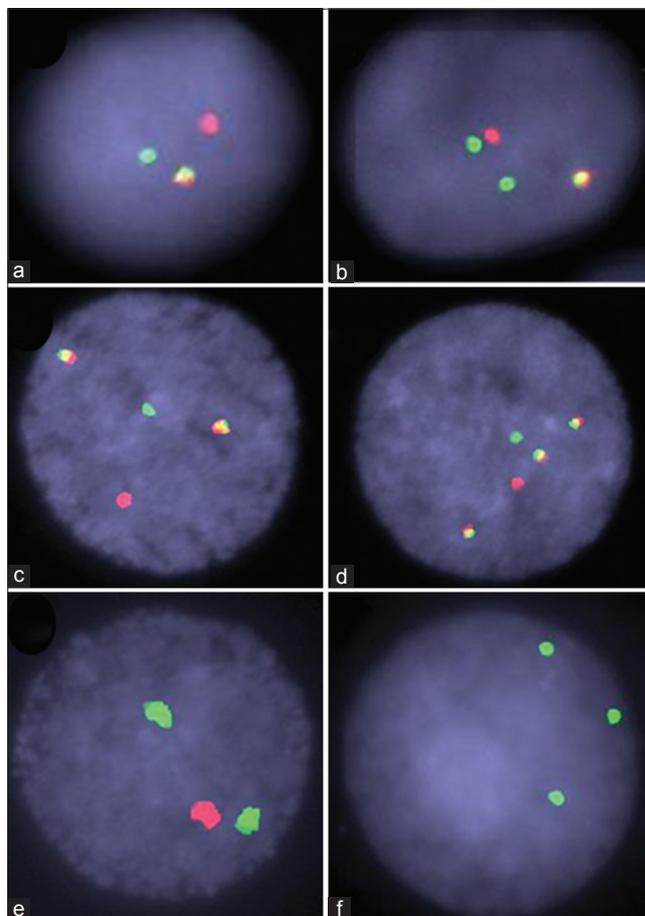


**Fig. 1:** (a) GTG-banded karyotype: 47,X,+der(X),-Y,del(1q), del(2)(p11),+3,del(8q),+2r(8),+der(9),der(12),-13,der(15), der(16),der(18),-19,-21,der(22) [arrows indicate abnormal chromosomes], (b) Spectral Karyotype: 47,X,+der(X)t(X;1), -Y,del(1q),del(2)(p11),der(2)t(2p;17),t(3p;18q),+der(3)t(3;8;18),del(8q),+2r(8),der(9)t(9;19),+der(9)t(9;2;19), der(12)t(Yq;12q),-13,der(15)t(13q;15q),der(16)t(16q;21q), der(18)t(18;?;1),-19,-21,der(22)t(??;2?;?)

The IGH break-apart probe showed a typical pattern in 40% of nuclei scored while an extra 5' signal was seen in 35% indicating clonal evolution. In concurrence, a classical fusion pattern and an extra fusion signal were observed in 40% and 35% of cells, respectively, with the FGFR3-IGH probe.

## DISCUSSION

MM is a clonal malignancy of differentiated B-cells with acquired genetic abnormalities of clinical importance unidentifiable by conventional cytogenetic analysis. Smol and Daudignon [12] compared IGH signal patterns using FGFR3-IGH dual-color, dual-fusion translocation probe, and IGH dual-color break-apart probe in 49 patients with MM. The  $t(4;14)$  translocation seen in 26% of cases was observed to be associated with multiple presentations of IGH, namely a loss or gain of the IGH locus and a deletion of the IGH variable segment. He *et al.* [13] reported several patterns of IGH deletion including monoallelic loss of whole IGH locus, monoallelic deletion of 3'IGH or 5'IGH and biallelic deletion of 3'IGH. Further, the authors observed IGH deletions to be more frequent with 13q deletion and without  $t(4;14)$ . Our proband showed  $t(4;14)$  translocation along with 13q deletion but without IGH deletion. In addition, a gain of 5'IGH variable segment was observed in the evolving sideline. The very high association of del(13q) abnormalities in  $t(4;14)$  patients and not vice versa led to the suggestion that chromosome 13 abnormalities precede the translocation [11,14]. Acquisition of additional abnormalities may reflect an underlying active or genetically



**Fig. 2:** Dual color IGH break-apart probe on interphase cells showing the pattern (a) F1G1R1 and (b) F1G2R1; LSI IGH-FGFR3 dual-fusion probe showing (c) F2G1R1 and (d) F3G1R1, (e) LSI D13S19 (13q14.3)/13q34 probe showing monoallelic deletion of locus 13q14.3 (one red signal) and two copies of locus 13q34 (two green signals); (f) CEP 9 probe exhibiting trisomy 9 (3 green signals). F yellow (fusion); G green; R Red

unstable clone driving the disease process at that point [15]. The translocation in association with del(13q) and complex chromosomal rearrangements denoted a poor prognosis in our patient who died within 6 months of diagnosis.

Application of SKY in our patient did not reveal the subtle t(4;14) translocation as in earlier reports describing new recurring translocations [8,16]. However, SKY could clarify the derivative chromosomes and confirm the numerical aberrations. Multicolor FISH techniques such as M-FISH and SKY provided improved characterization of complex and ill-defined karyotypes [2,3,8,9]. Sawyer *et al.* [9] using SKY identifying gain of 1q and loss of 8p frequently pointed out the importance of secondary aberrations in the progression of the disease. Several reports are available on the usefulness of SKY technique in the comprehensive analysis of chromosomes in hematopoietic disorders [17-19]. SKY will definitely serve as an adjunct to the conventional G-banding and iFISH techniques and aid in the identification of novel recurrent abnormalities in MM.

## CONCLUSION

A complex karyotype denotes rapid tumor progression, and the prognostic significance of individual abnormalities will be known through extended analysis over a larger sample size on confirmation using emerging technologies such as targeted next-generation sequencing.

## AUTHORS' CONTRIBUTIONS

P.G. performed the conventional cytogenetic and iFISH analyses; P.P. clinically examined, referred, and treated the patient; A.T. participated in iFISH analysis; P.S.K. and J.S.K. carried out SKY analysis; C.R.S. assisted in cytogenetic analysis; and P.G., C.R.S., and J.S.K. drafted the manuscript. All authors read and approved the manuscript.

## CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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