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Original Article

A diagnostic approach to detect cytogenetic heterogeneity and its prognostic significance in multiple myeloma

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الملخص

أهداف البحث: الورم النقوي المتعدد هو اضطراب دموي مع تكاثر غير متحكم به لخلايا البلازما النسيلية وتراكمها في نخاع العظم. حللت هذه الدراسة التردد والتغايرية الخلوية وارتباطها بالخصائص السريرية في مرضى الورم النقوي المتعدد.

طريقة البحث: تم تقييم نضح النخاع العظمي لسبعين وسبعين مريضا مصابا بـ الورم النقوي المتعدد باستخدام تقنيات الوراثة الخلوية التقليدية وتقنيات التهجين بين الطور البيني لمجموعة المسبار المناعي سلسلة ثقيلة، ومستقبلات عامل نمو "اف جي اف ار 3"، "ام أ اف بي"، حذف حذف الذراع القصيرة للكرموسوم 17 و حذف الذراع الطويلة للكرموسوم 13.

النتائج: كشفت الوراثة الخلوية التقليدية عن أنماط نواة غير طبيعية في 39 ٪ من المرضى الذين تم فحصهم. كانت نسبة حدوث 28% لنقصان ثنائي الصيغة الصبغية (72/20) ، وإفراط ثنائي الصيغة الصبغية كانت 10٪ (72/7). كشف تحليل تقنيات التهجين بين الطور البيني عن وجود نقل(11؛14) في 6٪ (72/4) و نقل(4؛14) في 11٪ (72/8) مريض. ارتبط المرضى الذين يعانون من نقصان ثنائي الصيغة الصبغية ومن إفراط ثنائي الصيغة الصبغية بالعديد من الأحاديات و التثلث. في تحليل كابلان ماير، لوحظ وجود فرق كبير بين المجموعات الإيجابية والسلبية لنقل(4 ؛ 14) والتثلث الصبغي 14 و أحاد الصبغي 13 ويرتبط ببقاء أقصر. أظهر التحليل النسبي "كوكس" أن نقل(4 ؛ 14)، والتثلث الصبغي 14 وأحاد الصبغي 13هي العوامل المهمة مع نسبة المخاطر 0,187 و 0.109 و 0.134.

الاستنتاجات: بالإضافة إلى التشوهات الوراثية الخلوية ، كشف تحليل تقنيات التهجين بين الطور البيني عن عدم التجانس الموجود بين مرضى الورم النقوي المتعدد. يجب اعتبار عدم التجانس الخلوي الخلوي في مرضى الورم النقوي المتعدد كعلامة تنبؤية رئيسية تساهم في تنوع المرض. ومن هنا تشير هذه النتائج إلى هذه التشوهات كعوامل تنبؤية مستقلة.

الكلمات المفتاحية: الوراثة الخلوية التقليدية؛ عدم التجانس الخلوي؛ مضان في الموقع التهجين؛ الورم النقوي المتعدد؛ التنبؤ

Abstract

Objective: Multiple myeloma (MM) is a hematological disorder involving the uncontrolled proliferation of clonal plasma cells and its accumulation in the bone marrow. This study analyzed the frequency, cytogenetic heterogeneity, and clinical characteristics of patients with MM.

Methods: Bone marrow aspirates were obtained from 72 patients with MM and evaluated by conventional cytogenetics (CCs) and interphase fluorescence *in situ* hybridization (iFISH) techniques for a panel of probes, including immunoglobulin heavy chain (IgH)/CCND1, IgH/fibroblast growth factor receptor 3 (FGFR3), IgH/MAFB, 13q deletion, and deletion 17p.

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Results: CCs revealed abnormal karyotypes in 39% of the patients examined. The incidence of hypodiploidy was 28% (20/72) while that of hyperdiploidy was 10% (7/72). iFISH analysis revealed t(11;14) in 6% (4/72) and t(4;14) in 11% (8/72) of patients. Patients with hyperdiploidy and hypodiploidy were associated with several monosomies and trisomies. Kaplan−Meier analysis revealed a significant difference between positive and negative groups for t(4;14), trisomy 14, and monosomy 13; this was associated with a shorter survival time. Cox proportional analysis identified t(4;14) ($P = 0.032$), trisomy 14 ($P = 0.004$), and monosomy 13 ($P = 0.009$), as significant factors with hazard ratio of 0.187 [confidence interval (CI): 0.041−0.862], 0.109 [CI: 0.024−0.500] and 0.134 [CI: 0.030−0.600].

Conclusion: In addition to cytogenetic abnormalities, iFISH analysis revealed significant heterogeneity among patients with MM. Cytogenetic heterogeneity in patients with MM should be considered as a major prognostic marker contributing to the variability of the disease. Our findings suggest that these abnormalities are independent prognostic factors.

Keywords: Conventional cytogenetics; Cytogenetic heterogeneity; Fluorescence *in situ* hybridization; Multiple myeloma; Prognosis

Introduction

Multiple myeloma (MM) is a clonal plasma disorder characterized by the accumulation of malignant plasma cells in the bone marrow. This disease evolves from premalignant monoclonal gammopathy of undetermined significance (MGUS) to smoldering multiple myeloma (SMM) and then to MM. This disease accounts for 10% of all hematological malignancies and is the second most common hematological malignancy.[1] MM is a malignant lymphoproliferative disorder with hypercalcemia, lytic bone lesions, renal impairment, and anemia with end-organ damage. This is a heterogenous disease that is mainly characterized by a spectrum of numeric and structural genetic aberrations. MM involves two oncogenic pathways; one is the non-hyperdiploidy pathway (involving translocations of immunoglobulin heavy chain locus [IgH]) and the hyperdiploidy pathway (involving the gain of an odd number of chromosomes).[2] Metaphase analysis by G banding provides us with a tool to detect aberrations at the cellular level in 30−50% of patients in advanced stages than in cases with newly diagnosed MM.[3] In patients with a low proliferative index, the identification of cryptic anomalies is better detected by interphase fluorescence in situ hybridization (iFISH) than by conventional cytogenetics (CCs). iFISH is a more sensitive method as this technique can facilitate detection on a cell-by-cell basis using region-specific probes.[4]

Recent cytogenetic analysis has shown that translocation involving (IgH) at 14q32, along with chromosomes 4, 6, 11, 16, 20, and the gain of chromosomes, are of prognostic importance in assessing the risk of patients with MM.[5] Translocation t(4;14), t(14;16), t(14;20), and del 17p, are considered to be high risk (HR), while t(6;14) and t(11;14) are considered to have standard risks for chromosomal abnormality.[6] The use a staging system, along with CCs and iFISH, provides significant insight into planning treatment strategies and predicting the outcome of this disease. Hallmark abnormalities, such as t(11;14), are associated with better outcomes, while t(4;14), del 17p, and t(14;16) are considered to be associated with adverse outcomes.[7]

The survival rate varies from several months to 10 years; this variation may be due to differences in prognosis, clinical presentation, and treatment response. Cytogenetics, molecular subtypes, and the clone size, can help us to understand the individualization and management of an individual's treatment. Genetic research is essential in molecular characterization and clinical translation. In the era of novel agents, treatment strategies vary from one individual to another based on staging, CCs, and iFISH analysis. Therefore, CCs and iFISH are recommended in the initial diagnostic workup of patients with MM.[8] The obstinate and resistant effect on patient treatment may be due to genomic complexity, and therefore, the role of these abnormalities in the disease needs to be investigated. In this study, we comprehensively evaluated the clinical characteristics, prevalence, and prognostic significance, of recurrent cytogenetic heterogeneity in association with myeloma treatment in patients with MM.

Materials and Methods

Patients

Bone marrow samples were acquired from 72 patients with MM who had been referred to the Department of Oncology, K.S. Hegde Charitable Hospital. The diagnosis was based on the International Myeloma Working Group Criteria (IMWG). Of the 72 patients, seven of them had relapsed MM. The Institutional ethics committee approved the study. Bone marrow was harvested in a heparin vacutainer and used for cytogenetic analysis following the morphological diagnosis of MM using bone marrow aspiration smears. All patients provided informed and written consent.

Conventional cytogenetics

An appropriate sample based on WBC count was added to 5 ml of Marrow Max media (Gibco, USA). For 24−48 h, the culture was incubated and then treated with 100 µL of KaryoMAX colcemid (0.08 µg/mL, Gibco), followed by hypotonic solution (KCl, 0.075 M) and Carnoy's fixative (methanol/acetic acid 3:1). The fixed pellet was then dropped onto slides and aged overnight at 60 °C. The GTG banding was performed using 0.05% trypsin and 1% Giemsa stain. Twenty well-banded spreads were captured using an Olympus BX53 microscope (Olympus, Tokyo, Japan) and

analyzed using GenASIs software (Applied Spectral Imaging, Edingen − Neckarhausen, Germany). The results were interpreted in accordance with the International System of Human Cytogenetic Nomenclature (ISCN 2013).[9]

FISH

iFISH analysis was performed on fixed pellets using probes targeting IGH/CCND1, IGH/FGFR3, IGH/MAFB, and RB1and P53 (Wuhan HealthCare Biotechnology) to detect t(11;14) (q13;q32), t(4;14) (p16;q32), t(14;20) (q32;q12), del(13q14), and del(17p13), respectively. The pellet and 10 μL of the probe were added to the hybridization area. The pellet and probe were co-denatured at 88 °C for 2 min and hybridization overnight at 45 °C. One hundred interphase nuclei were scored and signals were visualized using an OLYMPUS BX53 Fluorescence microscope equipped with DAPI and fluorescein isothiocyanate (FITC) and Texas Red filters. Metaphase and interphase nuclei were scored and the signals were captured using FISH View image Acquisition software (GenASIs, Applied Spectral Imaging). The results were interpreted according to the International System of Human Cytogenetic Nomenclature (ISCN 2013).[9]

Statistical analysis

To test the difference and association between groups, we performed Fisher's exact test for categorical variables. Overall survival was defined from the time of diagnosis until death. The distribution of survival times was estimated using the Kaplan−Meier, test and differences between groups were determined using the log-rank test. Cox regression analysis was used to evaluate the association between overall survival and cytogenetic events. The hazard ratio and confidence interval (CI 95%) were also determined. The patients were divided into four categories based on the therapy received: (a) chemotherapy, (b) chemotherapy and radiotherapy, and (c) stem cell transplantation. A P-value of <0.05 was considered statistically significant. Statistical analysis was performed with IBM SPSS (Statistical Package for the Social Sciences) statistical tool version 22.

Results

Patient clinical characteristics

The mean age of patients was 61.31 ± 8.67 years. Males were affected to a greater extent (42 patients 58%) than females (30 patients 42%). The male to female ratio was 1.4 (42/30).

The proportion (%) of bone marrow plasma cells and other clinical details are summarized in Table 1.

The International staging system (ISS) and Revised International Staging System (R-ISS) were used to stratify MM patients.[6,10]

Conventional metaphase cytogenetics

A total of 72 patients with MM were enrolled in this study. Of these, 40 patients (56%) had a normal karyotype while and 28 (39%) cases had an abnormal karyotype. Due to the inability to analyze metaphase, four cases (5%) were defined as culture failure. Of the abnormal cases, 20 patients (71%) were hypodiploid cases (17 hypodiploid and three pseudodiploid), seven (25%) were hyperdiploid cases, and one patient had interstitial deletion of chromosome 16 (Figure 1). Two hyperdiploid cases also had gains in structural rearrangements, such as the inversion of 1p and duplication of the 3q region. The common missing chromosomes among all hypodiploid karyotypes were chromosome 9, 12, 13, 16, 18, 20, 21, 22, and Y. Gain of chromosomes 5, 8, 15 and 19 were the most common manifestations (Figure 2).

iFISH results

Chromosomal analysis by iFISH showed no abnormality in 44 of the MM cases (61%) and abnormalities in 28 cases (39%). Hyperdiploid and hypodiploid groups were typically characterized by trisomy and monosomy. Of the 72 cases, IgH translocations were detected in 12 patients (17%), trisomy in 16 patients (22%) and monosomy in 13 patients (18%). Seven patients (8%) had only IgH translocations without trisomy and monosomy, 12 patients (17%) had only trisomy, and six patients (8%) had only monosomy. Four patients (6%) had both IgH translocations and trisomy, while six patients 6(8%) had both IgH translocations and monosomy.

Table 1: Clinical and laboratory characteristics.

Characteristics	
Age (years), mean ± SD	61.31 ± 8.67
Sex, male, n (%)	42 (58%)
Sex, female, n (%)	30 (42%)
ISS Staging, n	
I/II/III/undetermined	18/26/23/5
R-ISS Staging, n	
I/II/III/undetermined	23/19/26/5
IgG/IgA/undetermined	55/12/5
Light chain type, n, kappa/ lambda/undetermined	42/25/5
Plasma cells in bone marrow	
<10%	13
10−25%	21
>25%	38
Serum albumin (g/dL)[a]	3.52 (1.1−5.2)
Serum globulin (g/dL)[a]	3.65 (1.43−14.43)
Serum calcium (mEq/L)[a]	9.05 (5.4−16.8)
Creatinine (mg/dL)[a]	1.1 (0.44−6.99)
Sodium (mEq/L)[a]	135 (124−142)
Potassium (mEq/L)[a]	3.4 (2.74−40.4)
Alkaline phosphatases (U/L)[a]	94 (31−1136)
Hb (g/dL)[a]	9.5 (4.9−15)
TLC × 10^9[a]	6.2 (1.1−78.1)
ESR (mm/h)[a]	59.5 (3−150)
Platelet count × 10^9[a]	209.5 (32−504)

ESR, erythrocyte sedimentation rate; Hb, hemoglobin; TLC, total leukocyte count.
[a] Median (range).

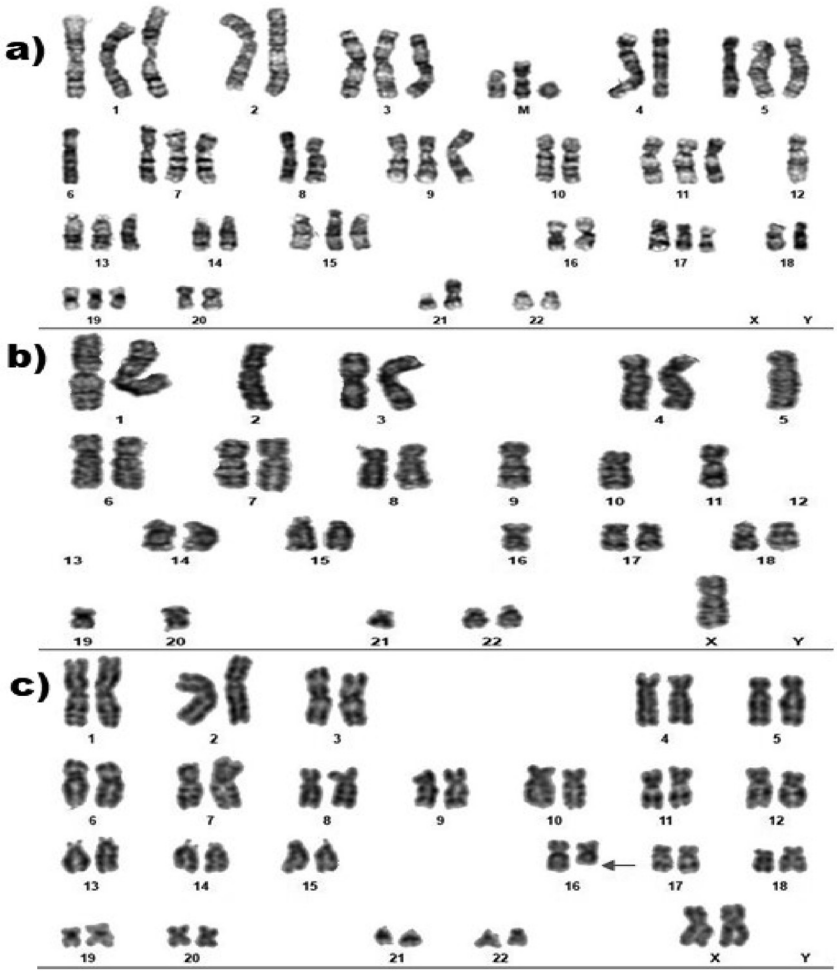

Figure 1: Representative image for hyperdiploid and hypodiploid karyotypes. (a) 55, XX, +1, +3, +5, −6, +7, +9, +11, −12, +13, +15, +17, +19, t(21;21), +3mar. (b) 32, XY, −2, −5, −9, −10, −11, −12, 12, −13, −13, −16, −19, −20, −21, −Y. (c) 46, XX, del(16) (q12q22).

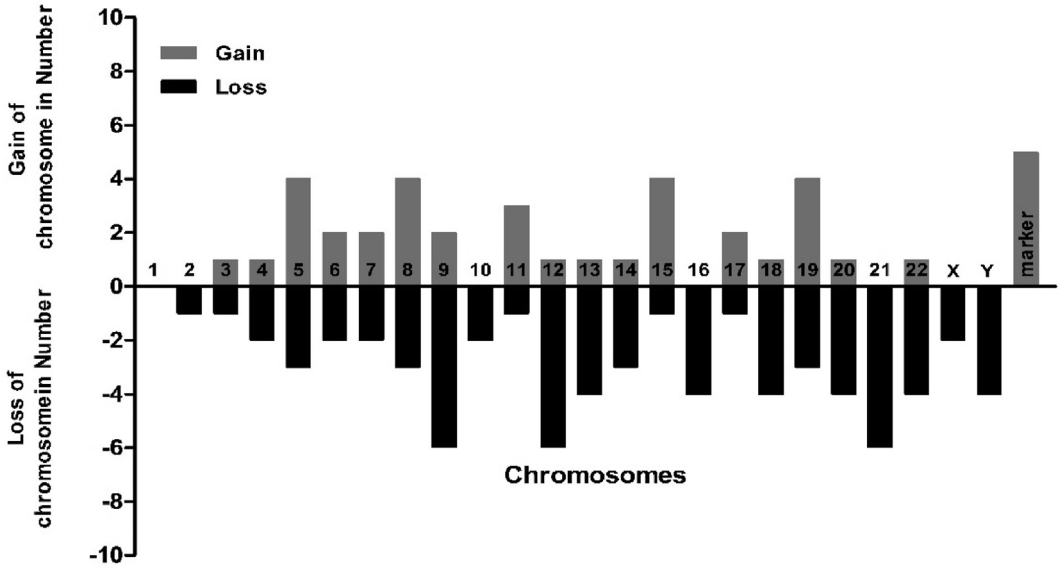

Figure 2: Bar graph showing the gain and loss of chromosomes.

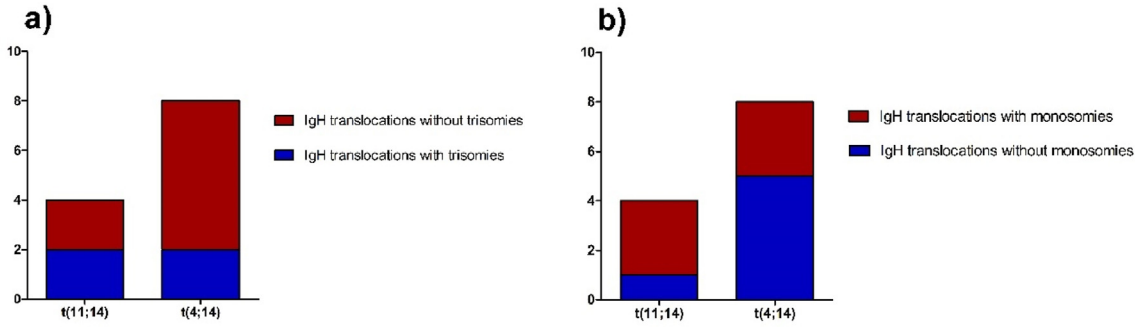

Figure 3: (a) Image of IgH translocation with and without trisomies. (b) Image of IgH translocation with and without monosomies.

t(4;14) was the most common primary IgH translocation detected in eight (11%) of all cases analyzed. Of these, six patients (8%) were found without trisomy while two patients (3%) had trisomy. Three patients (4%) were without monosomy, while five patients (7%) had monosomy. t(11;14) was detected in four patients (6%) of all cases analyzed. Two patients (3%) were shown to have trisomy and two patients (3%) had trisomy. One patient (1%) had monosomy while three patients (4%) did not have monosomy (Figure 3). No deletions were evident in any of the cases, although monosomy 13 was detected in 12 patients (43%). Monosomy 17 was detected in one patient while trisomy 17 was detected in two cases (Figure 4).

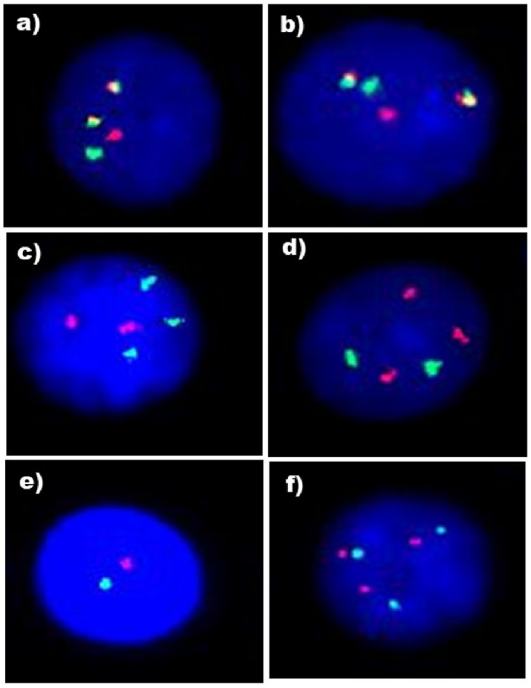

Figure 4: iFISH pattern of (a) cell showing 2 fusion, 1 green and 1 red signal indicating (11;14) (q13:q32) positive. (b) Cell showing 2 fusion, 1 green and 1 red signal indicating t(4;14) (p16;q32) positive. (c) Cell showing 3 green signals indicating trisomy of chromosome 14. d) Cell showing 3 red signals indicating trisomy 11. (e) Cell showing 1 green and 1 red signal indicating monosomy 13. (f) Cell showing 3 green and 3 red signals indicating trisomy 17.

Survival analysis

The median follow-up duration from the time of diagnosis until the last follow up for the total number of patients was 11.3 months with a range of 1–43.06 months (Figure 5). Out of the 72 MM cases, 35 patients (49%) received only chemotherapy; this included both VTD (bortezomib, thalidomide and dexamethasone) and VCD (bortezomib, cyclophosphamide and dexamethasone). Sixteen patients (22%) received both chemotherapy and radiotherapy, and 11 patients (15%) underwent stem cell transplants. The therapeutic patients of 10 patients (14%) was unavailable.

The abnormalities included with and without t(4;14) (p16;q32) (median OS: 10.93 vs 12.14 months: $P = 0.014$), trisomy 14 (median OS: 9.09 vs 11.78: $P = 0.0003$), trisomy 17 (median OS: 7.59 vs 11.78: $P = 0.0047$), monosomy 13 (Median OS: 10.14 vs 12.09: $P = 0.020$). No significant difference was detected for t(11;14) (q13:q32) (median OS: 14.07 vs 10.93, $P = 0.143$) and trisomy 11 (median OS: 17.06 vs 10.98, $P = 0.063$). Kaplan–Meier OS estimates of cytogenetic abnormalities are given in Figure 6.

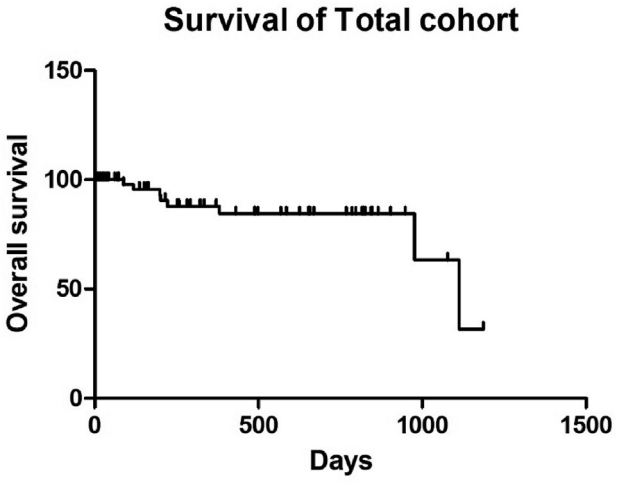

Figure 5: Kaplan–Meier plot for overall survival of the total cohort.

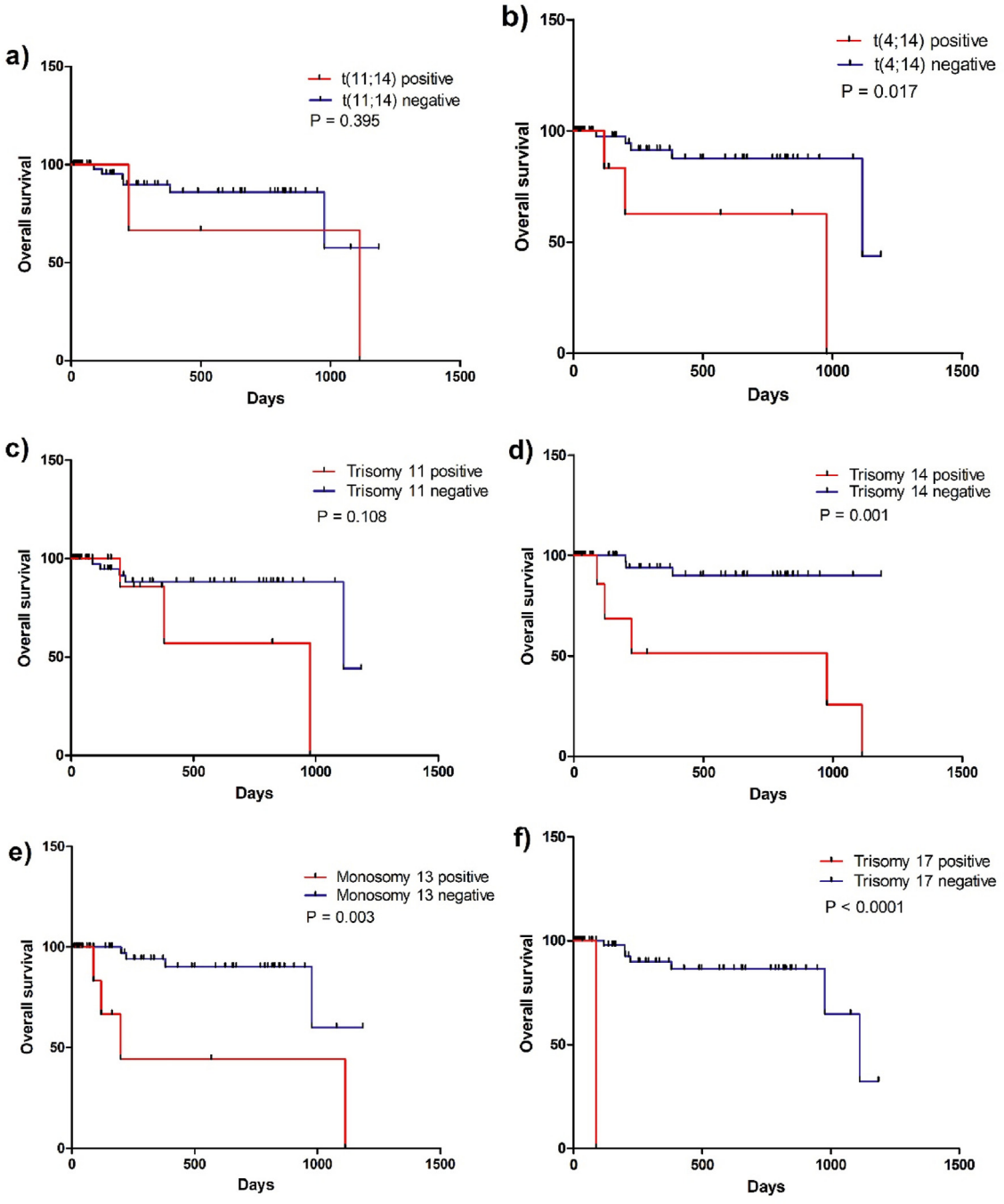

Figure 6: Kaplan–Meier plot for overall survival estimation.

Table 2: Cox regression model for cytogenetic abnormalities, overall survival and hazard ratios.

Parameters	Hazard ratio	95% CI	P
Age, ≥61 vs <61 years	2.08	0.487–8.920	0.322
Sex	2.33	0.470–11.622	0.300
Serum albumin ≥3.52 vs <3.52 g/dL	2.76	0.558–13.72	0.213
Serum globulin ≥3.65 vs <3.65 g/dL	0.425	0.082–2.200	0.308
Serum calcium ≥9.05 vs <9.05 mEq/L	0.432	0.101–1.843	0.257
Creatinine ≥1.1 vs <1.1 mg/dL	0.266	0.052–1.350	0.110
Sodium ≥135 vs <135 mEq/L	2.804	0.659–11.927	0.163
Potassium ≥3.40 vs <3.40 mEq/L	1.135	0.217–5.938	0.881

(*continued on next page*)

Table 2 (*continued*)

Parameters	Hazard ratio	95% CI	P
Alkaline phosphatases ≥94 vs <94 U/L	0.403	0.081−2.000	0.266
Hb ≥ 9.5 vs <9.5 g/dL	2.499	0.554−11.278	0.234
TLC × 10^9 ≥6.2 vs <6.2 × 10^9	1.582	0.354−7.080	0.548
ESR ≥59.5 vs <59.5 mm/h	1.937	0.370−10.127	0.433
Platelet count × 10^9 ≥209.5 vs <209.5 × 10^9	2.549	0.494−13.157	0.264
t(11;14) (q31;q32)	0.468	0.080−2.750	0.401
t(4;14) (p16;q32)	0.187	0.041−0.862	**0.032***
Trisomy 11	0.310	0.069−1.404	0.129
Trisomy 14	0.109	0.024−0.500	**0.004****
Monosomy 13	0.134	0.030−0.600	**0.009****

Cox regression hazard regression analysis test was done. $P < 0.05$ was considered to be significant (*$P < 0.05$, **$P < 0.01$). Bold digits in the table represents significance level *$P < 0.05$, **$P < 0.01$.
ESR, erythrocyte sedimentation rate; Hb, hemoglobin; TLC, total leukocyte count.

Multivariate analysis

Cox regression analysis showed that t(4;14) (p16;q32), trisomy 14, and monosomy 13, had significantly worse overall survival rates. Of the clinical parameters, sodium and albumin had the highest hazard ratio (2.80 and 2.76, respectively). Of the cytogenetic abnormalities, t(11;14) (q31;q32) and trisomy 11 had the highest hazard ratio (0.46 and 0.31), respectively. No significant differences were detected for the other parameters (Table 2).

Discussion

Cytogenetic changes are considered a hallmark feature of most malignancies, including MM. CCs and iFISH are the most frequently used techniques to identify genetic aberrations in patients with MM. However, despite their diagnostic and prognostic significance, these techniques also have certain limitations. In a few cases, CCs cannot be performed due to the low proliferative index of mitotic cells. Unlike CCs, iFISH is a highly sensitive technique that applies to cells in both metaphase and interphase. We aimed to study the prevalence and association between clinical parameters and their prognostic significance in patients with MM.

The mean age of all patients recruited in the study was 61 years (range: 42−75). IgG isotype and Kappa chain light chain (LC) were higher than other isotypes and LC. In the present study of 72 samples, CCs was successfully carried out in 94% (68/72) of samples. The prevalence of cytogenetic abnormalities was 39% (28/72). This frequency was comparable with that described in previously published work.[1,7,11] Most genetic abnormalities were detected in patients whose bone marrow aspirate had a plasma cell burden of more than 25%.

In the present study, most cases showed numerical abnormalities; only one patient had structural abnormality. The abnormal karyotypes were further classified into hyperdiploidy, non-hyperdiploidy and pseudodiploidy based on the number of chromosomes present; hypodiploidy was the most common condition.

In this study, 26% of cases had a hyperdiploid karyotype; this was similar to the report published by Aras et al.[12] Hyperdiploidy is characterized by the typical gain of an odd number of chromosomes and has been demonstrated to be an excellent prognostic marker.

Chromosomes 5, 8, 15, and 19 were the most common gains among the hyperdiploidy group. Our study showed higher survival rates in the hyperdiploidy group than in the non-hyperdiploidy group, although this was not statistically significant. Numerous gains were evident in the hyperdiploidy group, including trisomies 3, 7, 9, 15 and 19; these patients tended to have more prolonged survival than non-hyperdiploidy patients.[13]

Patients with hyperdiploid MM are known to have better survival rates than patients with non-hyperdiploid MM.[14] Loss of chromosomes 9, 12, 21, and Y were common in cases of hypodiploidy. However, the frequency of hypodiploid karyotype was higher in our study. Hypodiploid myeloma is known to have a clinically aggressive phenotype. Several studies have suggested that hyperhaploidy and hypodiploidy are associated with worse outcomes.

In a previous study, Soekojo et al. used conventional karyotyping to evaluate HR patients when FISH analysis was unavailable; this study showed patients with non-hyperdiploid myeloma had the worst outcomes which led to a worse OS in stage II R-ISS patients.[15] The loss of sex chromosomes in males and females is known to be related to aging. However, the loss of the Y chromosome in MM leads to genomic instability.[16] In our study, we observed the loss of the Y chromosome (10.9%) and X chromosome (2.7%). Loss of the X and Y chromosomes has a prognostic significance in MM.

In this study, we detected one case with interstitial deletion of chromosome 16. This frequency was very low in our population when compared with other studies. The partial or deletion of 16q is associated with worse overall survival. WWOX (WW domain-containing oxidoreductase gene) and CYLD are two genes associated with del(16q) and are responsible for disease outcome.[17]

The rapid identification of abnormalities can be performed using specific target region probes by iFISH. We used t(11;14), t(4;14), t(14;20), del (13q) and del(17p) as probes for FISH. The most common translocation was t(4;14); this was found in 11% (8/72) of all cases analyzed; a previous study reported that the frequency of this translocation was 11−

15%.[18] We found that t(4;14)-positive patients had a shorter median overall survival than negative patients. Furthermore, sub analysis of t(4;14) showed that monosomies were additional abnormalities for this translocation; thus, this was considered as a HR prognostic marker.[19,20] t(11;14) was detected in 6% of patients (4/72); this frequency was lower than that reported in the literature.[21,22] Sub-analysis showed that t(11;14)-positive patients carried additional chromosomal aberrations such as trisomies and monosomies. t(11;14) is regarded as standard risk; however, coexistence with other lesions can be considered a HR prognostic marker.[23]

t(14;20) is considered as a rare translocation. This translocation was not detected in our study; however, this is regarded as a poor prognostic marker.

Monosomies and trisomies are the results of hyperdiploidy and non-hyperdiploidy and were detected by both CCs and iFISH analysis. In a previous study, trisomy 11 was detected in 15% of all cases and was reported as recurrent cytogenetic aberrations in MM.[24,25] Trisomy 11 and trisomy 14 were the most common trisomies observed, with trisomy 11 being a typical chromosomal gain.

In the current study, we classified abnormalities into sole IgH translocations, those with both translocations and trisomies, and then those with monosomies and translocations.

Of all IgH translocations, most of the translocations were sole abnormalities and were not associated with either trisomies or monosomies. In MM, trisomies are the earliest abnormalities to form, these are then followed by translocations and monosomies.[14,26] In our study, the frequency of trisomy 17 was observed in 3% of cases; this was comparatively low when compared with other reports. This suggests that during the progression of the disease, the prevalence of trisomy 17 increases. Trisomies result from an increase in copies of a few chromosomes; thus increasing the copy number of gene loci mediating drug sensitivity, thereby signifying the impact of trisomies on gene expression, gene dose-effect, and survival outcom-es.[27,28]

Monosomy 13 was one of the most common abnormalities and was detected in 50–60% of MM cases by iFISH. In our study, monosomy for chromosome 13 was common, followed by monosomy 17. No cases of RB1(13q14) deletion were detected using either of these techniques. Monosomy 13 is considered an important prognostic factor in the diagnosis of MM. Monosomy 13 and 17 represent late events occurring during the progression of MM. The presence of these specific aberrations suggests the aggressive feature of this disease.[5] Most t(4;14) patients also possessed monosomy 13 as an additional cytogenetic abnormality. Kalff and Spencer previously hypothesized that chromosome 13 abnormality precedes t(4;14) during pathogenesis.[29]

We did not find any association between age and gender with cytogenetic abnormalities. No significant association between clinical characteristics and cytogenetic abnormalities was observed. We observed that certain cytogenetic anomalies exerted influence on OS. The OS of patients with abnormal cytogeny detected by iFISH had a significantly shorter survival than patients with normal results. The median OS of the study was 11.3 months. Patients who were positive for t(4;14), trisomy 14, trisomy 17, and monosomy 13 had a shorter OS survival. There was no significant difference in OS between t(11;14)-positive and -negative patients. A considerable difference between patients who were positive or negative for t(4;14), trisomy 14, and monosomy 13 was observed, thus suggesting that these abnormalities can be used as good prognostic markers.

M-Smart risk stratification guidelines from the Mayo clinic have suggested the existence of double hit myeloma (with two HR genetic abnormalities) and triple hit myeloma (with three HR abnormalities).[30,31] Only one case in our study had double hit myeloma with two HR abnormalities.

In a previous study, Abdallah et al. showed that patients with IgH translocations might benefit from proteasome inhibitors (PIs) and that patients with trisomy have a better response to immunomodulatory drug (IMD) combinations.[4] Irrespective of treatment modality, the deletion of 17p is considered an inferior outcome.[32]

This study has several limitations that need to be considered. For example, the study had a small sample size and no plasma cell enrichment prior to tests. CD138 labeling for plasma cell enrichment is also highly recommended to increase the rate of abnormality detection. Thus, studies on larger sample sizes should now be conducted to investigate the clinical significance of heterogeneity among patients with MM.

Conclusion

Our findings demonstrate the importance of both CCs and iFISH in elucidating diverse chromosomal aberrations in association with the clinical features of patients with MM. Although iFISH is a highly sensitive technique, both CCs and iFISH increase the detection rate of abnormalities. Therefore, CCs and iFISH are effective tools for identifying and detecting abnormalities. Identifying heterogeneity among individuals will help us to plan treatment strategies and target potential factors in patients with MM. Even though the life expectancy of patients with MM has increased with new modes of treatment, relapse is still common. Therefore, it is essential to detect different anomalies during the initial diagnostic workup.

We also found that different abnormalities can act as individual prognostic markers, thus providing evidence that MM is a group of cytogenetic anomalies; these findings account for the known variability of this disease.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The present study was approved by the Central Ethics Committee, NITTE (Deemed to be) University (Ref: NU/CEC/2019/0224).

Authors' contribution

DPS instigated and supervised the project. MA and RS helped in the methodology and analysis of the data. VS, KPS, and RK helped in conceptualization and provided research materials. AK and NK worked on data curation and investigation. AK wrote the initial and final draft of the article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Availability of data and materials

All the data and materials are true and valid. All figures and tables are original.

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References

1. Udupa CBK, Udupa KS, Pai A, Sherigar P. Cytogenetics and revised international staging system (R-iss): risk stratification in multiple myeloma-a retrospective study in indian population. **Iran J Pathol 2020**; 15: 182–188.
2. Binder M, Rajkumar SV, Ketterling RP, Greipp PT, Dispenzieri A, Lacy MQ, et al. Prognostic implications of abnormalities of chromosome 13 and the presence of multiple cytogenetic high-risk abnormalities in newly diagnosed multiple myeloma. **Blood Cancer J 2017**; 7: 600–606.
3. Govindasamy P, Pandurangan P, Tarigopula A, Mani R, Samuel CR. Cytogenetic abnormalities in multiple myeloma patients at a tertiary healthcare center in India. **Asian Pac J Cancer Prev 2019**; 20: 235–241.
4. Abdallah N, Rajkumar SV, Greipp P, Kapoor P, Gertz MA, et al. Cytogenetic abnormalities in multiple myeloma: association with disease characteristics and treatment response. **Blood Cancer J 2020**; 10: 1–9.
5. Shin SY, Eom HS, Sohn JY, Lee H, Park B, Joo J, et al. Prognostic implications of monosomies in patients with multiple myeloma. **Clin Lymphoma Myeloma Leuk 2017**; 17: 159–164.
6. Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al. Revised international staging system for multiple myeloma: a report from international myeloma working group. **J Clin Oncol 2015**; 33: 2863–2869.
7. Jian Y, Chen X, Zhou H, Zhu W, Liu N, Geng C, et al. Prognostic impact of cytogenetic abnormalities in multiple myeloma: a retrospective analysis of 229 patients. **Medicine 2016**; 95(1–7).
8. Barilà G, Bonaldi L, Grassi A, Martines A, Liço A, Macrì N, et al. Identification of the true hyperdiploid multiple myeloma subset by combining conventional karyotyping and FISH analysis. **Blood Cancer J 2020**; 10: 1–5.
9. Shaffer LG, McGowan-Jordan J, Schmid M, editors. *ISCN 2013: an international system for human cytogenetic nomenclature (2013)*. Karger Medical and Scientific Publishers; 2013.
10. Greipp PR, Miguel JS, Durie BG, Crowley JJ, Barlogie B, Bladé J, et al. International staging system for multiple myeloma. **J Clin Oncol 2005**; 23(15): 3412–3420.
11. Ashok V, Ranganathan R, Chander S, Damodar S, Bhat S, Nataraj KS, et al. Comparison of diagnostic yield of a FISH panel against conventional cytogenetic studies for hematological malignancies: a south Indian referral laboratory analysis of 201 cases. **Asian Pac J Cancer Prev 2017**; 18: 3457–3464.
12. Aras BD, Akay OM, Sungar G, Bademci G, Aslan V, Caferler J, et al. Conventional and molecular cytogenetic analyses in Turkish patients with multiple myeloma. **Turkish J Hematol 2012**; 29: 135–142.
13. Huang SY, Yao MI, Tang JL, Tsay WO, Lee FY, Liu MC, et al. Clinical significance of cytogenetics and interphase fluorescence in situ hybridization analysis in newly diagnosed multiple myeloma in Taiwan. **Ann Oncol 2005 Sep 1**; 16(9): 1530–1538.
14. Chng WJ, Santana-Dávila R, Van Wier SA, Ahmann GJ, Jalal SM, Bergsagel PL, et al. Prognostic factors for hyperdiploid-myeloma: effects of chromosome 13 deletions and IgH translocations. **Leukemia 2006**; 20: 807–813.
15. Soekojo CY, Wang G Miao, Chen Y, Casan J, Wolyncewicz G, Lin A, et al. Role of conventional karyotyping in multiple myeloma in the era of modern treatment and FISH analysis. **Clin Lymphoma Myeloma Leuk 2019**; 19: e470–e477.
16. Aydin C, Ulas T, Hangul C, Yucel OK, Iltar U, Salim O, et al. Conventional cytogenetics and interphase fluorescence in situ hybridization results in multiple myeloma: a Turkey laboratory analysis of 381 cases. **Indian J Hematol Blood Transfus 2020**; 36: 284–291.
17. Jenner MW, Leone PE, Walker BA, Ross FM, Johnson DC, Gonzalez D, et al. Gene mapping and expression analysis of 16q loss of heterozygosity identifies WWOX and CYLD as being important in determining clinical outcome in multiple myeloma. **Blood 2007**; 110: 3291–3300.
18. Kumar SK, Rajkumar V, Kyle RA, van Duin M, Sonneveld P, Mateos MV, Gay F, Anderson KC. Multiple myeloma. **Nat Rev Dis Prim 2017**; 3:17046.
19. Sonneveld P, Avet-Loiseau H, Lonial S, Usmani S, Siegel D, Anderson KC, et al. Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the International Myeloma Working Group. **Blood 2016**; 127: 2955–2962.
20. Rajkumar SV. Multiple myeloma: 2018 update on diagnosis, risk-stratification, and management. **Am J Hematol 2018**; 93: 1091–1110.
21. Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. **Leukemia 2009**; 23: 2210–2221.
22. Hu Y, Chen W, Chen S, Huang Z. Cytogenetic abnormality in patients with multiple myeloma analyzed by fluorescent in situ hybridization. **OncoTargets Ther 2016**; 9: 1145–1149.
23. Leiba M, Duek A, Amariglio N, Avigdor A, Benyamini N, Hardan I, Zilbershats I, Ganzel C, Shevetz O, Novikov I, Cohen Y. Translocation t (11;14) in newly diagnosed patients with multiple myeloma: is it always favorable? **Gene Chromosom Cancer 2016**; 55: 710–718.
24. Liebisch P, Wendl C, Wellmann A, Kröber A, Schilling G, Goldschmidt H, et al. High incidence of trisomies 1q, 9q, and 11q in multiple myeloma: results from a comprehensive molecular cytogenetic analysis. **Leukemia 2003**; 17: 2535–2537.
25. Chretien ML, Corre J, Lauwers-Cances V, Magrangeas F, Cleynen A, Yon E, et al. Understanding the role of hyperdiploidy in myeloma prognosis: which trisomies really matter? **Blood 2015**; 126: 2713–2719.
26. Kumar S, Fonseca R, Ketterling RP, Dispenzieri A, Lacy MQ, Gertz MA, et al. Trisomies in multiple myeloma: impact on survival in patients with high-risk cytogenetics. **Blood 2012**; 119: 2100–2115.

27. Jung HA, Jang MA, Kim K, Kim SH. Clinical utility of a diagnostic approach to detect genetic abnormalities in multiple myeloma: a single institution experience. **Ann Lab Med 2018**; 38: 196–203.
28. Mei J, Zhai Y, Li H, Li F, Zhou X, Song P, et al. Prognostic impact of hyperdiploidy in multiple myeloma patients with high-risk cytogenetics: a pilot study in China. **J Cancer Res Clin Oncol 2018**; 144: 2263–2273.
29. Kalff A, Spencer A. The t(4;14) translocation and FGFR3 overexpression in multiple myeloma: prognostic implications and current clinical strategies. **Blood Cancer J 2012**; 2: e89–e98.
30. mSMART Risk Stratification of Newly Diagnosed Myeloma, https://static1.squarespace.com/static/5b44f08ac258b493a25098a3/t/5b802d8270a6adbc6a79a678/1535126914646/Risk+Strat+3.0rev_svr.pdf.
31. Baysal M, Demirci U, Umit E, Kirkizlar HO, Atli EI, Gurkan H, et al. Concepts of double hit and triple hit disease in multiple myeloma, entity and prognostic significance. **Sci Rep 2020**; 10: 1–6.
32. An G, Li Z, Tai YT, Acharya C, Li Q, Qin X, et al. The impact of clone size on the prognostic value of chromosome aberrations by fluorescence in situ hybridization in multiple myeloma. **Clin Cancer Res 2015**; 21: 2148–2156.

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