ORIGINAL ARTICLE

Cytogenetic Abnormalities at Diagnosis and During Follow Up in Multiple Myeloma Patients and Their Prognostic Implications - A Preliminary Report

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ABSTRACT

Introduction: Multiple myeloma (MM) is characterized by genomic instability and cytogenetic abnormalities (CAs). The variation in rates of treatment response and overall survival is thought to arise from multiple genomic events that result in tumour development and progression. This study investigated CAs among the newly diagnosed MM (NDMM) patients at diagnosis and during follow-up and subsequently to assess their prognostic significance. **Methods:** This is a prospective cohort study where bone marrow (BM) samples of 16 NDMM patients were collected at diagnosis and during follow up and subjected to conventional cytogenetic analysis (CCA) and interphase fluorescence in situ hybridization (iFISH) analyses using standard procedures. Spectral karyotyping was performed on a selected patient who showed complex karyotype (CK) pattern on CCA. **Results:** Clonal abnormalities were identified in 56.3% of analysis by karyotyping and 68.8% by combination of karyotyping and iFISH. CCA showed normal diploid, hypodiploid and hyperdiploid karyotypes, whereas iFISH analysis could detect various IGH translocations involving 14q32 region, del(13q14) and del(17p13). Significant associations were observed between patients with complex karyotypes and abnormal karyotypes. Patients who had a CK or showed an abnormal CCA and iFISH results were associated with worse survival (p=0.011 and p=0.034 respectively). Structural abnormalities were found to be more common among hyperdiploid MM patients (p=0.001). Cytogenetic evolution was seen in the follow-up of BM cytogenetics. **Conclusion:** Karyotyping and iFISH are valuable assets in detecting prognostically relevant genomic abnormalities. The presence of cytogenetic evolution demonstrated the value of cytogenetics in monitoring treatment response and in management of MM.

Keywords: Conventional cytogenetics, iFISH, Spectral karyotyping, Complex cytogenetic abnormalities, High-risk cytogenetic abnormalities

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INTRODUCTION

Multiple myeloma (MM) is a cytogenetically heterogeneous malignant plasma cell (PC) disorder characterized by over-proliferation of monoclonal PCs in bone marrow (BM) leading to production of monoclonal protein and associated with organ dysfunction (1-3). According to GLOBOCAN 2018 (4) estimates, MM accounts for approximately 0.9% of all cancers and 1.1% of all cancer death globally. It also accounts for approximately 13% of all haematological malignancies (5, 6). The clinical presentations of newly diagnosed MM (NDMM) are highly heterogeneous. Presentation can range from asymptomatic with positive only for biomarkers of active disease to presence of one or more myeloma defining events (MDEs) namely hypercalcaemia, renal impairment, anaemia and bone lesions. Response to treatment and survival of NDMM are also heterogeneous with median overall survival (OS) ranging from two to >10 years (7). Studies over the years have revealed many factors that lead to the disease heterogeneity (8). The age and existing comorbid illnesses are important host factors as these will define the treatment strategy, especially the eligibility for transplant and tolerance to certain chemotherapy. Nevertheless, the most important tumour factor is genetic factors.

Host factors and genetic factors are two criteria which influence prognosis in MM patients. Genetic factors,

especially CAs in MM can affect every aspect of the disease, from tumour development, progression and evolution to clinical presentation, response to therapy and prognosis. Numerous numerical and structural chromosomal abnormalities such as chromosomal translocations, deletions and duplications can be found in the karyotypes of the malignant PCs. Genetic subtypes of MM have different underlying biologic features and show heterogeneity in clinical outcomes. A study conducted at the Mayo Clinic (9) found several important associations between CA and clinical presentation. Studies also have shown that presence of certain high risk genetic abnormalities namely del(17p), del(1p32), t(4;14) and 1q gains in the malignant PCs were associated with inefficient response to the lenalidomide-dexamethasone combination therapy but there was an improvement in survival by adding a proteasome inhibitor or a monoclonal antibody to the lenalidomide-dexamethasone regime (10-13).

Clinicians need to stratify MM patients into appropriate risk categories as the cytogenetic results guide treatment selection. However detecting cytogenetic abnormalities by conventional cytogenetic analysis (CCA) has been historically challenging in MM. Conventional cytogenetics is hindered by the low proliferation index of PC, resulting in cytogenetic abnormalities (CAs) detection rate of only 20 to 30% (7) and there is no assurance that a metaphase obtained is originated from the PC clone. Some cryptic CAs such as t(4;14), t(14;16), del(13q) and del(17p) are not detectable by CCA. In patients with a low proliferation index of myeloma cells, a normal karyotype if obtained, could likely be representing or corresponding to the genetic background of normal BM cells instead of malignant myeloma cells. Therefore, CCA is no longer the recommended first line routine test. A more sensitive technique using fluorescence in situ hybridization (FISH) carried out on interphase cells has been able to overcome the limitations of karyotyping. Since interphase FISH (iFISH) analysis does not require dividing cells, more cells can be screened, and this increases the sensitivity in detecting myeloma cells. With the availability of CD138-expressing PCs purification kit and dual staining probe for cytoplasmic immunoglobulin (Ig), the sensitivity has further increased. However, non-availability of probe combinations that are capable of covering all CAs is still a limitation. Many different probes might be needed to cover all possible numerical and structural abnormalities. Even then, the information collected might only represent a small fraction of the genome at one time and hence other CAs that are not covered by FISH probes go undetected. This study aimed to investigate the CAs among the newly diagnosed MM patients in Kelantan state, Malaysia at diagnosis and during follow-up, to stratify the patients based on their CAs and subsequently to assess their prognostic significance especially on how these cytogenetic risk groups affect the outcome of the patients.

MATERIALS AND METHODS

Patients

Ethical clearance was obtained from The Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/ 18030179) prior to the commencement of this study. This is a prospective cohort study involving 16 patients who were newly diagnosed with MM at Hospital USM from January 2017 to June 2018. Patients who fulfilled the diagnostic criteria for active MM defined by the revised International Myeloma Working Group (IMWG) diagnostic criteria for MM (2) only were included. All patients recruited had at least a BM cytogenetics with or without iFISH analysis performed during diagnosis. For patients without iFISH analysis performed during diagnosis, archive genetic material of these patients from the previous cytogenetic diagnostic test were used to perform iFISH analysis. Detailed information was extracted from patients' medical records including age of diagnosis, sociodemographic data, clinical presentation and relevant laboratory results. All patients underwent standard treatment with bortezomib-based triple-drug regimens recommended by the National Comprehensive Cancer Network (NCCN) Guidelines for MM (14). They were followed up until the end of the study period (12th June 2019). At least one CCA with iFISH analyses were performed during their follow up to evaluate their response to treatment based on the absence or presence of cytogenetic evolution. Cytogenetic evolution is defined as a change in previous baseline CAs such as acquisition of new CAs or regression of previous baseline cytogenetic features.

CCA, FISH analysis and FISH Spectral Karyotyping (SKY)

Chromosomal analysis was performed on overnight cultured BM samples and slides with metaphase chromosomes were prepared and stained by standard GTG-banding technique. A minimum of 20 GTG banded metaphases were karyotyped for each case. Chromosomal abnormalities were reported based on the International System for Human Cytogenomic Nomenclature 2016 (ISCN 2016) (15). The cultured BM samples were used for FISH analysis. FISH analysis using the MM FISH panel probes from Cytocell® FISH probes were performed on interphase nuclei to detect the most common MM specific cytogenetic abnormalities. A total of seven FISH probes from Cytocell Ltd. were investigated namely translocations of the immunoglobulin heavy (IGH) chain gene region 14q32, using dual-fusion FISH (D-FISH) probes for the five common IGH translocation partners [t(11;14)(q13;q32) {CCND1/IGH}, t(6;14)(p21;q32) {CCND3/IGH}, t(4;14)(p16.3;q32) {FGFR3/IGH}, t(14;16)(q32;q23) {IGH/MAF}, t(14;20)(q32;q12) {IGH/MAFB}] and the locus-specific probe for 17p13.1 deletion (D17Z1, TP53) and monosomy 13/deletion 13q14.3 (RB1,163C9) with del(13q14.3) referring to the loss of RB1 gene. For each probe set, at least 200

interphase nuclei were analysed where the probable abnormalities had to be present in at least 10% of the cells, to be reported as positive. SKY was performed on one selected patient who showed complex karyotype pattern on CCA, using GenASIs Hyper Spectral Karyotyping (HiSKY) Kit which contains 24-colour combinatorically labelled FISH probes.

Statistical Analysis

Baseline characteristics were presented as mean [standard deviation (SD)] and median [interquartile range (IQR)] for numerical data while categorical data were presented as frequency (percentage). Variation in CAs and biochemical profile among patients with MM were presented as frequency and percentage. Fisher's exact test was used to determine the association of CAs with other clinical parameters. The overall survival (OS) was defined as the duration from diagnosis to the date of death or last follow-up and were analysed using Kaplan-Meier analysis. Median survival time is the first observed time at which cumulative survival is 50% or less. All statistical analyses were performed using SPSS version 24. The results were considered significant if the p-value was <0.05.

RESULTS

Among the 16 patients included in this study, eleven (68.8%) were males and five (31.3%) were female patients with age ranging from 47 to 73 years old. At diagnosis, the mean age was 60.5 ± 7.38 years old. All the patients recruited in the present study were of Malay ethnicity. Majority (75%) of the patients were presented at the age of 65 and below as well as with advanced stage based on Durie Salmon Staging System (D-SSS) and International Staging System (ISS). According to D-SSS stage, 56.3% were in stage II and 43.7% were in Stage III. As per ISS stage, at least 50% were in stage III, 12.5% were in stage II, 6.3% were in stage I while the remaining 31.2% were unsure of status due to missing data (Table I). Among the hallmark sequelae of MM, anaemia and osteolytic bone lesion (87.5% respectively) were the most common presentations among the MM patients in the present study, followed by renal impairment (50%) and hypercalcaemia (43.8%) (Table I).

Among the 16 patients, the minimum number of 20 metaphases were not available in a few patients due to insufficient BM particles for culture. Clonal CA were identified in 56.3% of analysis by karyotyping and 68.8% by combination of karyotyping and iFISH. iFISH analysis was able to detect more clonal cytogenetic abnormalities among the MM patients such as t(4;14), t(14;16), del(13q) and del(17p) (Table I, Table II). Due to financial constraints, SKY could be carried out only in one patient who showed a complex karyotype (Fig. 1). SKY enabled identification of cryptic translocation and marker chromosomes in genetically complex malignant myeloma cells, present in this patient (Fig. 2).

Table I: Patients' baseline characteristics (n=16)

Particulars	n (%)
Sex	
Male	11 (68.7)
Female	5 (31.3)
Age at diagnosis (years)	60.5 (7.38)*
Durie-Salmon stage	
I A/B	0 (0.0)
IIA	8 (50.0)
IIB	1 (6.3)
IIIA	2 (12.5)
IIIB	5 (31.2)
ISS stage	
I	1 (6.3)
II	2 (12.5)
III	8 (50.0)
NA	5 (31.2)
Presence of Hypercalcaemia	7 (43.8)
Presence of Renal impairment	8 (50.0)
Presence of Anaemia	14 (87.5)
Presence of Bone lesion	14 (87.5)
Elevated LDH level	3 (18.7)
Abnormal Karyotype alone	9 (56.3)
Abnormal Karyotype + FISH	11 (68.8)
Complex karyotype (CK)**	10 (62.5)
HRCA***	9 (56.3)

Abbreviations: ISS- International Staging System, LDH- lactate dehydrogenase, HRCA- high risk cytogenetic abnormalities
*Mean(SD)
**A complex karyotype is defined as presence of 3 or more cytogenetic abnormalities including at least one structural abnormality.
*** A HRCA is defined as presence of either nonhyperdiploid karyotype or karyotype showing del(13q) through CCA or iFISH showing abnormalities such as del(17p13), t(4;14)(p16.3;q32), t(14;16)(q32;q23), t(14;20)(q32;q12) or bi-allelic del(13q14).

In the present study, based on combination of karyotyping and iFISH findings, 56.3% of the patients showed complex karyotype (CK). A CK is defined as presence of three or more CA including at least one structural abnormality (16). Fisher's exact test showed that there was significant association between abnormal karyotype pattern (detected by CCA alone) and CK pattern (p=0.001). Significant association was also seen between abnormal karyotype (detected by combination of CCA and iFISH) and CK (p= 0.001). Nine patients who had abnormal karyotypes detected by CCA alone as well as ten patients who had abnormal karyotypes detected by combination of CCA and iFISH, showed presence of CK.

There was a significant association between hyperdiploid clone and structural abnormalities (p=0.001) (Table III). All hyperdiploid clones detected from karyotyping showed at least one structural abnormality (Table II). Structural abnormalities detected by iFISH were not included in the analysis as some patients had mixture of hypodiploid and hyperdiploid clones. Therefore, it was not possible to distinguish whether the structural abnormalities detected by iFISH belonged to which clones, either hypodiploid or hyperdiploid clones.

The presence of abnormal karyotypes detected by

Table II: Karyotypes and FISH results of 16 MM patients

Case No.	Conventional cytogenetics	FISH analysis	Survival time (months)	Last status*
1.	46,XY[10]	del(13) -14.4% t(14,16)-10.0% del(17p)-10.7%	14.8	D
2.	46,XX[28]	All negative	23.3	C
3.	39-45,XY,-9[3],-14[3],-21[3] [cp12]/46,XY[29]	Monoallelic del(13)-29.9% Biallelic del(13)-70.1%	15.6	D
4.	48-54,XY,+3[2],+5[3],+7[3],i(8)(q10)[2],+11[4],+15[3],+19[5], +20[2],?add(20)(p13)[3],+21[2][cp5]/46,XY[12]	All negative	22.2	C
5.	46,XY[20]	Monoallelic del(13)-31.7% Monosomy 13-1.4% Biallelic del(13)-66.9% t(11;14)- 33.1%	21.0	C
6.	46,XY,?t(6;14)(p21;q32)[4]/51,XY,?t(6;14)(p21;q32),+5,+7,+9, +15,+19[1]/46,XY[5]	Monoallelic del(13)-36.7% Biallelic del(13)-63.3% t(6;14)-62.2%	3.2	D
7.	45,XY,add(2)(q32),t(11;14)(q13;q32),-13,-19,-20,+mar1,+mar2[3]/ 46,XY[17]	del(13)- 13.3% t(11;14)- 16.5%	23.5	C
8.	50,XX,+der(1;15)(q10;q10),+der(1;15)(q10;q10),+3,+9[8]/44- 45,XX,-13[2],-14[2],-22[4][cp4]/46,XX[10]	Monosomy 13-10.0%	19.7	D
9.	46,XY[16]	All negative	29.3	C
10.	55-56,XY,+der(2)t(2;11)(q21;p15)[3],+3[3],+5[3],+6[2],+7[3],+9[3],+der(11)t(2;11)(q21;p15)[3],+15[2],+17[2],+19[3],+20[2]/48,XY- ,+17,+19[1]/46,XY[31]	All negative	27.7	D
11.	46,XX [27]	All negative	29.0	C
12.	46,XY [20]	All negative	2.0	C
13.	53,Y,der(X)t(X;22)(q27;q11.2),+3,+5,+6,+9,+11,+15,+der(17) ins(17;1;3)(p11.2;?;?), +der(17)ins(17;1;3)(p11.2;?;?),-17,+19,-22 [15]/46,XY[3] (based on SKY findings)	Monoallelic del(13)-11.0% Biallelic del(13)-64.0% del(17p)-17.0%	4.5	D
14.	51-53,XX,t(2;3)(q21;p21)[8],+5[8],+7[3],+11[7],+14[4],+15[7], +17[3], +19[9],+21[8][cp9]/46,XX[4]	t(4;14)- 11.9% t(11,16)-15.7% del(13) -12.5% del(17p)-21.7%	24.0	D
15.	46,XX[32]	All negative	0.6	C
16.	43-45,X,-Y[4],-6[3],-21[3][cp16]/47-50,XY,+5[5],+7[4],+9[4],del(9) (q21q33)[4],t(14;16)(q32;q22)[2],+15[5],?add(15)(q26)[5] [cp5]/46,XY[28]	t(14;16)- 9.6%	29.3	C

*Last status – C=censored, D=death

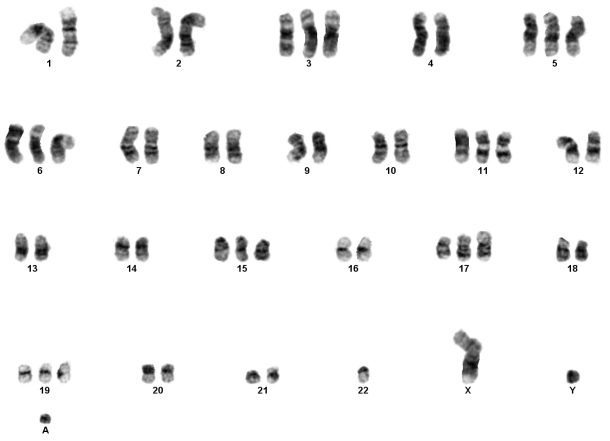

Figure 1: GTG banded metaphase of patient no.13 showing 53,Y,der(X)t(X;22)(q27;q11.2), +3,+5,+6,+9,+11,+15,der(17)ins(17;1;3)(q11.2;?;?),+der(17)ins(17;1;3)(q11.2;?;?),+19,-22 karyotype pattern.

combination of karyotyping and iFISH was found to have a significant association with shorter survival (death) (p=0.034) (Table III). Significant association was also observed between complex karyotypes and death (shorter survival) (p=0.001). During Kaplan Meier survival analysis in the current study, the median survival time of patients with and without CK could not be estimated due to short follow-up duration. However, there was a significant difference between patients with CK and without CK (p=0.041).

In MM, presence of either nonhyperdiploid karyotype or karyotype showing del(13q) through CCA or iFISH showing abnormalities such as del(17p13), t(4;14)(p16.3;q32), t(14;16)(q32;q23), t(14;20)(q32;q12) or biallelic del(13q14) are considered as high risk cytogenetic abnormalities (HRCA) (7). In the present study, the median survival of patients with HRCA was 19.7 months while for patients without HRCA, median survival time was not reached. There was no significant difference in survival time among patients with and without HRCA (p=0.054) (Fig. 3).

For follow up BM cytogenetic analysis, only three patients were available (patient no 4, 7, 8 from Table II). Out of these three patients, two patients had shown abnormal findings during follow up cytogenetics. Patient no.4 who previously had a CK by CCA and normal iFISH result during diagnosis, showed a normal

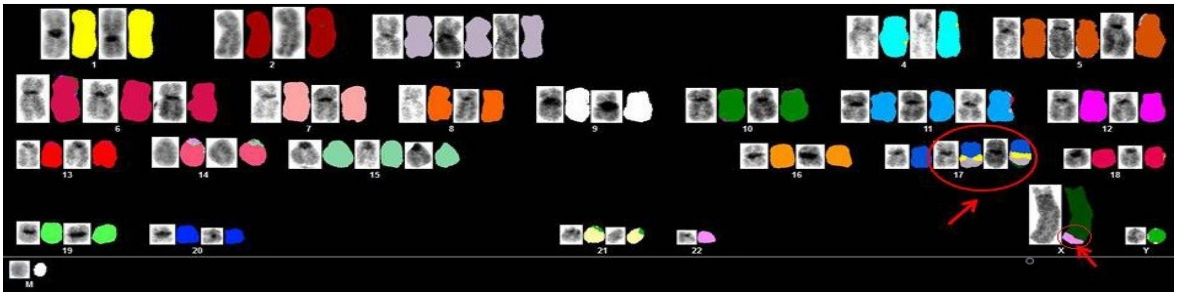

Figure 2: SKY for patient no.13 confirming the origin of der (17) resulting from insertion of translocated segments from chromosome 1 and 3 to chromosome 17, der(X) resulting from translocation of chromosome 22 to chromosome X and demonstrating marker originated from chromosome 9.

Table III: Summary of association between cytogenetic abnormalities, patients' last status, patients' staging and patients' clinical parameter using Fisher's exact test (n=16)

Complex karyotype (CK) [n (%)]			
	Non CK	CK	P value
Normal karyotype Abnormal karyotype	6 (37.5) 0 (0)	1(6.25) 9 (56.25)	0.001*
Normal karyotype + iFISH Abnormal karyotype + iFISH	5 (31.25) 1 (6.25)	0 (0) 10 (62.5)	0.001*
Last status [n (%)]			
	Censored	Death	P value
Normal karyotype Abnormal karyotype	6 (37.5) 3 (18.75)	1 (6.25) 6 (37.5)	0.060
Normal karyotype + iFISH Abnormal karyotype + iFISH	5 (31.25) 4 (25)	0 (0) 7 (43.75)	0.034*
Non CK CK	6 (37.5) 3 (18.75)	0 (0) 7 (43.75)	0.011*
No HRCA HRCA	6 (37.5) 3 (18.75)	1 (6.25) 6 (37.5)	0.060
Non advanced DSS (stage I &II) Advanced DSS (stage III)	6 (37.5) 3 (18.75)	3 (18.75) 4 (25)	0.615
Non advanced ISS (stage I &II)** Advanced ISS (stage III)**	2 (18.2) 5 (45.4)	1 (9.1) 3 (27.3)	>0.950
Normal LDH level*** Increased LDH level***	7 (46.7) 1 (6.7)	5 (33.3) 2 (13.3)	0.569
Hyperdiploidy [n (%)]			
	Hyperdiploid absent	Hyperdiploid present	P value
Absence of structural abnormality Presence of structural abnormality	8 (50) 1 (6.25)	0 (0) 7 (43.75)	0.001*
Absence of IgH translocation Presence of IgH translocation	6 (37.5) 3 (18.75)	4 (25) 3 (18.75)	>0.950
Advanced DSSS (stage III) [n (%)]			
	DSSS stage I &II	DSSS stage III	P value
Normal karyotype + iFISH Abnormal karyotype + iFISH	4 (25) 5 (31.25)	1 (6.25) 6 (37.5)	0.308
Non CK CK	4 (25) 5 (31.25)	2 (12.5) 5 (31.25)	0.633
No HRCA HRCA	5 (31.25) 4 (25)	2 (12.5) 5 (31.25)	0.358
Non advanced ISS (stage I &II)** Advanced ISS (stage III)**	3 (27.3) 4 (36.35)	0 (0) 4 (36.35)	0.236
Advanced ISS (stage III) [n (%)]**			
	ISS stage I & II	ISS stage III	P value
Normal karyotype + iFISH Abnormal karyotype + iFISH	1 (9.1) 2 (18.2)	3 (27.3) 5 (45.4)	>0.950
Non CK CK	1 (9.1) 2 (18.2)	4 (36.4) 4 (36.4)	>0.950
No HRCA HRCA	2 (18.2) 1 (9.1)	4 (36.4) 4 (36.4)	>0.950

Abbreviation: DSSS- Durie-Salmon Staging System, ISS- International Staging system, LDH-lactate dehydrogenase, HRCA- high risk cytogenetic abnormalities

**Only 11 patients had ISS stage. Therefore, analysis was performed among patients with ISS stage only.

***Only 15 patients had LDH level. Therefore, analysis was performed among patients with LDH level only.

karyotype by CCA and presence of del(13q14) by iFISH on the BM sample obtained after autologous stem cell transplantation (ASCT). For patient no. 7, follow up BM cytogenetics that was performed five months after he achieved at least very good partial response (VGPR), later showed similar CCA and iFISH abnormalities that were present during initial diagnosis. However, patient no.8 who had a CK by CCA and abnormal iFISH result during diagnosis, showed a normal iFISH on the BM sample obtained during follow-up analysis. Only iFISH results were available for patient no.8 as karyotyping was unable to be carried out due to non-availability of adequate and satisfactory metaphase spreads from her follow up BM sample.

DISCUSSION

In the present study, a slight preponderance of males accounting for 68.7% of the study subjects was observed compared to female MM patients. This higher incidence of MM among males is in agreement with GLOBOCAN 2018 estimates (4) which reported that the incidence of MM among males are higher as compared to females. The population of Kelantan state in Malaysia comprises predominantly of Malay ethnicity and accounts for approximately 92% of the whole Kelantan state population (17). This explains why all the patients

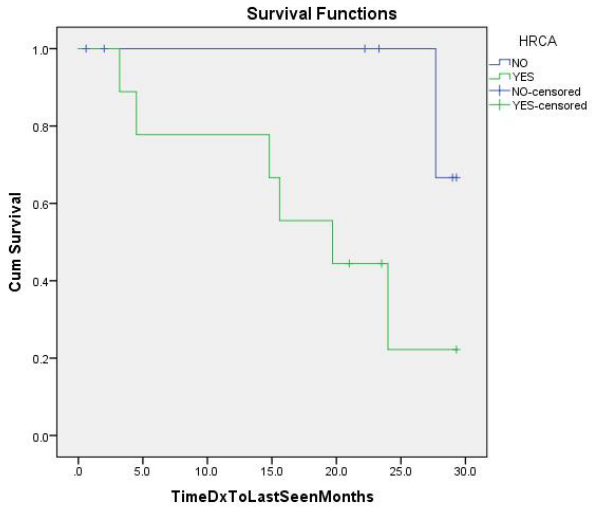

Figure 3: Survival plot of patients with HRCA

recruited in the present study were of Malay ethnicity.

Genomic instability in MM is characterized by CAs ranging from chromosome numbers to genetic translocations and genetic mutations. Based on the CCA in the present study, it is clear that the karyotype patterns of the MM patients can be highly heterogeneous. The abnormal karyotypes consisted of both numerical and structural abnormalities. The numerical abnormalities observed included hypodiploid clone alone, a mixture of hypodiploid and hyperdiploid clones or hyperdiploid clone alone. With regard to structural abnormalities, in addition to the chromosomal translocations involving the immunoglobulin H (IgH) locus (14q32) with several other common chromosomal translocation partners (chromosome 4, 6, 11 and 16), few other forms of complex structural abnormalities were also detected through CCA. If karyotyping employing CCA was not performed on these BM samples, these complex structural abnormalities would have been missed had it been done by iFISH analysis alone. This reiterates the importance of both CCA and iFISH analyses in MM.

Fisher's Exact test had demonstrated a significant association between CK and abnormal karyotypes detected either by karyotyping alone (p=0.001) or by a combination of karyotyping and iFISH (p=0.001). This shows that the patients who had abnormal karyotypes by CCA were likely to have a CK pattern. The IMWG consensus 2016, had grouped patients with combinations of ≥3CAs into ultra-high-risk group which only had less than two years of survival (7). This indicates that karyotyping other than iFISH, is also needed to predict patient outcome. Because of the sub-microscopic nature of some CAs such as del(13q14) and del(17p13), these cryptic abnormalities are usually underscored by CCA. By performing iFISH, these sub-microscopic CAs can also be detected and thus can further increase the CAs detection rate and this allows better prediction of outcome.

In the present study population, it was also noticed that structural abnormalities were more commonly observed in the hyperdiploid clone. All seven patients with hyperdiploid clone detected by CCA had at least one structural abnormality also within the same clone. Out of these seven hyperdiploid cases, five cases had high-risk CAs such as t(4;14), t(14;16), del(13q14) or monosomy 13 and del(17p13). The structural chromosomal abnormalities detected were not confined to the common chromosomal translocations involving the 14q32 IgH locus. Other forms of chromosomal rearrangements such as deletions and insertions etc were also detected. There was a strong association between hyperdiploid clone and structural abnormality (p=0.001). The current study results are not in agreement with Debes-Marun et al (18) who reported that non-hyperdiploid MM (NH-MM) harbour a higher prevalence of structural chromosome abnormalities other than the IgH translocations as compared to the hyperdiploid MM (H-MM) group. In the present study, the hyperdiploid clones were associated with a higher prevalence of other structural abnormalities leading to a complex karyotype pattern. In this context, the prognostic value of hyperdiploidy as good prognostic marker needs to be re-evaluated in large study. Comparison of the prognosis of H-MM patients with commonly occurring trisomies and H-MM patients with known high-risk genetic abnormalities should be carried out in larger samples. The finding in the present study, is in agreement with Barwick et al. (19) who reported that IgLambda (IgL) translocations, identified to be an independent negative prognostic marker, was more common in myelomas exhibiting hyperdiploidy. While 78% of IgL-MYC translocations co-occur with H-MM, t(IgL-MYC) is rarely identified clinically, suggesting that this subset of high-risk patients is being misclassified into standard-risk which confers a better prognosis. In general, hyperdiploidy alone do not confer poor prognosis (7, 20) and tend to have a better prognosis than IgH-translocated MM (21). The prognostic value of H-MM was related to trisomy 3 (7) and trisomy 5 (7, 13). Some studies (22, 23) had shown an ameliorating effect of concomitant trisomies in patients with newly diagnosed high risk MM while some did not (24, 25). The latest mSMART 3.0 classification of active multiple myeloma (26) had stated that presence of trisomies may ameliorate the effects of the high-risk (HR) genetic abnormalities if present individually, but do not ameliorate the HR effect of Double Hit Myeloma (presence of any 2 HR genetic abnormalities) and Triple Hit Myeloma (presence of 3 or more HR genetic abnormalities). The contrasting effect that was observed in the current study need to be further validated by other larger studies to confirm its prognostic significance. Because of the small sample size in the present study, the results might not be representative of the entire genetic background of the disease. Larger data sets are needed to determine the association between trisomies and other structural abnormalities.

On the other hand, findings from the current study are in agreement with Rajan and Rajkumar (27) who reported that detection of any CA on conventional metaphase cytogenetics indicates a more proliferative form of MM and have a poor prognosis. Due to the low proliferation index of PC, generally the CAs detection rate by CCA has been reported to be only 20 to 30% (7). Therefore, the presence of trisomies on CCA do not carry the same good risk implications as they do when detected by FISH. As demonstrated in the current study, the presence of an abnormal karyotype by conventional karyotyping itself might have been an indication of adverse prognosis. In the present study, all the patients who showed chromosomal abnormalities detected by CCA, had at least one additional structural abnormality also. According to Carballo-Zarate et al (28), the median OS of MM patients with H-MM was negatively correlated with the number of additional

structural chromosomal aberrations. The present study results are in disagreement with Barwick et al's finding (21) that H-MM is almost mutually exclusive with IgH translocations. In the current study, 18.8% of the patients with H-MM had IgH translocations demonstrated in their hyperdiploid clone. It is reasonable to suggest that small proportions of hyperdiploid myeloma do coexist with IgH translocations.

Sonneveld et al (7) had summarized the MM cytogenetic risk into two groups namely high risk (HR) and standard risk based on IMWG consensus (2016). The HR group included patients with t(4;14)(p16.3;q32), t(14;16)(q32;q23), t(14;20)(q32;q12), del(17/17p), gain(1q), karyotype del(13), nonhyperdiploid karyotype and gene expression profiling (GEP) high-risk signature. The standard risk was categorized as all other abnormalities including t(6;14) (p21; q32) and t(11;14) (q13;q32). In the present study, bi-allelic deletion of RB1 gene detected by iFISH was also included into the HR group. Chavan et al. (29) had reported that bi-allelic inactivation of RB1 gene, which commonly results from the homozygous deletion of RB1 gene, is an independent negative prognostic marker associated with relapse and a poor prognosis. Bi-allelic RB1 deletion is not commonly seen in NDMM. In the current study, there were four NDMM patients with bi-allelic RB1 gene deletion. This probably could be because of the late presentation and also delay in establishing diagnosis from the initial suspicion of the disease. Many of the patients in the current study population preferred to seek alternative therapy (traditional treatment) prior to presentation to the hospital which might have led to delay in presentation. Some of the patients in the present study also refused BM trephine biopsy (BMAT) during the initial presentation and subsequently went for alternative therapy. These groups of patients usually came back to the hospital a few months later when the alternative therapy failed or when the symptoms worsen. By then, the disease might have progressed along the way. Among the three patients with bi-allelic RB1 deletion who had passed away, one of them had the diagnosis established only after four months from the initial presentation to hospital, as the patient refused BMAT even after repeated counselling. He also had at least six months history of bone pain prior to the first presentation to hospital. Another patient also had a similar history where he had two years history of bone pain before he sought medical attention. This phenomenon is not uncommon in the present study hospital setting. At the end of this study, only one patient with bi-allelic RB1 deletion is still alive. Because of the short follow up period in this patient (<2 years), it is anticipated that in this patient also disease may progress in near future because of the bi-allelic RB1 deletion which is a HR poor prognostic marker.

Data from previous studies had shown that almost all cases with a t(4;14) have monosomy of chromosome 13 leading to loss of RB1 which emerged as a prognostic marker (30-33). Chavan et al. (29) had confirmed that the negative prognostic effect of RB1 deletion found in their study was not due to association with t(4;14). Further analysis of the results after dividing the samples based on presence/absence of each alteration, showed that patients with either the t(4;14) or alteration of RB1 were associated with a poor prognosis. When both lesions were present together, the prognosis was worse. With the advancement of technology such as micro array and sequencing, more studies had reported such co-occurrences or oncogenic dependencies between genomic markers (33). Mono-allelic RB1 deletion has been reported to be present in up to 50% of NDMM patients (21). In the current study, 50% of the NMDD showed either monosomy 13 or del(13q14) involving either mono-allelic or mixture of mono-allelic and bi-allelic deletions of the RB1 gene. Presence of these abnormalities in most of the cases indicates that del(13q14) could be an early event and RB1 gene deletion could be a driver mutation in MM pathogenesis. Based on double hit theory, it is reasonable to suggest that the presence of mono-allelic RB1 deletion during the initial presentation will place the patient at higher risk and a second hit, either by mutation or deletion of remaining RB1 gene, will render bi-allelic RB1 inactivation that is associated with poor prognosis and relapse.

In the current study, two out of three patients who were available for follow-up BM cytogenetic analysis, showed abnormal findings. One was a post-ASCT patient (patient no.4) who showed emergence of new CA (deletion 13q14) in low percentage detected by iFISH while another patient showed re-emergence of the previous baseline CA detected by CCA and iFISH. Patient no.4 showed disappearance of previous baseline complex CAs detected by CCA but showed the emergence of new CA. Even though this patient appeared clinically and biochemically to be normal, emergence of new CA maybe an early warning sign of disease progression. She requires close monitoring to enable early detection of disease progression and subsequently early intervention. Patient no.7 showed re-emergence of the baseline CAs indicating that the malignant myeloma cells had developed drug resistance to the previous responsive chemotherapy. It is reasonable to suggest that patients who show re-emergence of baseline CAs during follow-up CCA require more aggressive treatment and early ASCT as disease refractoriness is the most common terminal pathway leading to death. Patient no.8 who had a relapse showed normal iFISH results for all the IgH translocations and del(17p13) and del(13q14) during her relapsed BM sample analysis. But it is reasonable to presume that she might have shown other complex CAs, had her metaphases been available for karyotyping. Comparative Genomic Hybridization (CGH) would have been an alternative method to detect the genomic abnormalities in this patient; unfortunately, CGH and arrayCGH studies were beyond the scope of the present study.

The results of the follow-up BM cytogenetics demonstrated the value of cytogenetics in monitoring the treatment response and in the management of the disease. Detection of cytogenetic evolution such as the emergence of new CA and disappearance, re-emergence or persistence of previous baseline CAs during follow up allow monitoring of patient's treatment response and early detection of disease progression. This will allow in planning the frequency of patient monitoring, predicting the aggressiveness of treatment and outcomes and designing more aggressive treatment or rescheduling therapy accordingly.

The Kaplan Meier survival analysis in the present study showed significant difference between patients with CK and without CK (p=0.041). Although no significant difference in median survival time was seen between the HRCA and non-HRCA group (p=0.054), the HRCA group had lower median survival time compared to the non-HRCA. The one death that occurred in the non-HRCA group had a CK even though the patient did not have the defined HRCA. Because of the short duration of follow up time (within the short study period), the OS data generated need not be accurate. The median OS has been reported to range from <2 years in ultra-high-risk patients to >10 years in standard risk patients (7). Despite this, the results from the present study are in agreement with IMWG consensus 2016. Among the ten patients with complex karyotypes, seven patients had passed away. Six patients (60%) had passed away within two years and one patient (10%) had passed away within three years. Five out of eight (62.5%) patients with HRCA passed away within two years of diagnosis.

The present study has a few limitations such as a short duration of study period, short follow up duration, small samples size and missing data. Therefore, the duration of follow up for each patient recruited was relatively short which led to underestimate the survival time. The sample size was small because MM is one among the rare haematological malignancies. The short duration of the study period also limited the number of patients recruited. The data on a few clinical parameters, which are required for staging system were missing for a few patients in the present study. This led to a failure in establishing an association between the CA and clinical parameters such as ISS stage and LDH level. Likewise, due to the limited amount of financial support, FISH probes for detecting 1q21 gain and t(8;14) were not included in the study. Therefore, patients who were likely to have these CA would have missed. SKY could not be carried out in all the patients due to financial limitations. Besides that, due to financial constraints, CD138-expressing PCs purification kit or dual staining probe for cytoplasmic immunoglobulin (Ig) that can increase the sensitivity of myeloma cell detection rate were not available. Therefore, the percentage of each CA detected might have been lower than the actual percentage. In the event of the lower sensitivity of detection rate when these kits or probes were not used, there is the possibility of false negative results in patients with low myeloma cells count. These limiting factors might have deterred from getting significant results for some of the abnormalities detected by CCA or iFISH.

The results obtained from this study prompts to recommend that this study be replicated in larger sample size, longer study duration, longer follow-up period and utilizing more sophisticated tools such as GEP, arrayCGH and next generation sequencing to identify other similar or novel CA that can prognosticate the outcome of MM.

CONCLUSION

A better definition of MM subgroups is essential not only to provide a framework for patient counselling but also to provide more effective personalized therapies. The CAs detected by karyotyping and iFISH analysis play a crucial role in risk stratification and thus very important in prognostication as well as monitoring of treatment. CCA and iFISH analysis, which are valuable assets in detecting prognostically relevant genomic abnormalities, can be combined with ISS staging to help define prognostically relevant new subgroups that may help in the implementation of stratified/ personalized approaches in the management of MM patients.

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