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Cytogenetics and Revised International Staging System (R-ISS): Risk Stratification in Multiple myeloma - A Retrospective Study in Indian Population

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ABSTRACT

Background & Objective: Cytogenetic abnormalities in Multiple myeloma (MM) has emerged as the most important factor that determine the prognosis and survival. Fluorescence in situ hybridization (FISH) can detect a greater number of cytogenetic abnormalities as compared to conventional karyotyping and hence has become the standard test in determining genetic abnormalities in MM. The present study was planned as there is an unmet need to find out various cytogenetic abnormalities and to implement them in prognostic stratification by Revised International Staging System (R-ISS) among Indian population.

Methods: A single institution retrospective study was conducted among a total of 117 patients newly diagnosed as Multiple Myeloma. They were analyzed for various cytogenetic abnormalities by using interphase FISH (iFISH) and were staged according to Revised International Staging System (R- ISS).

Results: Out of the 117 patients studied, deletion 17p13 (p53) was present in 16 patients (13.67%). Thirty patients (25.64%) showed deletion 13q14.3. Three patients (2.56%) were detected to have t(4:14).Two patients (1.7%) had t(11:14) and t(14:16), respectively. Total of 19 patients (16.23%) in our study exhibited high risk cytogenetics and two among them had more than one high risk cytogenetic abnormalities. There was a 66.4% moderate correlation between ISS-III and high-risk cytogenetics which was statistically insignificant. Of the total 117 patients, 37 (31.62%) were staged R-ISS III.

Conclusion: High risk cytogenetics was found in 16.23 % of our study population and del 17p13 was the most common high-risk cytogenetic abnormality. Of the studied subjects, 31.62% had R-ISS III, which is significantly higher compared to western population.

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Introduction

Multiple myeloma is a neoplasm of terminally differentiated plasma cells that produce monoclonal proteins. It accounts for 10% of all hematological malignancies and 1% of all malignancies. The prognosis of myeloma was determined earlier by International staging system (ISS) based on two parameters namely beta-2 Microglobulin and Albumin.

Cytogenetic abnormalities in Multiple Myeloma has emerged as one of the most important prognostic factors which determines the resistance to treatment and the outcome of the disease (1). Due to low proliferative nature of malignant plasma cells, conventional cytogenetics miss a lot of cytogenetic abnormalities and hence interphase FISH (iFISH) is the preferred methodology which can detect >60% of genetic changes (1-3). Hence a new staging system incorporating cytogenetic abnormalities and lactate dehydrogenase (LDH) in addition to the parameters in ISS was developed by International Myeloma working group called Revised International staging system (R-ISS) for better prognosis of the disease (Table 1).

Table 1. Comparison of ISS and R-ISS

Stage	ISS	R-ISS
I	Serum β_2-microglobulin < 3.5 mg/L, AND Serum albumin ≥ 3.5 g/dl	ISS stage I and standard-risk CA by iFish AND Normal LDH
II	Not ISS stage I or III	Not R-ISS stage I or III
III	Serum β_2-microglobulin ≥ 5.5 mg/L	ISS stage III and either High-risk CA by iFISH or High LDH
Chromosomal abnormalities (CA) by iFISH		
High risk	Presence of del(17p) and/or translocation t(4;14) and/or translocation t(14;16)	
Standard risk	No high-risk CA	
LDH		
Normal	Serum LDH < the upper limit of normal	
High	Serum LDH > the upper limit of normal	

Materials and Methods

A retrospective study was conducted on 117 patients who were diagnosed as Multiple Myeloma according to International Myeloma Working Group criteria (4,5). Patients who underwent both cytogenetic analysis and R-ISS staging were included. Institutional ethics committee approval was taken prior to the study and written consent was obtained from all the participants. The retrospective recruitment was conducted at the Department of Medical Oncology at Kasturba Medical College, Manipal, between January 2014 and January 2019. Cytogenetic abnormalities were detected by using interphase FISH (iFISH) done on plasma cells which were enriched through Magnetic bead separation process using CD138 antibody in Onquest Laboratories, New Delhi.

2-3 mL bone marrow sample was taken and enriched for Plasma cells by EasySep Kit (STEMCELL technologies, USA), as follows. Desired cells were targeted with Tetrameric Antibody Complexes (TACs) recognizing CD138 and dextran-coated magnetic particles. This cocktail also had an antibody to human Fc receptor to minimize nonspecific binding. Labeled cells separation was done using an EasySep® (STEMCELL technologies, USA), magnet without the use of columns. Cells of interest remain in the tube while unwanted cells are poured off. The sample was evaluated for percentage of Plasma cells before and after enrichment.

The cell suspension was washed twice with cold freshly prepared fixative (methanol: acetic acid in the ratio 3:1) and either stored in refrigerator or directly taken for FISH staining. The sample was stained and studied using FISH probes for 13q14.3, 17p, IgH/CCND1, IgH/FGFR3, IgH/MAF (all probes were sourced from Vysis, Abbott Molecular).

Glass slides were appropriately labelled and cell suspension was dropped onto the slides. The slides were aged by incubating at 80ºC for 1 hr. Thereafter, the sample was dehydrated by incubating the slides in increasing gradations of alcohol (70%, 85% and absolute Ethanol). The sample was given enzyme digestion by incubating the slides in solution A (150 µL of 1% pepsin solution and 500 µL of 1N hydrochloric acid added to 49 mL MQ water) at 37ºC for 5 min. After rinsing in 1X PBS, the slides were incubated in Solution B (1.34 mL of 37% Formaldehyde added to 48.5 mL MQ water) at 2-8ºC for 5 min.

The sample was dehydrated in alcohol and air dried. Thereafter, appropriate volumes of the FISH probes (as indicated by the manufacturer) were added to the sample in dark.

The slides were placed in a humidified Thermobrite hybridization chamber and incubated at 80ºC for 10 min followed by 37ºC for 14-16 hrs.

After incubation, the slides were removed from the humidified chamber and processed for washing, to remove unbound probe by using low and stringency SSC (Sigma) washes.

The slides were air-dried and then mounted in a solution of 5 µL DAPI II. The slides were visualized on a Fluorescence microscope equipped with Cytovision software and scored as per guidelines.

The haematological and immunobiochemical parameters, namely, Hemoglobin, Albumin, LDH, Creatinine and β2 Microglobulin were also analyzed in the study group. Patients with either one or more cytogenetic abnormalities including 17p13 (p53), t(4:14) and t(14:16) were labelled having high risk cytogenetics. International staging system (ISS) for Myeloma was calculated for all 117 patients using serum albumin and β2 Microglobulin levels and revised ISS (R-ISS) was calculated by using LDH and cytogenetic abnormalities in addition to the parameters in ISS (6,7).

Statistical Analysis

Descriptive statistics such as mean and standard deviation were used for continuous variables. Frequency counts and percentages were used for categorical variables. Chi-square test was employed to evaluate the associations between ISS staging and cytogenetic abnormalities.

Results

Of 117 patients included in our study, 78 patients were males and 39 patients were females with mean age of 59.11 years (SD ± 10.43 years). Demographic profile of our study population is shown in Table 2.

ISS and Revised ISS Staging of our patient cohort is shown in Table 3. Of our patients, 49 (41.88%) were staged ISS-III whereas 37 patients were staged R-ISS III (31.62%).

Table 2. Demographic profile

Demographic parameters	Values	Standard deviation S.D
Mean age, in years	59.11	(±10.43)
Mean Haemoglobin, in g/dL	11.32	(±2.2)
Mean Albumin, in g/dL	3.5	(±0.854)
Mean Creatinine, in mg/dL	1.79	(±2.102)
Mean LDH, in IU/L	254	(±112.49)
β2 Microglobulin, in ng/mL	5528.7	(±4477.7)

Table 3. Number of patients in ISS and R-ISS

Stages	ISS Stage	R-ISS Stage
Stage – I	36	21
Stage – II	32	59
Stage – III	49	37

Cytogenetic abnormalities were detected in 39 patients. Total of 19 patients (16.23%) in our study had high risk cytogenetic abnormalities and 16 of them had del 17p13 (p53). Del 13q14 was the most common cytogenetic abnormality and was found in 30 cases (25.64%) in our study population.

Various cytogenetic abnormalities detected in our study and its correlation with ISS and R-ISS are illustrated in Tables 4, 5 and 6.

High risk cytogenetics was observed in 13 of our patients staged as ISS-III, four patients staged as ISS-II and two patients staged as ISS-I. There was a 66.4% moderate correlation between ISS -III and high risk cytogenetics which was statistically insignificant with P-value of 0.213.

Table 4. Total number of cytogenetic abnormalities

Cytogenetic abnormality	n
del 17p13	16
del 13q14	30
t (4:14)	3
t (11:14)	2
t (14:16)	2
Trisomy on chromosome 17	2

Table 5. Multiple Coexisting cytogenetic abnormalities

Cytogenetic abnormality	n
del 17p alone	5
del 17p & 13q	8
del 17p & t(4:14)	1
del 17p & t(11:14)	1
del 17p, del 13q, t(14:16)	1
del 13q alone	17
del 13q & t(4:14)	1
del 13q & t(11.14)	1
del 13q & t(14:16)	1
t(4:14) alone	1
Trisomy on chr 17	1
Trisomy on chr 17	1

Table 6. Table comparing cytogenetic abnormalities, ISS stage and revised R-ISS stage

Cytogenetic abnormality(n=total number of patients)	ISS Stage	n	R-ISS Stage	n
del 17 alone (n=5)	1 2 3	2 0 3	1 2 3	0 2 3
del 17p & 13q (n=8)	1 2 3	0 3 5	1 2 3	0 3 5
del 17p & t(4:14) (n=1)	1 2 3	0 0 1	1 2 3	0 0 1
del 17p & t(11:14) (n=1)	1 2 3	0 1 0	1 2 3	0 1 0
del 17p, 13q, t(4:16) (n=1)	1 2 3	0 0 1	1 2 3	0 0 1
del 13q alone (n=17)	1 2 3	1 5 11	1 2 3	1 5 11
del 13q & t(4:14) (n=1)	1 2 3	0 0 1	1 2 3	0 0 1
del 13q & t(11:14) (n=1)	1 2 3	0 0 1	1 2 3	0 0 1
del 13q & t(14:16) (n=1)	1 2 3	0 0 1	1 2 3	0 0 1
t(4:14) (n=1)	1 2 3	0 0 1	1 2 3	0 0 1
Trisomy on chr 17 (n=1)	1 2 3	1 0 0	1 2 3	1 0 0
Trisomy 17 & del 13q (n=1)	1 2 3	1 0 0	1 2 3	1 0 0

Discussion

Multiple Myeloma is a hematological neoplasm caused by proliferation of malignant plasma cells which produce monoclonal proteins. iFISH is used for identifying multiple and complex genetic abnormalities in Myeloma patients. We studied 117 patients with Multiple Myeloma for various cytogenetic abnormalities including del 17p13 (p53), del 13q14.3, t(4:14), t(14:16) and t(11:14).

Cytogenetic abnormalities were found out in one third (33.3%) of our study population which is less compared to various other studies where 50-90% genetic abnormalities have been reported in Myeloma patients (9,9). High risk cytogenetic abnormalities were present in 19 patients (16.23%) which is less as compared to studies by Amare *et al.* and Shaji *et al.* who in their studies had 21% and 24% high risk cytogenetic abnormalities respectively (10,11).

The presence of del 17 p signifies high risk cytogenetics and is associated with aggressiveness of the disease, hypercalcemia, extra-medullary disease and poor survival. In our study del 17p13 (p53) is found in 16 patients (13.67%) which is comparable to various Indian and western studies (11-14).

Translocation (4:14) was present in 3 (2.56%) patients which is less compared to various Indian and western studies. Amare *et al.* and Shaji *et al.* quoted 10% of their study group having t(4:14). The translocation is more prevalent in IgA subset and is associated with poor prognosis (10,11).

Two (1.7%) of our patients had t(14:16) which is comparable to other studies. This abnormality is usually missed by conventional karyotyping and is identified by iFISH. There is limited data regarding prognostic implication of this translocation but seems to have poor prognosis (15). Based on the findings of Chung *et al.* translocation is associated with chromosome 13 deletion (16) which was in accordance with our study.

Del 13q 14.3 (25.64%) was the most common cytogenetic abnormality found in our study which is comparable to Indian studies but less than what is quoted in western literature (10,17). In our study we had 2 patients with plasma cell leukemia and both had del 13q14.3. This is similar to the results of Garcia-Sanaz *et al.* (18).

In our study, two (1.7%) patients had t(11:14) which compared to that found in other western and Indian studies, is less (10,19,20). In most of the studies this translocation is associated with good prognosis except for plasma cell leukemia where the outcome is poor.

In our study, 49 (41.88%) patients were staged ISS-III which was more compared to other Indian study by Jacob LA *et al.* (2017) and western studies by Greipp PR *et al.* (2005) and Attal M *et al.* (2015) where frequency of ISS-III was 39%, 39% and 18% respectively (21,6,22).

According to R-ISS, 37 (31.62%) cases were staged as R-ISS III which is significantly more compared to the study by Palumbo A *et al.* (2015) and Chang H *et al.* (2004) where patients staged as R- ISS III were only 10% and 20% respectively (7,23). In Studies by Samu Kurki et al. and Kastritis E et al. also proportion of R-ISS III patients was less compared to that found in our study (24,25).

There was a 66.4% moderate correlation between ISS-III and high-risk cytogenetics which was statistically insignificant with P-value of 0.213. In a study by Amere *et al.* (2016), there was no correlation found between high risk cytogenetics and ISS-III and the incidence of high-risk cytogenetic abnormalities was similar in groups having ISS -III and a combined group having ISS-I and ISS-II (10).

The drawback of the study was that we were not able to analyze t(14:20) and chromosome 1q abnormalities. We also did not do conventional karyotyping in addition to iFISH testing which would have diagnosed additional cytogenetic abnormalities. We have not analyzed the outcome of these patients which would have helped us to understand the prognosis and survival of our patients.

Our study has clearly shown that the cytogenetics and R-ISS characteristics of Indian patients are different than western patients. Hence the future goal should be to conduct large scale multicentric, randomized control trials which will help us to clearly understand cytogenetic abnormalities and treatment outcome among Indian patients.

Conclusion

In our study, high risk cytogenetic abnormalities were less as compared to other Indian and western studies and del 17p13 was the most common high risk cytogenetic abnormality. This is the first Indian study where R-ISS system is used to stage the disease and found that patients with R-ISS stage III were significantly higher compared to western literature.

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Conflict of Interest

The authors declared that there is no conflict of interest regarding the publication of this article.

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