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Pattern of Cytogenetic Abnormality by Fluorescence in Situ Hybridization (FISH) in Multiple Myeloma Patients in a Tertiary Hospital

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Abstract

Background: Multiple myeloma is a plasma cell neoplasm with acquired genetic abnormalities of clinical and prognostic importance, with survival duration ranging from a few months to more than 10 years. Cytogenetic abnormalities (CA) detected by fluorescence in situ hybridization (FISH) are of major prognostic significance since e.g. patients with del(17p), t(4;14) or gain 1q21 show dismal outcome. Objective: To evaluate the cytogenetic patterns by fluorescence in situ hybridization (FISH) of clinically diagnosed cases of multiple myeloma. **Methods:** This cross-sectional study was conducted in Department of Haematology, Dhaka Medical College Hospital, Dhaka, from January 2018 to December 2018. A total number of 30 patients with multiple myeloma were analyzed cytogenetically by interphase fluorescence in situ hybridization (iFISH). The collected data were analyzed by using the Statistical Package for Social Science (SPSS-24) for windows version 10.0. **Results:** Out of 30 diagnosed Multiple Myeloma cases the mean age was 56.37±10.38 years and male to female ratio was almost 3:1. Sixteen (56.7%) of 30 patients. Among 30 cases of 8 cases were thyrogenicity positive of 7(23.3%) patients was detected del 13q positive. Isolated del 13q was found in 4 cases. 2 cases were found coexistence of del 13q and del 17p positive ;1 case was found coexistence of del 13q and t(4;14) positive and rest of 1 case had del 17 p positive. There was no detectable t (11; 14) and t(14;16) in any of 30 cases. **Conclusion:** FISH panel for Multiple Myeloma including del (13q); t(11;14); t(4;14), del(17p), t(14;16) is very important molecular test for the prognosis , risk stratification, treatment modality of the patient. On the basis of cytogenetic abnormality Multiple Myeloma risk stratification is modified now a day. This Revised International Staging system R-ISS is a simple and powerful prognostic staging system.

Keywords:- Cytogenetic; Fluorescence in Situ Hybridization, Myeloma, plasma.

INTRODUCTION

Multiple myeloma accounts for 1% of all adult cancers and approximately 10% of all hematologic malignancies.[1] According to a retrospective multi- center study of haematological malignancy in Bangladesh has evidenced that the incidence of Multiple

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Myeloma is 10.5%, median age 55years.[2] The annual age-adjusted incidence in the United States has remained stable for decades at approximately 4 per 100,000.[3] Multiple myeloma is slightly more common in men than in women, and is twice as common in African-Americans compared with Caucasians.[4] The median age of patients at the time of diagnosis is about 65 years.[5] The 3 world regions with the highest age standardized incident rate (ASIR) of MM were Australasia, North America, and Western Europe.[6] Multiple myeloma (MM) is a cytogenetically heterogenous plasma cell malignancy.[7,8,9] Nearly all myeloma patients have abnormal chromosomes by fluorescence in situ hybridization (FISH), including deletions, aneuploidy, and translocations. Although abnormal karyotypes are seen only in one third of cases. This apparent discrepancy is explained by the generally low proliferative rate of myeloma cells and the requirement of obtaining plasma cells (and not just the rapidly dividing normal myeloid precursors) in metaphase to generate conventional cytogenetics.[10,11] There are two broad types of cytogenetic abnormalities in MM : primary and secondary. There are two main types of primary cytogenetic abnormalities in MM: trisomies and translocations involving the immunoglobulin heavy chain (IgH) gene. The trisomic form of MM is characterized by an extra copy of one or more odd-numbered chromosomes (chromosomes 3, 5, 7, 9, 11, 15, 17). The IgH-translocated form of MM includes several distinct subtypes, the most common being t(11;14), t(4;14), t(6;14), t(14;16) and t(14;20). In each of these translocations, an oncogene from a partner chromosome is translocated to the IgH region on chromosome 14q32. Thus the genes dysregulated in these translocations are: 11q13 (CCND1 (cyclin D1 gene)), 4p16.3 (FGFR-3 and MMSET), 6p21 (CCND3 (cyclin D3 gene), 16q23 (c-MAF), and 20q11 (MAF-B), respectively.[12,13,14]

One of the earliest described secondary cytogenetic abnormality is monosomy 13 or del (13q).[15] Approximately 50% of newly diagnosed MM patients have del 13 or del 13q by FISH. It has been shown that del 13q is associated with specific biologic features, including a higher frequency of λ-type MM, slight female predominance, higher plasma cell labeling index (PCLI) and a higher frequency of a serum M component of less than 10 g/L.[16] Deletion of the TP53 gene locus as a result of monosomy 17 or deletion of 17p is seen in 10% of patients at diagnosis and is associated with very short median OS.[17] In contrast, inactivating mutation of TP53, locus 17p13, is rare in freshly explanted myeloma cells but is common in human myeloma cell lines and in patients with a terminal phase of myeloma. Such mutations have been observed in ~5% of cases of early MM versus 20% to 40% of cases of plasma cell leukaemia (PCL).[18] Another set of new cytogenetic prognostic markers are abnormalities involving chromosome 1 either gain of 1q21 or loss of 1p21, and have been associated with poor outcome. Abnormalities of both the short and long arms of chromosome 1 have been noted since the first cytogenetic studies of myeloma. Hanamura et al. demonstrated that the frequency of 1q21 amplifications increases from monoclonal gammopathy of undetermined significance (0%), to overt MM (43%), and finally to relapse (72%).[19] There was a strong association between chromosome 1p and/or 1q

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abnormalities and deletion of chromosome 13 or 13q. Patients with 1p/q abnormalities had a significantly shorter overall survival (OS) than patients with normal karyotypes. Amplifications of 1q21 are concurrent with dysregulated expression of c-MAF, MMSET/FGFR3, or deletion 13.[20] Other secondary cytogenetic abnormality including RAS mutation, secondary translocation involving MYC.[21] A standard myeloma FISH panel will contain probes for the common translocations and structural abnormalities and will detect them regardless of the proliferative rate of the plasma cells. FISH, however, provides no information on the proliferative index of the myeloma cells.[22] The detection and interpretation of cytogenetic abnormalities in MM is of critical importance for prognosis and risk stratification of MM.[23] The median survival is approximately 6–7 years; in patients eligible for ASCT 4 year survival rates exceed 80%. Tumor burden in multiple myeloma has traditionally been assessed using the Durie-Salmon Staging (DSS)and the International Staging System (ISS).[24,25,26] Disease biology best reflected based on the molecular subtype of multiple myeloma and the presence or absence of secondary cytogenetic abnormalities such as del(17p), gain(1q), or del(1p).[21] The Revised International Staging System (RISS) combines elements of tumor burden (ISS) and disease biology (presence of high risk cytogenetic abnormalities or elevated lactate dehydrogenase level).[27]

MATERIAL AND METHODS

This cross-sectional study was conducted in Department of Haematology, Dhaka Medical College Hospital, Dhaka, from January 2018 to December 2018. A total number of 30 patients with multiple myeloma were analyzed cytogenetically by interphase fluorescence in situ hybridization (iFISH). The collected data were analyzed by using the Statistical Package for Social Science (SPSS-24) for windows version 10.0.

Operational definitions: Fluorescence in Situ hybridization (FISH) A standard myeloma FISH panel will contain probes for the common translocations and structural abnormalities and will detect them regardless of the proliferative rate of the plasma cells. FISH, however, provides no information on the proliferative index of the myeloma cells.[22] The detection and interpretation of cytogenetic abnormalities in MM is of critical importance for prognosis and risk stratification of MM.[23] The median survival is approximately 6–7 years; in patients eligible for ASCT 4 year survival rates exceed 80%. However, there is major variation in survival depending on host factors, tumor burden (stage), biology (cytogenetic abnormalities), and response to therapy.26 Tumor burden in multiple myeloma has traditionally been assessed using the Durie-Salmon Staging (DSS) and the International Staging System (ISS).[24,25,26] Disease biology best reflected based on the molecular subtype of multiple myeloma and the presence or absence of secondary cytogenetic abnormalities such as del(17p), gain(1q), or del(1p).21, The Revised International Staging System (RISS) combines elements of tumor burden (ISS) and disease biology (presence of high risk cytogenetic abnormalities or elevated lactate dehydrogenase level) It is important to note that in order to ensure uniform availability,[27] only 3 widely available cytogenetic markers are used in the RISS.

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RESULTS

[Table 1] shows the demographic profile distribution of the study population, age >60 years was observed in 16 (53.3%) patients. The mean age was 56.37±10.38 year with range 33 to 75 years. Twenty two (73.3%) patients were male and 8(26.7%) patients were female. [Table 2] shows the variable distribution of the study population, it was observed that 17(56.7%) patients had Hb <10 (gm/l). Eighteen (60.0%) patients had serum calcium >11 (mg/dl). Serum creatinine >2 (mg/dl) was found in 12(40.0%) patients. Hb: Haemoglobin. [Table 3] shows the distribution of the study population by lytic bone lesion. It was observed that 15 (50.0%) patients had 3 lytic bone lesions, 4(13.3%) patients had 2 lytic bone lesions and 2(6.7%) patients had 1 lytic bone lesions. There was no lytic bone lesion in 9(30%) patients. [Table 4] shows the variable distribution of the study population. Serum albumin >3.5 gm/dl was observed in 18 (60.0%) patients and <3.5gm/dl was in 12(40%) patients. The mean serum albumin was 3.17±0.53 gm/dl with range 2.2 to 3.8 gm/dl. Elevated serum LDH (>222 U/l) was found in 13(43.5%) patients and 17(56.5%) patients had normal LDH (<222U/l). The mean serum LDH was 208.87±180.68 U/l with range 26 to 631U/l. β2 microglobulin >5.5 µg/mL was detected in 12 (39.9%) patients. Seven (23.2%) patients had β2 microglobulin >3.5to <5.5 µg/mL and rest of 11(36.7%) patients had β2 microglobulin <3.5 (normal). The mean β2 microglobulin was 5.95±6.77 µg/mL with range 1.6 to 37.28 µg/mL. [Table 5] shows the risk stratification of the study population according to cytogenetics detected by FISH. It was observed that 22(73.4%) patients had normal cytogenetics and 8(26.6%) patients had abnormal cytogenetics by FISH. High risk cytogenetics was found in 3 patients, 1 patient had intermediate risk cytogenetics and 4 patients had standard risk cytogenetics. FISH: Fluorescence in Situ Hybridization. Table VII shows the distribution of the study population according to individual cytogenetic abnormality by FISH. It was observed that 22(73.4%) patients had no cytogenetic abnormality and 8(26.6%) had abnormal cytogenetics detected by FISH. Among these 13 q del. 4(13.3%) and coexistence of 13p del+17p del in 2(6.7%) and 13q del + t (4; 14) in 1(3.3%) patient respectively. So total 13q del in 7 (23.3%) patients, 17p del total in 3 (10.0%) patients and t(4;14) in 1 patient. t(11;14) and t(14;16) were not detected in any patient. risk, another 8(36.3%) cases were intermediate risk and rest of 6(27.3%) cases was standard risk according to RISS (Revised International staging system). Cyto -ve std risk: cytogenetic negative standard risk, cyto –ve int. risk: cytogenetic negative intermediate risk, cyto-ve high risk: cytogenetic negative high risk.

Table 1: Distribution of the study population by demographic profile (n=30)

Age (year)	**N=30**	%
≤40	3	9.9
41-59	11	36.7
≥60	16	53.3

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Mean±SD	56.37±10.38	
Range	33-75	
Sex Distribution		
Male	22	73.3
Female	8	26.7

Table 2: Distribution of the study population by variable (n=30)

Variable	**N=30**	%
Hb (gm/l)		
>10	13	43.3
<10	17	56.7
Serum calcium (mg/dl)		
<11	12	40.0
>11	18	60.0
Serum Creatinine (mg/dl)		
<2	18	60.0
>2	12	40.0

Table 3: Distribution of the study population by lytic bone lesion (n=30).

	N=30	%
Lytic bone lesion		
≥3	15	50.0
≥2	4	13.3
1	2	6.7
No bone lesion	9	30.0

Table 4: Distribution of the study population by variable (n=30).

Age (year)	**N=30**	%
Serum Albumin (gm/dl)		
<3.5	12	40.0
>3.5 (Normal)	18	60.0
Mean±SD	3.17±0.53	
Range	2.2-3.8	
Serum LDH (U/I)		
<222 (Normal)	17	56.5
>222	13	43.5
Mean±SD		
Range		
β2 microglobulin (µg/mL)		

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<3.5 (Normal)	11	36.7
>3.5 to <5.5	7	23.2
>5.5	12	39.9
Mean±SD	5.95±6.77	
Range	1.6-37.28	

Table 5: Risk stratification of the study population according to cytogenetic abnormality detected by FISH (n=30).

	n=30	%
FISH		
No abnormal cytogenetics	22	73.4
Standard risk cytogenetics	4	13.3
Inter mediate risk cytogenetics	1	3.3
High risk cytogenetics	3	10.0

Table 6: Risk stratification of the study population by ISS and RISS (n=30)

Variable	**n=30**	%
International staging system (ISS)		
Standard risk	8	26.7
Intermediate risk	7	23.3
High risk	15	50.0
Revised International staging system (RISS)		
Standard risk	8	26.7
Intermediate risk	7	23.3
High risk	15	50 .0

Table 7: Distribution of the study population on the basis of specific cytogenetic abnormality by FISH (n=30)

	n=30	%
Cytogenetic abnormality By FISH		
Negative/no abnormality	22	73.4
13 q del	4	13.3
17p del	1	3.3
13p+17p del	2	6.7
13q del + t (4,14)	1	3.3
t(11;14)	0	0
t(14;16)	0	0

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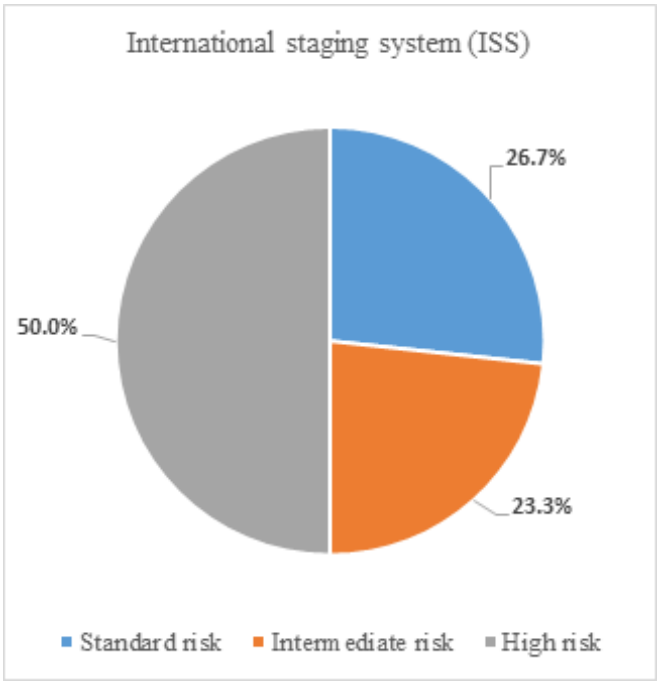

Figure 1: Pie chart showing risk stratification of the study population by International staging system (ISS)

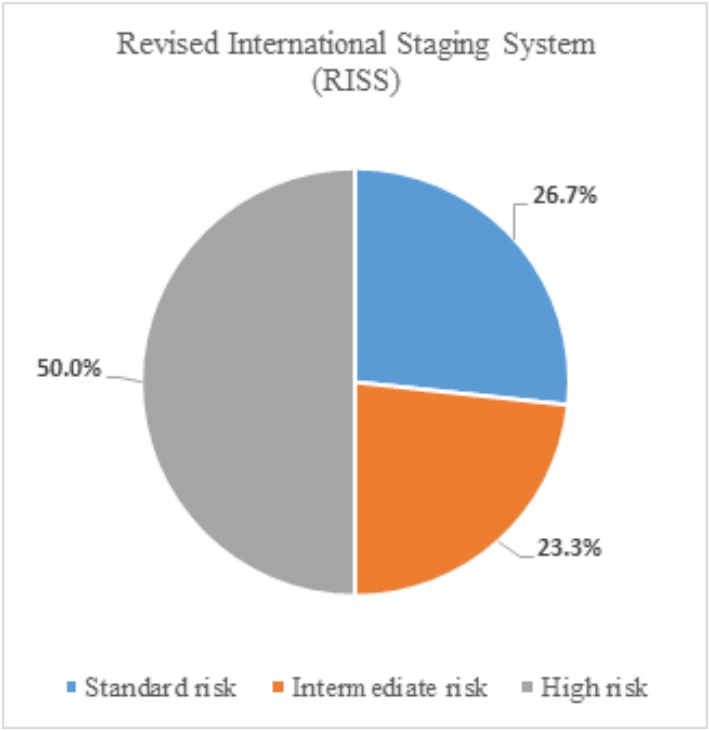

Figure 2: Pie chart showing risk stratification of study population by revised international staging system (RISS)

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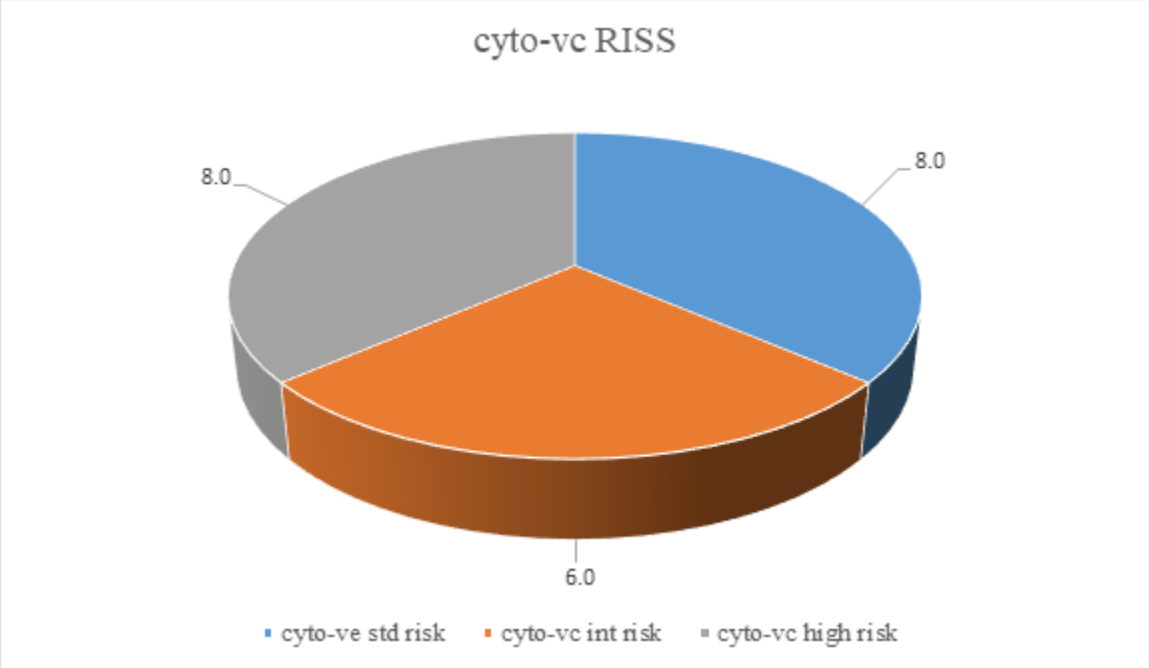

Figure 3: Pie chart showing risk stratification of the study population by RISS who had no abnormal cytogenetics by FISH (fluorescence In Situ Hybridization).

DISCUSSION

This cross-sectional observational study was carried out with an aim to evaluate diagnostic workup of multiple myeloma patients by bone marrow, serum Protein electrophoresis, Immunofixation and other biochemical markers and to detect del(13q); t(11;14); t(4;14), del(17p), t(14;16) from bone marrow aspirate of multiple myeloma patients by FISH. The cytogenetic abnormalities can be used for the classification of MM into cytogenetically distinct subtypes, laboratory testing strategy for practice, influence of cytogenetics on disease presentation, risk stratification and prognosis of MM and selection of therapy. For the clinical practice, overall management depends upon cytogenetic study. A total of 30 patients with multiple myeloma in the Department of Haematology, Dhaka Medical College Hospital, Dhaka, from January 2018 to December 2018 were included in this study. Bone marrow aspiration was done and sent for cytogenetical analysis by interphase Fluorescence In Situ Hybridization (FISH). Diagnosed cases of multiple myeloma including new and relapsed cases were enrolled in this study. Patients of Multiple myeloma who were partially treated, patients who had other haematological diseases concomitantly and MGUS or Smouldering MM were excluded from this study. The present study findings were discussed and compared with previously published relevant studies.

In this present study, it was observed that 16(53.3%) patients were ≥60 years of age. The mean age was 56.37±10.38 year with range 33

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to 75 years. In Bangladesh haematological malignancy has evidenced that the incidence of Multiple Myeloma is 10.5% with median age 55 years.[2] The median age of patients at the time of diagnosis is about 65 years.[3] Palumbo et al.[27] study found the median age was 62 years varied from 18 to 91 years and 65.0% of patients were under 65 years old and 35.0% were older than age 65 years. The above findings are consistent with the present study. In this present study it was observed that out of 30 cases 22 (73.3%) patients were male and 8 (26.7%) were female and male to female ratio was almost 3:1. Multiple myeloma is slightly more common in men than in women, and is twice as common in African-Americans compared with Caucasians.[4] On the contrary Palumbo A et al; Kumar S, et al; Gadhia P and Vaniawala S had observed male predominance in multiple myeloma patients.[11,27] Organ involvement in multiple myeloma is attributable by anaemia, hypercalcaemia, lytic bone lesion, renal involvement or high serum creatinine. Durie Salmon staging also dependent on these factors which reflects the prognosis of the disease.[24] In this current study, it was observed that 16 (56.7%) patients had Hb <10 (gm/l), 18(60.0%) patients had Serum calcium >11 (mg/dl) and 12(40.0%) patients had serum creatinine >2 (mg/dl). In this study, it was also observed that half 15(50.0%) patients had ≥3 lytic bone lesions; 13.3% had ≥2 lytic bone lesions and 6.7% had 1 lytic bone lesion. In a study with 50 patients of MM Hu Y et al observed anaemia in 56.7% of patients, hypercalcaemia in 26.7% patients.

In this current study, it was observed that serum albumin <3.5 gm/dl was found in 12(40.0%) patients. 13(43.5%) patients had serum LDH >222 U/I. 12 (39.9%) patients had β2 microglobulin >5.5 µg/mL. In a study Hu Y et al described increased LDH, increased β2 microglobulin and decreased albumin in 20.8%, 73.3% and 56.7% patients respectively.[28] Drach et al also showed that >6 µg/mL β2 microglobulin was found only in 15.2% case. Increased LDH (>240 U/I) was observed in 18.1% cases.[29] These findings of Hu Y et al and Drach et al were inconsistent with the current study. High serum β2 microglobulin level reflects high tumour mass and reduced renal function and low serum albumin mainly caused by inflammatory cytokines mainly IL-6 secreted by myeloma microenvironment.[27] So it can be asserted that elderly patients with progressive disease, high tumour burden and high plasma cell proliferative rate were more in the current study than others. In this present study, it was observed that 22 (73.4%) patients' cytogenetics revealed normal and only 8(26.6%) patients' cytogenetic abnormalities were detected by iFISH. Out of which, 4(13.3%) patients revealed standard risk cytogenetics, 1(3.3%) case showed intermediate risk and 3(10%) cases showed high risk cytogenetics. This FISH panel included 17p del, 13q del, t(11;14), t(4;14) and t(14;16).

In this study insufficient plasma cells were found in 7 of 37 (18%). These 7 cases were not included in iFISH analysis. But this result is very much informative about poor or insufficient plasma cells. 11Lai J L et al found 15% insufficient plasma cells.50Other studies showed very few cases with insufficient plasma cells.[11,16,27] On the basis of serum albumin, β2 microglobulin 30 patients were stratified into high risk, intermediate risk and

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standard risk group by international staging system (ISS). In this study 15(50%) cases were in high risk group. Again revised international staging system (RISS) reflected the same results including 15(50%) high risk, 7(23.3%) intermediate risk and 8(26.7%) standard risk group. Palumbo et al studied with 4445 patients and ISS stage I, II and III were 36%, 37% and 22% respectively.[27] High risk cytogenetics was found in 19% case. In this study high risk cytogenetics was found only in 3 (10%) cases. In this study a panel of 5 cytogenetic abnormalities including del 13q, t (11; 14),t (4;14), t(14;16) and del 17p were detected by iFISH. Eight (8) of 30 cases showed abnormal cytogenetics by iFISH. Among these 8 cases del 13q was most common. Seven (7) of 30 (23.3%) cases were del 13q positive either singly or coexistantly with 17p del. Gadhia P and Vaniawala observed del13q14 in 48.3% cases.44 Tricot G et al described del 13 as poor prognostic. Similar results were found from Hu Y, et al and Gozetti A and Beau MML []28,29. Kassem N A et al found 13q14 del in 40/100 (40%) of the patients, of which 10(25%) went into remission and 30(75%) were resistant to VAD treatment or died.

55/100 (55%) of the patient were negative for 13q14 deetion, of which 45 (81. %) went into remission and 10(18.2%) were resistant to VAD or died. Kassem et al postulated that 13q14 detected by iFISH has an adverse prognostic outcome on the patients. 13q del was in intermediate risk .when detected by metaphase karyotyping.[16] Del 13q is restratified in standard risk group according to International myeloma working group (IMWG).[21]

In this study 17p deletion was detected by iFISH in 3 of 30 (10%) patients of multiple myeloma. Drach et al had detected 25 of 74 (33.8%) patients of MM. Among them 33.8% were newly diagnosed MM and 54.5% in relapsed MM.46Hu Y et al obsereved 7 of 33 (21.21%) cases of MM.47 Gadhia P and Vaniawala S found 17p deletion in 13.8% patients of MM. It was also postulated that del 17p is considered to be worst prognosis due to loss of TP 53 tumour suppressor gene44 Another high risk cytogenetics for MM is t (14; 16). In a retrospective study Loiseau AV et al observed t (14; 16) in 32 of 1003(3.2%) cases. Gadhia P and Vaniawala S also found t (14, 16) in 1 of 58 (1.7%) cases.44 In this current study t (14; 16) was not detected in any of the 30 cases of MM. Probably the low incidence of t (14; 16) and small sample size are the contributing factors for this findings. In this study, t (11; 14) was not found in any of the 30 cases. Trisomies, t(11;14) and t(6,14) are standard risk cytogenetics and comprises 75% of all cytogenetic abnormalities.[21] Above data are inconsistent with this current study. Intermediate risk cytogenetics is 1q gain and t (4; 14). In newly diagnosed patients ,intermediate risk cytogenetics are positive only in 10% case.[21] Gadhia P and Vaniawala S had shown 3of 58 (5.2%) cases of MM having t(4;14) [30]. In this current study t (4; 14) was detected in one of 30(3.3%) cases of multiple myeloma. This finding is comparable with data of above study.

In this study, 22 of 30(73.4%) patients were detected no abnormal cytogenetic by FISH. Revised International Staging system included serum albumin, β2 microglobulin and serum LDH or cytogenetic.[21] Cytogenetic abnormality negative these twenty two (22) cases were stratified into standard,

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intermediate or high risk group on the basis of serum albumin, β2 microglobulin and serum LDH. Eight (8) of 22 (36.3%) patients were in high risk group and another 8 of 22(36.3%) were in intermediate risk group. Rest of the 6 of 22(27.3%) patients was in standard risk group. From these above data and comparison with others it is observed from the current study that cytogenetic abnormality analyzed by iFISH is comparable with other studies but positive tests are lower than others. Small sample size and short duration of study may be the confounding factors. All the data were compared with the study of different study population and geographical areas. But no similar studies were found in our country to compare with this current study which could reflect the actual scenario.

Limitations of the Study

The study population was selected from one selected hospital in Dhaka city, so that the results of the study may not reflect the exact picture of the country. Small sample size was a limitation of the present study. Therefore, in future further study may be under taken with large sample size.

CONCLUSIONS

This study was undertaken to evaluate the cytogenetic patterns by fluorescence in situ hybridization (FISH) of clinically diagnosed cases of multiple myeloma. Most of the patients were in 6th decade and above. Predominant patients were male (73.3%). Risk stratification on the basis of serum albumin and β2 microglobulin had shown predominant high risk 15(50.0%) according to International staging system (ISS) .Revised International staging system (RISS) was followed on the basis of serum albumin ,β2 microglobulin and serum LDH or cytogenetics. RISS also showed similar result. The main focus of the study was to observe the abnormal cytogenetics by iFISH which was lower than the other studies. Eight of 30 (26.6%) patients had cytogenetic abnormalities detected by iFISH. Out of these eight (8) cases, standard risk cytogenetics was four (4); intermediate risk was one (1) and high risk cytogenetics revealed in three (3) cases. In this study most common cytogenetic abnormality was del 13q which was found coexistence with del 17 p or isolated. Deletion17p was detected in three (3) cases. Small sample size, short study period, insufficient plasma cell yield, quality control including plasma cell sorting technique, and probe selection, transportation, collection, refrigeration and culture of the plasma cell are the probable factors that may yielded poor result. In addition to the cytogenetic findings, hypoalbuminaemia, high β2 microglobulin and high serum LDH level were detected in the majority of the patients majority of the patients were aneamic, Serum Calcium belonged to >11mg/dl and S. Creatinine belonged to <2mg/dl. Majority of the patients had □3ytic bone lesions. These data suggest that majority of the patient had myeloma related organ or tissue impairment. There was no similar study in Bangladesh with large sample size to compare with this current study.

Recommendation

Cytogenetic test by FISH for Multiple Myeloma can be used as prognosis of the disease. Risk stratification and treatment and its outcome also depend upon cytogenetic categorization of Multiple myeloma. In our

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socioeconomic context this molecular test is costly. BM plasma cell sampling technique, its transportation, preservation, cell sorting method should be monitored properly. So prospective studies can be undertaken including large number of patients.

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