Frequency of t(4;14) in Multiple Myeloma and Its Clinicopathological Correlation

Saima Zaheer, Helen Mary Robert, Asad Mahmood, Rafia Mahmood, Ayesha Khurshid, Nabeela Khan

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS), Rawalpindi Pakistan

ABSTRACT

Objective: To determine the frequency of t(4;14) in multiple myeloma in Pakistani population and study the clinic-pathological correlation of this translocation in myeloma patients.
Study Design: Cross sectional study.
Place and Duration of Study: This study was conducted at Armed Forces Institute of Pathology from Jun 2017 to May 2018 using non probability convenience sampling technique.
Methodology: A total of 53 newly diagnosed cases of multiple myeloma were included in the study. Patients were diagnosed as having multiple myeloma based on diagnostic criteria of international myeloma working group. Fish analysis was done for t (4; 14). Workup for end organ damage /myeloma defining events was done.
Results: Out of 53 patients, 16 (30%) were females and 37(70%) were males; the mean age of the patients was 59.81 ± 11.34 range from 37 to 87 years. Fish for t(4;14) was positive in eight (15%) patients while negative in forty-five (85%) patients. Patients with positive results have significantly deranged renal function tests and raised beta 2 micoglobulins levels as compare to t(4;14)negative patients.
Conclusions: Detection of t (4:14) in multiple myeloma patients not only has diagnostic value but is important in risk stratification of these patients and thus effect treatment decision.

Keywords: Fluorescence in situ hybridization, Multiple myeloma, T(4;14) immunoglobulin heavy chain translocation.

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INTRODUCTION

Multiple myeloma is a post germinalcentreB cell neoplasm which results in accumulation of plasma cells in the bone marrow.[1] Its annual incidence is 4 per 100,000. Multiple myeloma is slightly more frequent in males and median age at diagnosis is 65-70 years.[2]

It is characterized by proliferation of a specific clone of plasma cells with one or more of the following myeloma defining events including bony lytic lesion, hypercalcaemia and anemia.[3] Multiple myeloma isgenomically unstableand characterized by translocations mainly involving IGH locus on chromosome 14q32, hypodiploidy or hyperdiploidy, methylation and dysregulated expression of cyclin D genes.[4] Nearly half of multiple myeloma patients show IGH translocation involving five recurrent chromosomal pattern i.e., t(4;11) , t(4;14), t(14;16), t(6;14) and t(14;20).[4,5]

Different prognostic scores have been employed for risk stratification of Multiple myelomapatients.[6] The Durie-Salmonstagingsystem was introduced in 1975.[3] It demonstrates association between the percentage of myeloma and the damage it has caused, such as bone disease, renal failure or anemia.[3-5] In 2005, a new staging system,International staging system (ISS) was urbanized by the International Myeloma Working Group (IMWG).[7] This system include serum beta 2 microglobulin and serum albumin as strong predictors of disease burden and progression.[6,7] With further evolution and establishment of different genetic factors contributing to disease development and progression, in 2015 IMWG incorporate genetic factors as assessed by fish and level of LDH in risk stratification (R-ISS).[6,7]

Genetic aberrations are important in risk stratification of multiple myeloma patients and its therapeutic approach.[6,8,9] t(4;14) is primary event in multiple myeloma and is seen approximately 15% of multiple myeloma cases.[10,11] This translocation is identified by fluorescence in situ hybridization.[1,8] It causes immediatede regulation of fibroblast growth factor receptor 3 gene on der (14) and Multiple myeloma SET domain on der (4) which lead toover expression of FGFR3 and MMSET genes on plasma cells.[10] These mutations initiate proliferation and prevent apoptosis.[10,11] Multiple studies has identified t(4;14) as the only unfavorable prognostic factor for both progression free survival and overall survival.[10]

The t(4;14) is also important in risk stratification of multiple myeloma patients. Fish is the gold

Correspondence: Dr Saima Zaheer, Registrar Haematology, Armed Forces Institute of Pathology Rawalpindi Pakistan
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diagnostic modality of choice for its detection.[8] Uptill now, all data come from western population. Armed Forces Institute of Pathology is a referral center offering Fish facilities in Pakistan. In our study we have analyzed the diagnosed patients of multiple myeloma for frequency of t(4;14) in our population and compared the clinico-pathological features inthese patients with and without t(4;14).

METHODOLOGY

This cross-sectional study was conducted at Armed forces institute of Pathology from June 2017 to May 2018 using non probability convenience sampling technique. The protocol was approved by the Local Institutional Review boards/ethics committees (IRB/ 17/402), and the study was conducted in accordance with the International Conference on Harmonization. Written informed consent was taken from patients or guardians.

Inclusion Criteria: Newly diagnosed cases of multiple myeloma (diagnosed according to the IMWG diagnoastic criteria) were included in the study.

Exclusion Criteria: Diagnosed patients with MGUS and other plasma disorders were excluded from the study. Patients already on treatment were also excluded from the study.

Detailed history and complete physical examination was done. Complete blood counts were performed on Sysmex XE-5000. Baseline investigations including renal function test were carried out. Serum and urine protein electrophoresis and immunofixation were done. Each patient was tested for RFTS, serum calcium, serum albumin, and beta 2 microglobulin. Bone marrow aspiration and trephine biopsy was done to see plasma cell number and morphology. Diagnosis of multiple myeloma was made as per IMWG diagnostic criteria of MM.[11,12]

Blood or bone marrow samples were analyzed for Interphase FISH studies. Samples were processed by standard methods for culture.

3 ml of peripheral blood or bone marrow sample was collected in sodium heparin and 0.5 ml of blood is added to 7 ml of culture media (100ml RPMI + 10 ml FBS + 1ml of amphotericin B+1 ml of pencillin/ streptomycin). It was incubated at 37 C for 24hours. After 24 hours 2.5 ml Colchicine was added and incubated at 37 C for 45min.Then centrifugation at 1500 RPM was done for 8 min and supernatantwas removed. KCL and Distilled water was added and incubated for 10 min. Again supernatant was centrifuged and discarded. Fixation with glacial acetic acid was done. Repeated washing with fixative 3-5 times until it became colorless. Slide was prepared with pellet. Fixation of slides with 20 SSC for 2 min and then increasing concentration of ethanol was done. The slidewere dry and Fish probeapplied and potted with rubber solution. Denaturation was done at 74 C for 15 min and hydrization at 37 C for 18 hours. The solution was washed with increasing concentration of alcohol followed by 10% SSC solution. Counter staining was done with Dappiz counter stain. Then it was placed at 20 C for 30 min. In each probe set, total of 500 nuclei were analyzed with an orange green spectrum filter using Fluorescence microscope. T(4;14) is a dual fusion probe. The orange labelled probes indicate the breakpoint at the FGFR3 gene at gp 16. Green labeled probes indicate the breakpoint at 14q32 proximal and distal to the IGH gene region. One green,one orange and two green-orange fusion signals indicate t(4;14) *p*. Fish analysis is shown in Figure.

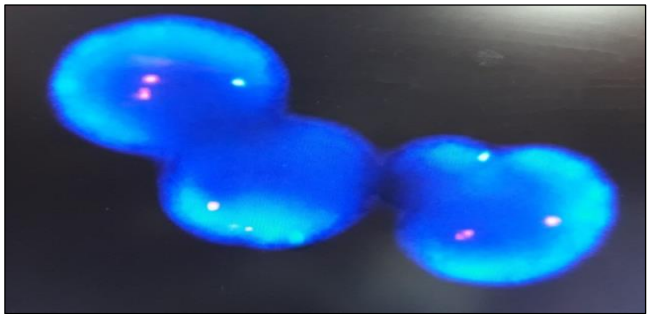

Figure: FISH analysis.

Analysis was done by statistical software SPSS-20. Mean and standard deviation was determined for quantitative variables. Frequency and percentage were calculated for qualitative variables. Chi square and Fisher exact test were applied for qualitative data and independent t-test was used for continuous variables. A *p*-value ≤0.05 was considered as significant.

RESULTS

Out of 53 patients, 16 (30%) were females and 37 (70%) were males; the mean age of the patients was 59.81 ± 11.34 ranging from 37-87 years. The most common clinical presentation was weakness, back ache and bone pains followed by fractures and renal failure. Fish for t(4;14)was positive in eight (15%) patients while negative in forty-five (85%) patients.

In patients with t(4;14) the Mean ± SD of creatinine was 286.50 ± 192.899 and in negative patients it was 165.61 ± 85.867, there was statistically significant difference in creatinine levels between t(4;14) positive

and negative patients (p=0.005).In patients with t(4;14) the mean ± SD of bone marrow plasma cells on presentation was 48.125 ± 20.157 and in patients without t(4:14) it was 42.0667 ± 18.79, there was statistically insignificant difference between percentage of plasma cells in patients with t(4;14) positive and negative patients (p=0.411). In patients with t(4;14) the Mean ± SD of calcium was 2.164 ± 0.447 and in negative patients it was 2.3256 ± 0.454, there was statistically insignificant difference between calcium levels of patients with and without t(4:14) (p=0.005).

In positive patients the mean ± SD of serum Albumin was 38.38 ± 7.99 and in negative patients it was 37.74 ± 5.598, there was statistically insignificant difference between serum Albuminand t(4;14) (p= 0.786). In patients with t(4;14) the mean ± SD of B2 Micro was 7.07 ± 3.64 and in patients without t(4;14) it was 4.096 ± 2.064, there was statistically significant difference between B2 Microglobulin and t(4;14) (p=0.029).

In patients with t(4;14) the mean ± SD of Hemoglobinwas 8.950 ± 1.267and in negative patients it was 9.25 ± 2.209 g/dl, there was statistically insignificant difference in Hemoglobin levels between t(4;14) positive and negative patients (p=0.711). In patients with t(4;14) the mean ± SD of Total leucocyte countwas 8.88 ± 3.50 and in negative patients it was 6.87 ± 2.71x109, there was statistically insignificant difference in total leucocyte count in patients with t(4;14) and without t(4;14) (p=0.071). In patients with t(4;14) the Mean ± SD of platelet count was 176 ± 74.143 x 10^9 and in patients without t(4;14) it was 214.33 ± 99.761 x 10^9, there was statistically insignificant difference in Platelet count of patients with t(4;14) and without t(4;14) (p=0.306) as shown in Table-I & II.

Table-I: Comparison of multiple myeloma with FISH.

Laboratory Results	T (4;14) Positive (n=8)	T (4;14) Negative (n=45)	*p*-value
	Mean ± SD	Mean ± SD	
Age (Years)	47.50 ± 9.24	62.00 ± 10.29	0.001
Hemoglobin (g/dl)	8.950 ± 1.267	9.25 ± 2.209	0.711
TLC (109/l)	8.88 ± 3.50	6.87 ± 2.71	0.071
Platelet (109/l)	176.00 ± 74.143	214.33 ± 99.761	0.306
Creatinine	286.50 ± 192.899	165.61 ± 85.867	0.005
Bone Marrow Plasma Cells at presentation (%)	48.125 ± 20.517	42.0667 ±18.79	0.411
Calcium	2.146 ± 0.447	2.3256 ± 0.454	0.357
B2 Micro	7.07 ± 3.64	4.96 ± 2.061	0.029
Albumin	38.38 ± 7.99	37.74 ± 5.598	0.786
Serum Protein Electrophoresis			
IgG K	7 (88)	31 (69)	0.415
Negative	1(12)	14(31)	

Table-II: Comparison of presenting complaints with t(4;14) and without t(4;14).

Presenting Complaints	T (4:14) By FISH		*p*-value
	Positive	Negative	
Weakness	4 (50)	19 (43)	0.921
Backache and Bone Pains	3 (37)	16 (36)	
Fracture	1 (13)	7 (16)	
Renal Failure	0	2 (5)	

DISCUSSION

Multiple myeloma is characterized by various cytogenetic abnormalities which also effect patient presentation and disease progression.[8] Cytogenetic analyses has important role played in the prognostic assessment in Multiple myeloma. (F8,9) But conventional cytogenetic interpretation has very limited role due to decreased in-vitro Plasma Cell proliferation index and less plasma cell infiltration which leads to culture failure,FISH analysis does not require metaphase for analysis,so proved to be more useful and sensitive.[9]

In our study the frequency of patients with t(4:14) by FISH in our population is 15% which is comparable with other studies. A study conducted by Smol *et al* frequency of patients with t(4:14) is 11.5%,[12] and 15% in a study conducted by A Kalff*et al*,[13] and 13% in a other international study by K. Naben *et al*,[14] and 18%[9]

Age of our patients range from 37-87 years with mean age of 59.8 years which is similar to the study conducted locally in Sindh by Sadia *et al*,[15] and 55 years in Indian population ,a study conducted by Kaur *et al*, 2014.[16]

Male to female ratio in our patients was 2.3:1, which was similar to local study by Shaheen *et al*,[18] and a bit higher than other regional studies by Kaur *et al*, 2014.[16]

Most of the patients Presented with symptoms of weakness, fatigue, bone pains, pathological fractures and renal failure similar to others studies by Saadia *et al*,[14] and presenting complaints were almost similar in both groups.

Creatinine was significantly raised in patient with t(4;14) with SD value of 286.50 ± 192.899 and in negative patients with SD value of 165.61 ± 85.867. These results are similar to studies conducted by Radocha *et al*.[11,19]

Beta 2 microglobulin levels were high in patients with t(4;14) with mean ± SD of 7.07 ± 3.64 and in patients without t(4;14) it was 4.096 ± 2.064, (p=0.029) and

these results were comparable with study conducted by Radocha *et al*.[19,5]

In positive patients the mean ± SD of S albumin was 38.38 ± 7.99 and in negative patients it was 37.74 ± 5.598, there was statistically insignificant difference between *S albumin* and t(4;14) (p=0.786). These results does not matched with results of Radocha *et al*.[19,5]

Most of the patients with t(4;14) presented with advanced stage according to ISS scoring.Whereas in t(4;14) negative patients 30% were in stage I, 37% in stage II, and 33% in stage III. These results were comparable with results of study conducted by Ja min byun in which according toISS, 54 patients (33.5%) were in stage I, 61 (37.9%) in stageII and 46 (28.6%) in stage III.[20,5]

CONCLUSION

The application of FISH has brought revolution in the genetic analysis of MM. Thet (4;14) is one of the high risk cytogeneticabnormalities which is an important indicator of disease progression.Patient with this translocation usually present with more aggressive disease and usually in younger ageand can lead to dismal outcomes. Thus detection of t(4;14) in multiple myeloma patients not only has diagnostic value but is important in risk stratification of these patients and thus effect treatment decision.

Conflict of Interest: None.

Author's Contribution

SZ: Direct Contribution to Conception, design, analysis and interpretation, HMR:, RM: Intellectual Contribution to analysis, literature review and manuscript preparation, AM: Intellectual contribution to analysis and data interpertation, AK:, NK: Manuscript Preparation.

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