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Chromosomal abnormalities in primary myelodysplastic syndrome

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Chromosomal Abnormalities in Primary Myelodysplastic Syndrome

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Abstract

Objective: To determine the frequency of cytogenetic abnormalities in patients diagnosed as primary myelodysplastic syndrome (MDS) using conventional karyotyping.

Study Design: Case series.

Place and Duration of Study: The Clinical Laboratory, The Aga Khan University Hospital, Karachi, between January 2006 - June 2012.

Methodology: Patients of all ages and either gender who fulfilled WHO criteria for MDS were included. Cytogenetic analysis was conducted at the time of diagnosis. Patients who had secondary MDS were excluded from analysis. Chromosome identification and karyotype description was done according to the International System for Chromosome Nomenclature (ISCN, 1995) and described as frequency percentage.

Results: Out of the 122 cases of MDS, 71 patients had their karyotype done at the time of diagnosis, including 42 males (59.2%) and 29 females (40.8%) with median age of 60 years. Forty one (57.7%) showed normal karyotype and 30 (42.3%) showed clonal karyotypic abnormalities at diagnosis. Out of which 14 (19.7%) had single, 11 (15.5%) had complex and 6 (8.5%) had double cytogenetic abnormalities. The common abnormalities found were: trisomy 8 in 7 cases (9.9%), -7/del (7q) in 3 cases (4.2%), -Y and complex 5q in 2 cases (2.8%) each, complex trisomy 8, del 11q , inversion 9, trisomy 19 and del 20q were found in 1 case (1.4%) each. Other abnormalities were found in 11 cases (15.5%).

Conclusion: Trisomy 8 was the most common disorder/abnormality found in this study population followed by the complex cytogenetics.

Key Words: *Primary myelodysplastic syndrome. Cytogenetic abnormality. Hematological malignancy. Karyotyping.*

INTRODUCTION

Myelodysplastic syndrome (MDS) is a group of disorder characterized by peripheral blood cytopenias in the presence of hypercellular/normocellular bone marrow with dysplastic features and increased risk of leukemic transformation.[1] Pathogenesis of MDS is poorly understood. Apart from its clonal nature, immunological abnormalities and increased apoptosis mediated by cytokines has also been proposed.[1,2] The diagnosis of MDS is usually made based upon an evaluation of the bone marrow and peripheral smear in an appropriate clinical context.[3] Certain cytogenetic abnormalities result in the diagnosis of MDS in patients with otherwise unexplained refractory cytopenia and no morphologic evidence of dysplasia.[4] The patients are prone to develop symptomatic anemia, recurrent infections and bleeding because of cytopenia. The condition can be broadly classified into primary and secondary, depending on whether MDS arises *de novo* or arises as a result of previous exposure to chemotherapy, ionizing radiation and various chemicals.[5] According to the United States cancer surveillance program in 2001, the overall incidence rate for MDS is 3 - 5 per 100,000 annually that increases markedly with age.[6] It usually affects older individuals more than 60 years of age but MDS has also been reported in pediatric population.[7] Specific cytogenetic abnormalities identified by conventional karyotype analysis or Fluorescence In Situ Hybridization (FISH) analysis have prognostic significance for patients with primary MDS and affects treatment planning.[8] Cytogenetic abnormalities are found in approximately 40 - 50% of primary MDS and nearly about 80 - 90% in secondary MDS.[9] Cytogenetics is an essential part of International Prognostic Scoring System (IPSS) published in 1997, but has also been incorporated in WHO Classification-based Prognostic Scoring system (WPSS).[10] Determination of clonal abnormality on diagnosis not only predicts the response to treatment but also its risk of transformation to acute leukemia. Allogenic bone marrow transplantation is the only curative treatment for MDS and its outcome also depends on the cytogenetic abnormality.[11] Though a number of therapeutic agents like lenalidomide, dasatinib and azacytidine have been proposed to have good response in patients with specific cytogenetic abnormality like deletion 5q (del5q) and monosomy/deletion 7 (-7/del7).[12]

Few local studies have been published, encompassing the clinicopathological spectrum of MDS but cytogenetic abnormalities in MDS have not yet been reported from our region.[13,14]

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This study was undertaken to determine the frequency of cytogenetic abnormalities in patients diagnosed as primary myelodysplastic syndrome using conventional karyotyping.

METHODOLOGY

The subjects in this study were consecutive patients referred to The Aga Khan University Hospital, Karachi between January 2006 - June 2012. The patients who fulfilled WHO criteria for MDS were included. Cytogenetic analysis was conducted at the time of diagnosis. The patients with an ambiguous diagnosis of MDS, those who had previously received chemotherapy or radiotherapy, and those with MDS secondary to a previous malignancy were excluded from the analysis. Data was collected using in house questionnaire. Informed consent was taken before performing the bone marrow procedure and cytogenetic analysis.

Chromosome identification and karyotype description was done according to the International System for Chromosome Nomenclature (ISCN, 1995).[15] Bone marrow cells were cultured for 24 hours in F-10 Nutrient mixture (Gibco Cat. No. 11550-035) together with fetal bovine serum. After 24-hour incubation, 75 ul of colcemid was added and incubated for 30 minutes at 37°C. The cells were then treated with hypotonic KCl (0.075 M) for 12 - 15 minutes and fixed with methanol/acetic acid (3:1). Metaphase chromosomes were banded using the conventional GTG banding technique and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN) 1995. At least twenty metaphases were analyzed. A karyotype was considered simple if there was involvement of one chromosome, double if two chromosomes and complex if there was an involvement of three or more chromosomes.[6]

Statistical Package for Social Sciences (SPSS) version 19 was used for statistical analysis. Data was presented as frequencies and percentages.

RESULTS

A total of 122 patients were diagnosed as primary myelodysplastic syndrome. Out of them, 71 patients had their karyotype done at the time of diagnosis.

Out of these 71 patients, 42 were males (59.2%) and 29 were females (40.8%). The median age was 60 ± 20 years. Only one patient was under 15 years of age. Moreover, out of the 71 patients, 37 (52.1%) were classified as refractory cytopenia with multilineage dysplasia (RCMD), 17(23.9%) as refractory anemia with excess blast-II (RAEB-II), 8 (11.3%) as refractory anemia with excess blast-I (RAEB-I), 6 (8.5%) as refractory anemia (RA), 2 (2.8%) as refractory anemia with ringed sideroblast (RARS) and 1 (1.4%) as refractory cytopenias with multilineage dysplasia-ringed sideroblast (RCMD-RS) (Table I).

Table I: Patient clinical characteristics (n=71).

Age	
Median (range) in years	60 (10-85)
Sex	
Male	42 (59.2%)
Female	29 (40.8%)
Hemoglobin (gm/dl)	
< 10	60 (84.5%)
> 10	11 (15.5%)
Absolute neutrophil count (x10^9/L)	
> 1.8	38 (53.5%)
< 1.8	33 (46.5%)
Platelet count (x10^9/L)	
< 100	49 (69%)
> 100	22 (31%)
Marrow blast cell percentage (%)	
< 5	49 (69%)
5-10	13 (18.3%)
11-20	8 (11.3%)
21-30	1 (1.4%)
Cytopenias	
Two	30 (42.3%)
Three	22 (31%)
One	18 (25.4%)
None	1 (1.4%)
Morphology	
RCMD	37 (52%)
RAEB-II	17 (23.9%)
RAEB-I	8 (11.3%)
RA	6 (8.5%)
RARS	2 (2.8%)
RCMD-RS	1 (1.4%)
Cytogenetic abnormalities	
Normal	41 (57.7%)
Abnormal	30 (42.3%)

Among 71 patients, 41 (57.7%) showed normal karyotype and 30 (42.3%) showed clonal karyotypic abnormalities at diagnosis. Out of which 14 (19.7%) had single, 11 (15.5%) had complex and 6 (8.5%) had double cytogenetic abnormalities. The highest number of chromosomal abnormalities were found in RAEB-II i.e. 10 (58.8%) followed by RAEB-I, n=4 (50%). The frequency of the different chromosomal abnormalities and their relationship to the WHO classification is shown in Table II.

The common abnormalities found were trisomy 8 in 7 cases (9.9%), -7/del (7q) in 3 cases (4.2%), -Y and complex 5q in 2 cases (2.8%) each, complex trisomy 8, del 11q , inversion 9, trisomy 19 and del 20q were found in 1 case (1.4%) each and other abnormalities in 11 cases (15.5%). The latter included various trans-locations, hyperdiploidy, hypotetraploidy, additions and monosomies.

DISCUSSION

Myelodysplastic syndrome consist of group of clonal hematological disorder characterized by peripheral blood cytopenias in the presence of hypercellular bone marrow with features of dysplasia. The degree of dysplasia, cytopenia, number of blast cells and need for

Table II: Cytogenetic profile of patients with MDS (n=71).

WHO subtype	Total cases	Normal karyotype (%)	Abnormal karyotype (%)	Chromosomal abnormalities	Number of cases
RCMD	37	23 (62)	14 (37.8)	• del 7	• 1
				• complex5q	• 1
				• t(8;12) (q24.3;q15)	• 1
				• +19	• 1
				• del 11 (q23;q25)	• 1
				• hypotetraploidy	• 1
				• t (16;16) (q22;24)	• 1
				• inv (9)	• 1
				• 46,XXY	• 1
				• +1, +2, +3, +5, del6, +7, +11, +12, -14, +22	• 1
				• add (2), -3, -5, add (6), -7, -13, -16, -5, der6, t (6;10), -10, -13	• 1
				• +8	• 1
				• del20q	• 1
				• der19,t (1;19)	• 1
RAEB-II	17	7 (41)	10 (58.8)	• +8	• 4
				• Hypotetraploidy (88~91)	• 1
				• Inv8, hyperdiploidy	• 1
				• -Y	• 1
				• +8, +21	• 1
				• Del7	• 1
				• Del5, del11	• 1
RAEB-I	8	4 (50)	4 (50)	• del 7	• 1
				• Complex 5q del	• 1
				• +8	• 1
				• add (11), add (18), der11, t (1;11)	• 1
RA	6	4 (66)	2 (33)	• -Y	• 1
				• +8	• 1
RARS	2	2 (100)	0		
RCMD-RS	1	1 (100)	0		
Total	71	41	30		

blood transfusions predict the outcome of myelodysplastic syndrome but are prone to subjective variation. In such cases, the cytogenetic studies form the mainstay of evaluating the prognosis, treatment and leukemic transformation. Cytogenetic abnormality can be carried out by conventional karyotyping or by FISH analysis both are sensitive for detecting the cytogenetic abnormality.[16,17]

In this study, which consisted of 71 patients with *de novo* MDS, the male to female ratio of 1.5:1 is consistent with the well-known male predominance which has been reported from other Asian and European regions. The median age was 59 years as found previously in Pakistani MDS patients[14], compared to 63 years in Chinese, 60 years in Tunisians,[18] 42 years in Indians[19] and 67 years in USA[20] and other Western population.

RCMD was most commonly reported WHO classified entity followed by RAEB-II in this study, comparable to that reported from Pakistan and China.[13,21] Clonal cytogenetic abnormalities were identified in 30 patients (42.3%), which was lower than 54.5% in India,[19] 67.5% in China,[21] higher than Greece (26%) but comparable with 45% in USA.[20]

Single chromosomal abnormality was found in 19.2% followed by complex cytogenetics in 16.4%. Patients with RAEB-II had highest number of chromosomal abnormalities i.e. 58.8% comparable to that reported from India[19,21] and USA.[19] In China, patients with MDS - Unclassifiable have the most number of chromosomal abnormalities.[22]

In contrast with del5q which is the most reported cytogenetic abnormality in India,[19] Arab Emirates[18] and USA,[20] trisomy 8 was found to be most common in this study population followed by the complex cytogenetics. The difference could not be clearly explained due to small sample size and availability of the cytogenetic studies in this study.

Two patients showed loss of Y-chromosome which is associated with neutral or favourable survival response.[23]

A number of novel translocations in MDS have been reported in literature by various authors[24] but in this study, some other translocations were found in isolation and as a part of complex karyotype namely t (8;12), t (6;10), t (1;19) and t (1;11). To the best of authors' knowledge and literature searched, these translocations are not yet been reported.

Clinico-haematological characteristics of MDS has been defined locally by Ehsan *et al*. and Irfan *et al*.,[13,14] but this is the first study encompassing the most comprehensive cytogenetic characterization.

Karyotypic abnormalities have an essential role in the diagnosis and determination of prognosis of MDS.

Trisomy 8 has been identified by IPSS as an intermediate risk factor which is associated with poor survival,[6] hence, such patients can be offered allogenic bone marrow transplant as an upfront treatment modality. Though advances have been made in determining the molecular defects including FLT-3 and JAK2 mutation, the importance of cytogenetic studies, still holds the position in IPSS and WPSS in developing countries.

Although cytogenetic investigations in Pakistan are performed in only a few hospitals at present, prospective studies on a large number of patients are warranted to elucidate more precisely the demographic and ethnic differences in the pathogenesis of MDS amongst the Pakistani population.

CONCLUSION

Trisomy 8 was the most common disorder/abnormality found in this study population followed by the complex cytogenetics.

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