ORIGINAL ARTICLE

CYTOGENETIC ABNORMALITIES IN ACUTE MYELOID LEUKAEMIA PATIENTS

Mehwish Roshan Shaikh, Ghulam Haider, Paras Memon, Raja Rahool, Maryum Nouman, Shumaila Beg, Khalil Meher, Saima Zahoor, Abdus Sami, Bhunisha Pavan

Department of Medical Oncology, Jinnah Postgraduate Medical Center, Karachi-Pakistan

Background: Acute myeloid leukaemia (AML) is malignant neoplasms of myeloid cells categorized by clonal expansion of hematopoietic blasts of myeloid lineage in peripheral blood and bone marrow. The aim of current study is to identify the common cytogenetic abnormalities in AML patients presenting at a tertiary care hospital of Pakistan. **Methods:** It was a cross-sectional study conducted at the department of Medical oncology of the Jinnah Postgraduate Medical Center, Karachi from Jun 2017- Jan 2019. The non-probability consecutive sampling technique was used to select patients. Total 92 cases of AML of age 15–55 years of either gender were included in the study. The detection of cytogenetic abnormality was done on the bone marrow biopsy. The cytogenetic abnormalities were classified into the three cytogenetic risk groups as favourable, intermediate and unfavourable. For analysis of data SPSS 23 version was used. **Results:** The cytogenetic abnormalities were detected in 34 (37%) of the AML patients while 58 (63%) patients had normal cytogenetic. Thirty-two females (34.8%) had a normal cytogenetic (46; XX), and 15 females (16.3%) had various cytogenetic abnormalities. Twenty-six males (28.3%) had normal cytogenetic (46; XY) and 19 males (20.7%) had various cytogenetic abnormalities. Most of the patients were in intermediate risk group (67.4%), followed by favourable (17.4%) and unfavourable risk group (15.2%). The most frequent chromosomal abnormalities observed were complex cytogenetic which was detected in 5 AML patients. **Conclusion:** In the present study cytogenetic abnormalities were found in 37% of AML patients. Sixty-seven of the AML patients were in intermediate risk group and five patients had complex cytogenetic. Hence the cytogenetic analysis provides significant information regarding prognosis of AML patients and the cytogenetic abnormalities are less than international literature.

Keywords: Cytogenetic abnormalities; Acute myeloid leukaemia; Normal cytogenetic; Complex cytogenetic

Citation: Beg S, Meher K, Zahoor S, Sami A, Pavan B. Cytogenetic abnormalities in acute myeloid leukaemia patients. J Ayub Med Coll Abbottabad 2020;32(1):33–7.

INTRODUCTION

Acute myeloid leukaemia (AML) is malignant neoplasms of myeloid cells categorized by clonal expansion of hematopoietic blasts of myeloid lineage in peripheral blood and bone marrow.[1] In US for 2019 it is estimated about 21,450 AML cases will be diagnosed with a number of 10,920 cases of death. Acute myeloid leukaemia is the disease of elder age and rare under the age of 45. Acute myeloid leukaemia is mostly common in males than females, but average lifetime odds of having AML is half of 1% in both genders.[2]

Researches showed that more than 50% of the patients with AML had abnormal karyotype at the time of diagnosis.[3] The response to treatment and outcome in patients of AML is determined by cytogenetic abnormalities which are considered as potential prognostic indicators.[4] According to the cytogenetic abnormalities, the AML patients can be divided into 3 risk groups, 20% in favourable, 30% unfavourable and 50% in intermediate group. The favourable cytogenetic risk group consist t (15;17), t (8;21) or t (16;16) or inv (16).[5] The unfavourable cytogenetic risk group consist of t (3:3), inv (3), t (9;22), 7q- & 5q-. The intermediate cytogenetic risk group consist del (9q), del (20q), t (9;11), del(7q), -Y, +8, +11, +13, and +21 or normal karyotype.[6] The potential factors for the development of AML consists of past history of myelodysplastic syndrome, history of treatment with radiotherapy or chemotherapy, exposure to ionizing, chemicals, radiation, genetics & benzene.[7] Down syndrome is also related with 10–20 fold increased risk of leukaemia.[8]

The prevalence of cytogenetic abnormalities in AML patients includes a very wide range from 30% observed in Malaysian population to 60% in Chinese population.[5,9] The frequency of abnormal karyotype was 39% in adult AML patients of Pakistan among them 8.3% were identified as t (8;21), (q22; q22) and 4.9% as t (15;17), (q22;q12) moreover adverse prognostic cytogenetic subcategories including complex karyotype, monosomy 7 and t (6;9) (p23; q34) were identified in 9%, 1% and 0.7% patients respectively.[10] The aim of the present study was to identify the common cytogenetic abnormalities in acute myeloid leukaemia in our Pakistani population. This study will help in risk stratification and determining prognosis of the disease.

MATERIAL AND METHODS

It was a cross-sectional study conducted at the department of Medical oncology of Jinnah Postgraduate Medical center, Karachi from Jun 2017–Jan 2019. Sample size was estimated as 92 by using Open Epi sample size calculator taking statistics for abnormal cytogenetic as 38.9%, margin of error as 10% and 95% confidence level.[10] The non-probability consecutive sampling technique was employed. All the newly diagnosed cases of AML of age 15–55 years of either gender were included in the study. Patients who were on chemotherapy or who have received treatment and relapsed AML were excluded from the study.

The ethical review committee approval was sought before the conduct of study. Informed written and verbal consent was taken from all the patients. Information regarding socio-demographic & presenting signs and symptoms & other related history were obtained from all the patients. The diagnosis of cytogenetic abnormality was done on bone marrow biopsy sample only. The cytogenetic abnormalities were classified according to WHO 2016 update; the three cytogenetic risk groups were defined as favourable, intermediate and unfavourable.[11]

The favourable cytogenetic risk group consist t (15;17), t (8;21) or t (16;16) or inv (16), the unfavourable cytogenetic risk group consist inv (3), t (9;22), 7q- & 5q- and complex karyotype and the intermediate cytogenetic risk group consist del(9q), del(20q), t(9;11), del (7q), -Y, +8, +11, +13, and +21 or normal karyotype. Data was entered and analysed by SPSS 23. Descriptive statistics such as mean & SD was computed for numeric variables whereas frequencies and percentages were computed for qualitative variables. The chi-square test was applied between the variables. p-value<0.05 was taken as statistically significant.

RESULTS

Total ninety-two patients of AML were included. The average age (years), platelets count ($X10^9$), WBC ($X10^9$) & Hb level (g/dl) of the patients were reported as 32.19±11.86, 102±67.1, 106±49.9 & 9.04±4.5. Majority of the patients belonged from Sindh (42.4%). Fever (60.8%) was the most frequent presenting complains followed by fatigue (47.8%) & weight loss (20.6%). About 86.9% of the AML patients had no addiction history (Smoking/Gutka/Areca nut consumption). (Table-1) The cytogenetic abnormalities were detected in 34 (37%) of the AML patients while 58 (63%) patients had normal cytogenetic. Thirty-two females (34.8%) had a normal cytogenetic (46; XX), and 15 females (16.3%) had various cytogenetic abnormalities. Twenty-six males (28.3%) had normal cytogenetic (46; XY) and 19 males (20.7%) had various cytogenetic abnormalities. Most of the patients were in intermediate risk group (67.4%), followed by favourable (17.4%) and unfavourable risk group (15.2%). The most frequent chromosome abnormalities observed were complex in 5 AML patients. (Table-2) Using the FAB subtype, 40 cases were M2, followed by 30 M1, 14 M4, 5 M5, 2 M0 and 1 M3. (Figure-1)

The proportion of the gender and age groups with cytogenetic status were analysed (Table-3). Age group (15–30 years) had the highest frequency of intermediate risk compared to the other age group, i.e., 31–40 years, 41–50 years and >50 years however the difference was statistically insignificant (p>0.05). The proportion of the gender with cytogenetic status were also analysed. Female had the highest frequency of intermediate risk as compared to males however the difference was statistically insignificant (p>0.05).

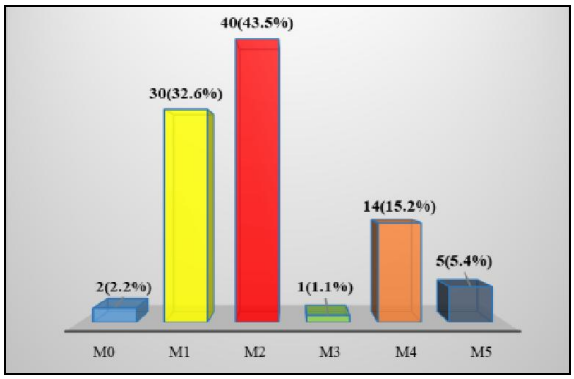

Figure-1: FAB Subtype

Table-1: Baseline characteristics of AML patients

Quantitative Variables	**Mean±SD**
Age (years)	32.19±11.86
Platelets ($X10^9$)	102±67.1
WBC ($X10^9$)	106±49.9
Hb level (g/dl)	9.04±4.5
Qualitative variables	**n (%)**
Ethnicity	
Sindhi	39 (42.4)
Punjabi	10 (10.9)
Pathan	10 (10.9)
Balochi	5 (5.4)
Urdu	20 (20.1)
Other	8 (8.6)
Presenting signs & symptoms	
Motion & vomiting	2 (2.1)
Gum swelling or bleeding	5 (5.4)
Night sweats	2 (2.1)
Body or joint pain	13 (14.1)
Loss of appetite	2 (2.1)
Fever	56 (60.8)
Fatigue	44 (47.8)
Dyspnoea	6 (6.5)
Bleeding	8 (8.6)
Weight loss	19 (20.6)
Cough/Sore throat	7 (7.6)
Fits	1 (1.1)
Decreased vision	1 (1.1)
Oral ulcer	1 (1.1)
Smoking/Gutka/Areca nut consumption	
Yes	12 (13.1)
No	80 (86.9)

Table-2: cytogenetics abnormalities in AML patients

Cytogenetic status	Type of cytogenetic	Cytogenetic abnormality	n (%)	Gender
Favourable	46XY, t (8:21)	Abnormal	3 (3.3)	Male
(n=18)	46XX; t (8:21)	Abnormal	4 (4.3)	Female
	45X-Yt (8:21) [10] /46XY [10]	Abnormal	1 (1.1)	Male
	Trisomy 21 with inv 16	Abnormal	1 (1.1)	Male
	46XX (3) t (15:17)	Abnormal	1 (1.1)	Female
	45, X-X, t (8:21) (q22: q2) [20]	Abnormal	3 (3.3)	Female
	T (8:21), (inv16) [200]	Abnormal	1 (1.1)	Female
	46~52-hyperdiploidy 52, XY+Mar, +8, +9, +11+21 (15)	Abnormal	1 (1.1)	Male
	46~74 hyperdiploidy [14] /46XX [06]	Abnormal	1 (1.1)	Female
Intermediate	46XX	Normal	32 (34.8)	Female
(n=62)	46XY	Normal	26 (28.3)	Male
	47XY, Trisomy 21	Abnormal	1 (1.1)	Male
	46XY, t (10:12) (8)/46XY (3)	Abnormal	1 (1.1)	Male
	47XX, +8(3)/46XX (+6) (trisomy 8)	Abnormal	1(1.1)	Female
	47XY, +8 (18)/46XY (3) (Trisomy 8)	Abnormal	1 (1.1)	Male
Unfavourable	Complex	Abnormal	5 (5.4)	Male
(n=14)	46XY, del (6q21) (-q23) (8)/46XY	Abnormal	3 (3.3)	Male
	46XY, t (9:22) (3)	Abnormal	2 (2.2)	Male
	47, XX, +msv [13]/47, XX, -5, +mar1+mar2 [2]	Abnormal	1 (1.1)	Female
	46XX, del (3), der (19), t (1:19) [15] /46XX	Abnormal	1 (1.1)	Female
	trisomy+monosomy	Abnormal	2 (2.2)	Female

Table-3: Stratification with respect to gender and age of cytogenetic status

Cytogenetic status	Gender		Age groups			
	Male	Female	15–30 years	31–40 years	41–50 years	>50 years
Favourable	6	10	6	3	5	2
Intermediate	29	33	33	12	14	3
Unfavourable	10	4	9	3	2	0
p-value	0.150		0.634			

DISCUSSION

Acute myeloid leukaemia is a heterogeneous disease and different parameters are required to isolate it into biologic entities to understand its pathogenesis mechanism and plan explicit treatment strategies. The examination of cytogenetics has become the most significant prognostic tool for AML patients. On cellular level the detection of specific chromosol anomalies has reinforced the view that leukaemia is a hereditary disease and has also directed the best approach to mapping and cloning of genes engaged with the leukemic process.[12,13] Hence, the goal of the present study was to identify the common cytogenetic abnormalities in acute myeloid leukaemia patients in our Pakistani population.

Acute myeloid leukaemia can probably occur at any age and its incidence raised with increasing age.[14] In the current study we have assessed 92 patients with AML. The average age of the patients was 32.19±11.86 years ranging from 15 to 55 years. Sultan S *et al* evaluated the demographical & clinical characteristics of adult AML Pakistani patients and found that mean age of the AML patients as 38.8±20.1 years ranging from 15 to 85 years.[15] In another research by Venkateswaran SP *et al*, found the average age as 29.58 years of the patients with AML.[16] A similar study by Chauhan *et al* carried out at India showed the mean age of AML patients as 32 years at the time of disease presentation.[17] However, dissimilar results have been found in German and Swedish population, where the median age were 60 & 71 years.[18,19] Hence this difference may be due to geography and genetic makeup across different populations.

Acute myeloid leukaemia is found to be two times more common in men as compared to females but average lifetime odds of having AML is half of 1% in both genders.[2] In a study conducted by Shaikh MS et al. also found predominance of males (63%) as compared to females (37%).[10] However in the present study, dissimilar findings have been observed as cytogenetic abnormalities were more in females (n=21) as compared to males (n=13). This may be due to smaller sample size.

The analysis of the karyotype at the time of diagnosis gives critical prognostic information however up to 50% AML had non-specified or normal cytogenetic findings.[20] In the present study, the cytogenetic abnormalities were detected in 34 (37%) of the AML patients while 58 (63%) patients had normal cytogenetic. Almost similar results have been found such as 30.4% patients had abnormal karyotype 70% of the AML patients had a normal karyotype in a study conducted by Meng CY *et al.*[5] Complex abnormalities have been estimated in up to 12% of patients with AML and it is considered as high- risk cytogenetic aberration with a poor

prognosis.[21] In the present study the most common cytogenetic abnormalities observed in AML patients were complex (5.4%) followed by 46XX; t (8:21) in 4.3% of the cases. The cytogenetic abnormalities such as 46XY, t (8:21), 45, X-X, t (8:21) (q22: q2) [20] & 46XY, del (6q21) (-q23) (8)/46XY (3) were observed in 3.2% of the AML patients. In a study conducted by Kumar CC found t (8;21) & t (15;17) as the most frequent abnormalities (10%) followed by inv[16] (5%) of the AML patients[22].

The risk stratification is divided into three groups such as favourable, intermediate and unfavourable cytogenetic based on the presence of particular chromosomal abnormalities. Literature have shown that 55% of the patients with favourable cytogenetic had survival rate of five years whereas 24% for patients with intermediate risk and only 5% for patients with poor-risk cytogenetic.[23] With increasing age the adverse cytogenetic abnormalities also increased within each cytogenetic risk stratification group however the prognosis with standard therapy gets worse with age.[22] In the present study according to risk stratification, majority of the AML patients were in intermediate group (67.4%) and only 17.4% & 15.2% were in favourable and unfavourable risk groups.

The FAB classification system was developed by "French-American-British Cooperative Group" grounded on conservative, cytochemical and readily accessible morphologic strains.[24] In Pakistan majority of the diagnostic laboratories use FAB classification due to its cost effectiveness.[25] In a study conducted by Udapa MSN *et al* found M2 as the most frequent FAB subtype in AML patients.[26] Similarly, in a study conducted by Sadiq MA et al. also found AML-M2 as the most common FAB AML subtype among 42% of the paediatric and adults patients followed by AML M4 in 18% patients.[25] In the present study, we also found M2 as the most frequent subtype in 43.5% of the AML patients followed by M1 (32.6%), M4 (15.2%), M5 (5.4%), M0 (2.2%) and M0 only in 1.1% of the cases.

In the present study, age group (15–30 years) had the highest frequency of intermediate risk compared to the other age groups 31 years onwards however the difference was statistically insignificant (p>0.05). Females had the highest frequency of intermediate risk as compared to males however the difference was statistically insignificant (p>0.05). In a study conducted by Shaikh MS *et al*[10] found majority of the AML patients were under the age of 30 years and 66.4% were in intermediate risk group, however the proportion of favourable and unfavourable cytogenetic abnormalities was statistical significant in the different age groups (p>0.05) and males proportion was higher as compared to females however frequency of favourable and unfavourable cytogenetic abnormalities with respect to the gender was statistically insignificant (p>0.05).

CONCLUSION

In the present study cytogenetic abnormalities were found in 37% of AML patients. Sixty-seven of the AML patients were in intermediate risk group and five patients had complex karyotype. Hence the cytogenetic analysis knowing about karyotype and genetic profile at the time of diagnosis can be important in order to take decisions in management and overall response of the AML patients.

AUTHORS' CONTRIBUTION

MRS: Conceptualization of study design, data collection, data analysis, write-up, proof reading. GH: Write-up and proof reading. All other authors contributed equally.

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Submitted: 30 October, 2019	*Revised: 27 December, 2019*	*Accepted: 18 January, 2020*

Address for Correspondence:

Mehwish Roshan Shaikh, Department of Medical Oncology, Jinnah Postgraduate Medical Center, Karachi-Pakistan
Cell: +92 307 346 4920
Email: mehwishshaikh338@gmail.com