Cytogenetic Pattern in Adult Patients with de novo Acute Myeloid Leukaemia: A Single Centre Study in Bangladesh

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Citation: Baqi SMA, Munmun UK, Islam S, Rahman F, Khan MR, Shah MS, Aziz MA, Begum M. Cytogenetic Pattern in Adult Patients with de novo Acute Myeloid Leukaemia: A Single Centre Study in Bangladesh. Haematol J Bangladesh 2020; 4 (1): 08-12.

DOI: <http://doi.org/10.37545/haematoljbd202047>

Received: 09 April 2020

Accepted: 16 April 2020

Published: 20 June 2020

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Funding: This study was carried out with the support of Research Grant of Bangabandhu Sheikh Mujib Medical University (BSMMU).

ABSTRACT

Background: Cytogenetic analysis performed at diagnosis is the most important prognostic factor in AML. **Objective:** The purpose of this study was to observe the pattern of cytogenetic abnormalities in adult patients with de novo AML. **Method:** Total fifty-two newly diagnosed de novo AML patients were selected for the study. Six cytogenetic abnormalities including t(8;21), t(15;17), inv(16), BCR-ABL1, FLT3-ITD and NPM1 mutations were studied by Real-Time PCR. **Results:** In this study, 36 (69.2%) patients showed different cytogenetic abnormalities. The t(15;17) was found to be the most common. t(15;17), t(8;21) and inv(16) were found only in M3, M2 and M4 FAB subtypes, respectively. **Conclusion:** Cytogenetic study should be performed routinely in all cases of AML for proper diagnosis, prediction of prognosis and implementation of effective therapeutic measures.

Key words: acute myeloid leukaemia, cytogenetics.

Introduction

Acute myeloid leukaemia (AML) is a clonal, malignant disease of haematopoietic tissue which is characterized by accumulation of abnormal (leukaemic) blast cells, principally in the bone marrow, and impaired production of normal blood cells.¹ The incidence of AML is higher in the white population (3.8 per 100,000 person) than that of the Asian population (3.2 per 100,000 person).² AML is the most common acute leukaemia in adult and it is more common in males. Majority of the AML patients are older than 60 years.³ Cytogenetic abnormalities were first described in AML in the 1960s.⁴ In 1988, the Morphologic, Immunologic and Cytogenetic (MIC) classification was published. Since then, cytogenetic investigations have become more important in AML classification.⁵ AML is heterogeneous in terms of morphology, immunophenotype, cytogenetics and molecular genetics.⁶ Using the World Health Organization (WHO) criteria, the diagnosis of AML is established by the presence of 20% or more of leukaemic myeloblasts

in the peripheral blood or bone marrow. However, in a subgroup of AML, the presence of recurrent genetic abnormalities alone is sufficient for the diagnosis of AML regardless of the blast percentage.⁷ Geographical,

ethnic, and environmental influences should be considered in determining the cytogenetic and morphological features of this disease.⁸ Cytogenetic abnormalities are identified in 50-60% of adult newly diagnosed AML

patients.⁹ Age and cytogenetic abnormalities are the most important prognostic factors in AML.¹⁰ There is an increase in the proportion of patients with unfavourable risk cytogenetics and a decrease in favourable risk cytogenetics with advancing age.¹¹ Besides the prognostic importance, cytogenetic analysis is also recommended to monitor minimal residual disease (MRD) in case of AML patients with abnormal cytogenetics.¹²

From these points of view, the current study was designed to observe the pattern of cytogenetic abnormalities in adult patients with de novo AML, to categorize the patients into three risk groups and to observe the association of cytogenetic findings with FAB subtypes, age, sex and other laboratory findings.

Methods

This observational study was conducted in the department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), from February 2018 to January 2019. Total fifty-two newly diagnosed adult patients with de novo AML had been enrolled for the study by purposive type of sampling technique. Patients with myelodysplastic syndrome and chronic myeloid leukaemia transforming into AML and therapy-related AML were excluded from the study. Informed consent was taken from the subjects. Ethical clearance was obtained from institutional review board (IRB) of BSMMU. Complete blood count was done by automated cell counter machine and checked manually. Morphological diagnosis of AML had been done by bone marrow study. For cytogenetic study, 3-5 ml of peripheral blood or bone marrow aspirate was collected in EDTA tube and was sent to the laboratory as early as possible with appropriate precautions. Cytogenetic investigations were done on Dr Lal Path Labs Ltd. India. Six cytogenetic abnormalities including t(8;21), t(15;17), inv(16), BCR-ABL1, FLT3-ITD and NPM1 mutations were detected by Real-Time Polymerase Chain Reaction (RT PCR) technique. The ABI 7500 RT PCR system (using the Taqman chemistry) was used for doing the cytogenetic analysis. The ABI 7500 RT PCR Instrument with SDS Software is a real-time nucleic acid amplification and five-color fluorescence detection system used for in vitro diagnostic. At the start of RT PCR, the temperature is raised to denature the double-stranded DNA. In the next step, the reaction temperature is lowered to allow the primers and probe to anneal to their specific target sequences. Taq DNA polymerase synthesizes new strands using the unlabeled primers and the template. When the polymerase reaches a TaqMan probe, its endogenous 5' nuclease activity

cleaves the probe, separating the fluorescence dye from the quencher which is then measured by a CCD camera at each cycle of amplification.

A pre-designed semi-structured data collection sheet was used for data collection. The statistical analysis was carried out using the Statistical Package for Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Qualitative variables of this study were expressed as percentage. Quantitative variables were expressed as mean $\pm$ standard deviation. Chi-Square test was used for categorical variables. To compare means of two groups' unpaired t-test was used in case of normally distributed data and Mann-Whitney U test was used where the data were not normally distributed. For all statistical tests, p-value less than 0.05 was considered as statistically significant.

Results

The mean age of the patients was 37 ($\pm$ 13.29) years. Maximum (30.8%) patients belonged to 18-27 years age group. Female was slightly predominant with a male: female ratio was 1: 1.08. Maximum patients belonged to FAB M2 (32.7%), followed by M3 (26.9%) and M1 (17.3%). Among the 52 cases, 36 (69.2%) cases showed different cytogenetic abnormalities and 16 (30.8%) cases had normal cytogenetics. The t(15;17) was found to be the most common, detected in 13 (25%) patients. NPM1 mutation was found in 10 (19.23%), t(8;21) in 8 (15.38%) and FLT3-ITD mutation in 8 (15.38%) cases (Table I).

Table I: Cytogenetic pattern in study subjects. (n:52)

Cytogenetic pattern	n (%)
Normal cytogenetics	16 (30.8)
t(8;21)	7 (13.5)
t(15;17)	12 (23.1)
inv(16)	2 (3.8)
BCR-ABL1	1 (1.9)
NPM1	6 (11.5)
FLT3-ITD	2 (3.8)
NPM1 and FLT3-ITD	4 (7.7)
t(8;21) and FLT3-ITD	1 (1.9)
t(15;17) and FLT3-ITD	1 (1.9)
Total	52 (100)

M0 and M6 subtypes did not show any cytogenetic abnormality. Most of the M1 cases also had normal cytogenetic pattern. The t(15;17), t(8;21) and inv(16) were found only in M3, M2 and M4 FAB subtypes respectively. Only one case showed BCR-ABL1 mutation which belongs to M1 subtype. A combination of NPM1

and FLT3-ITD mutation was found in M4, M1, M3 and M2 subtypes (Table II).

Table II: Distribution of specific cytogenetics among FAB subtypes of AML. (n:52)

Cytogenetic pattern	FAB subtypes; n (%)						
	M0	M1	M2	M3	M4	M5	M6
t(8;21)			7 (13.5)				
t(15;17)				12 (23.1)			
inv16					2 (3.8)		
BCR-ABL1		1 (1.9)					
NPM1		1 (1.9)	2 (3.8)		2 (3.8)	1 (1.9)	
FLT3-ITD					2 (3.8)		
NPM1 and FLT3-ITD		1 (1.9)	1 (1.9)	1 (1.9)	1 (1.9)		
t(8;21) and FLT3-ITD			1 (1.9)				
t(15;17) and FLT3-ITD				1 (1.9)			
Normal cytogenetics	2 (3.8)	6 (11.5)	6 (11.5)			1 (1.9)	1 (1.9)
Total	2 (3.8)	9 (17.3)	17 (32.7)	14 (26.9)	7 (13.5)	2 (3.8)	1 (1.9)

No significant difference observed between age among different cytogenetic pattern. In only one case with 78 years of AML M1 had BCR-ABL1 mutation. (Table III).

Table III: Relationship of cytogenetic abnormalities with age (years). (n:52)

Cytogenetic pattern	Age in years; n (52)				
	n (%)	Present Mean age $\pm$ SD	Absent n (%)	Mean age $\pm$ SD	p-value
t(8;21)	8 (15.4)	30.00 $\pm$ 10.30	44 (84.4)	38.11 $\pm$ 13.49	0.107
t(15;17)	13 (25.0)	31.92 $\pm$ 8.42	39 (75.0)	38.51 $\pm$ 14.27	0.150
inv16	2 (3.8)	40.50 $\pm$ 7.77	50 (96.2)	36.72 $\pm$ 13.50	-
BCR-ABL1	1 (1.9)	78.00	51 (98.1)	36.06 $\pm$ 12.07	-
NPM1	10 (19.2)	42.80 $\pm$ 11.76	42 (80.8)	35.45 $\pm$ 13.37	0.073
FLT3-ITD	8 (15.4)	35.13 $\pm$ 13.52	44 (84.6)	37.18 $\pm$ 13.39	0.713

Favourable cytogenetics were more frequent in younger age groups. Intermediate and unfavourable cytogenetics were common in relatively older age groups. These findings were statistically significant (p:0.004) (Table IV).

Table IV: Distribution of the cases according to different age groups and cytogenetic risk groups. (n:52)

Age groups (years)	Cytogenetic risk groups; n (%)			p-value
	Favorable	Intermediate	Unfavorable	
18-27	9 (56.3)	6 (37.5)	1 (6.3)	
>27-37	8 (57.1)	5 (35.7)	1 (7.1)	
>37-47	7 (70.0)	3 (30.0)	0 (0.0)	0.004
>47-57	3 (27.3)	8 (72.7)	0 (0.0)	
>57	0 (0.0)	0 (0.0)	1 (100)	

FLT3-ITD mutation was significantly associated with increased WBC count (p: 0.042) (Table V).

Table V: Relationship of cytogenetic abnormalities with WBC count ($\times 10^9/L$). (n:52)

Cytogenetic pattern	WBC count ($\times 10^9/L$)				
	n (%)	Present Mean $\pm$ SD	Absent n (%)	Mean $\pm$ SD	p value
t(8;21)	8 (15.4)	45.71 $\pm$ 86.99	44 (84.4)	26.12 $\pm$ 32.98	0.603
t(15;17)	13 (25.0)	26.33 $\pm$ 39.69	39 (75.0)	30.07 $\pm$ 46.82	0.492
inv16	2 (3.8)	7.55 $\pm$ 8.55	50 (96.2)	30.00 $\pm$ 45.47	-
BCR-ABL1	1 (1.9)	8.50	51 (98.1)	29.54 $\pm$ 45.14	-
NPM1	10 (19.2)	30.90 $\pm$ 40.19	42 (80.8)	28.72 $\pm$ 46.26	0.546
FLT3-ITD	8 (15.4)	44.93 $\pm$ 41.17	44 (84.6)	26.26 $\pm$ 45.27	0.042

No significant difference observed between bonemarrow blast percentage with different cytogenetic pattern.. (Table VI).

Table VI: Relationship of cytogenetic abnormalities with bone marrow blast (%). (n:52)

Cytogenetic pattern	bone marrow blast (%)				
	n (%)	Present Mean $\pm$ SD	Absent n (%)	Mean $\pm$ SD	p value
t(8;21)	8 (15.4)	72.50 $\pm$ 8.86	44 (84.4)	72.11 $\pm$ 17.73	0.560
t(15;17)	13 (25.0)	76.53 $\pm$ 9.43	39 (75.0)	70.71 $\pm$ 18.26	0.675
inv16	2 (3.8)	40.00	50 (96.2)	73.46 $\pm$ 15.59	-
BCR-ABL1	1 (1.9)	80.00	51 (98.1)	72.01 $\pm$ 16.73	-
NPM1	10 (19.2)	65.30 $\pm$ 24.71	42 (80.8)	73.80 $\pm$ 13.96	0.452
FLT3-ITD	8 (15.4)	64.75 $\pm$ 18.02	44 (84.6)	73.52 $\pm$ 16.19	0.081

Patients with normal cytogenetics showed significant association with relatively increased bone marrow blast percentage (p:0.033). No other parameter (sex, haemoglobin level, platelet count) showed any significant association with cytogenetic pattern.

Discussion

AML is characterized by a spectrum of clinical, morphological, immunophenotypic and associated cytogenetic abnormalities. In this study the mean age at presentation of AML was 37 years. Studies in most of the other countries showed much higher mean age.^{6,8,13-15} The reason for this difference may be due to inclusion of only de novo cases of AML in this study or may be geographic/ethnic influence.

Cytogenetic abnormalities were detected in 69.2% of patients with an increased frequency of t(15;17) in this study. These findings are consistent with the results of several other studies conducted by Ayesh *et al.* (2012) and Enjeti *et al.* (2004).^{13,16} Here, favourable cytogenetic risk group belonged to young patients and frequency of unfavourable cytogenetics were higher in older age groups. Similarly, Meng *et al.* (2013) found 75% favourable cytogenetics in young patients and mostly complex karyotype in elderly patients.⁶

Male: female ratio in this study was 1:1.08. This finding differs from most of the studies in other countries where male predominance was found.^{6,13,17} This difference is probably due to greater proportion of female bed in our department. Geographic or ethnic influence may also be responsible. An increased frequency of t(15;17) (28%) was found among the male patients in this study. Enjeti *et al.* (2004) also found increased frequency of t(15;17) among male patients in a study from Singapore.¹³ No other significant difference was found in this study among male and female patients in relation to cytogenetics.

Cases with FLT3-ITD mutation were found in this study to have increased WBC count. Peng *et al.* (2008) also demonstrated a correlation between the presence of FLT3 mutation and the increased WBC count.¹⁸ Similarly, Schnittger *et al.* (2002) and Haferlach *et al.* (2012) reported NPM1 and FLT3 mutations to be correlated with higher peripheral WBC count.^{19,20}

The t(15;17) was found to be the most common (25%) cytogenetic abnormality in this study. The frequency of t(15;17) found in this study is much higher than other parts of Asia, like Taiwan (15%), compared to those observed in North America and Europe (3-10%).^{21,22} The t(15;17) was found in 85.7% of M3 subtype patients in the current study, which is very close to the findings in Singapore (82.5% of M3 patients), compared to Japan (75.4%) and Europe (72.5%).^{8,13,22}

On the other hand, t(8;21) was found in 41.2% of AML M2 patients in this study. This percentage is closer to the incidence found in Japanese and Taiwanese reports (33.1% and 34% of their AML M2 patients).^{8,21} The t(8;21) is less frequent among the AML M2 patients in Singapore (14.5%), Australia (15.3%) and North America (22%).^{13,23,24}

In this study, significant difference was found in case of inv(16) pattern between M4 and non-M4 subtypes. Enjeti *et al.* (2004) also found the highest frequency of inv(16) among their M4 cases.¹³

FLT3-ITD mutation was found more frequently in M4 subtype in this study. Similarly, another study carried out by Koczkodaj *et al.* (2016) in Southeastern Poland found the highest frequency of FLT3-ITD mutation in AML M4 cases.²⁵ In an Iranian study, Rezaei *et al.* divided the patients into FAB M3 and non-M3 groups and analysed the FLT3-ITD and NPM1 mutational status among these patients.²⁶ They also found that these mutations were

more frequent in non-M3 patients. On the other hand, Smith *et al.* (2011) found a higher frequency of FLT3-ITD mutations among M3 patients.²⁷ Falini *et al.* (2005) observed that FLT3-ITD mutations occur twice as often in the cases with simultaneous NPM1 mutations as in the cases without this mutation.²⁸ The current study also shows that co-existent FLT3-ITD and NPM1 mutations are more frequent than FLT3-ITD mutation alone. These differences in the frequency of cytogenetic/molecular genetic abnormalities might be due to geographic and ethnic heterogeneity, variation in the sample size or inclusion of paediatric patients in some studies. Paediatric patients were excluded in our study.

Conclusion

Significant number of patients showed different cytogenetic abnormalities. Therefore, cytogenetic study should be performed routinely in all cases of AML for proper diagnosis, prediction of prognosis and implementation of effective therapeutic measures.

Limitations

Samples were collected from only one center and sample size was small; hence it may not represent the whole population of the community. Duration of the study was short. Six cytogenetic abnormalities were analyzed in this study because of resource constraints.

Recommendations

Cytogenetic study should be performed routinely in all cases of AML for proper diagnosis, prediction of prognosis and implementation of effective therapeutic measures. A cytogenetic and molecular genetic laboratory should be established at Haematology department of BSMMU. Future studies with a larger sample size and more cytogenetic markers are recommended.

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