RESEARCH PAPER

Cytogenetic Profiles of 472 Indian Children with Acute Myeloid Leukemia

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Objective: To analyze the cytogenetic abnormalities of a large cohort of consecutive pediatric Acute Myeloid Leukemia (AML) patients, treated on a uniform protocol.

Design: Review of case records**.**

Setting: Pediatric Cancer Center of tertiary care hospital between June 2003 and June 2016.

Participants: 617 consecutive *de novo* pediatric AML patients were screened and 472 patients were found eligible. Eligibility criteria included non M3 patients, successful cytogenetic profile and availability of complete records

Main outcome measure: Cytogenetic profile.

Results: Gum-hypertropy, chloromas and rate of complete remission were significantly different between European Leukemia Network classification (ELN) cytogenetic risk groups (P<0.01). *t* (8;21) (141, 29.8%), loss of Y chromosome (61,12.9%) and trisomy 8 (39, 8.3%) were the most common abnormalities. Among the chromosomal gains, trisomy 8 and trisomy 21 (both P<0.01) were significantly different among the three ELN risk groups. Among the chromosome losses, monosomy 5, 7 (both P<0.01) and 9 (P=0.03), loss of X and loss of Y (both P<0.01) were statistically different amongst three cytogenetic risk groups. Event-free survival (P<0.01) and overall survival (P<0.01) were found to be significantly different among the three risk groups.

Conclusions: The higher frequency of *t* (8; 21) and its association with chloroma in Indian pediatric patients is different from other studies around the world.

Keywords: *Childhood cancers*, *Chloroma, Chromosomal translocation, Karyotype*.

Acute myeloid leukemia (AML) is a heterogeneous disease from morphologic, cytogenetic, immunophenotypic, molecular, and clinical perspectives. AML accounts for 15% to 20% of all childhood leukemia [1]. Reliable figure for incidence of AML in Indian children is lacking.

Cytogenetic and molecular data are recognized as the most valuable prognostic factors in AML both in National Comprehensive Cancer Network (NCCN) and European Leukemia Net (ELN) risk stratification models [2,3]. Most of the studies on cytogenetic profiling of AML are from Western countries [4] and similar data from the Indian subcontinents is lacking. We conducted this retrospective study to analyze the cytogenetic abnormalities in AML patients at a single cancer centre in India.

METHODS

This is a single center, retrospective, observational study conducted at a tertiary cancer center in Northern India. Children with AML who were registered between June 2003 and June 2016 were included. This study was approved by the Institutional Ethics Committee. We included all patients aged ≤18 year with *de novo* AML. The patients who had acute promyelocytic leukemia (M3 AML), secondary AML, therapy related AML and incomplete records were excluded from the study. All patients were treated with common protocol (3+7 induction + 3 high dose cytarabine). Allogenic stem cell transplant in first complete remission (CR1) was not done. However, at relapse, stem cell transplantation was offered in second remission (CR2). Their medical records were comprehensively reviewed for the demographics, baseline disease characteristics, cytogenetic profile, treatment, and outcomes. Cytogenetic analysis was considered successful if they qualified ISCN guidelines (evaluation of 20 metaphases for normal cytogenetic and 10 metaphase for abnormal cytogenetic) [5].

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The diagnosis of AML was made according to the World Health Organization (WHO) classification of hematopoietic neoplasm, which requires identification of 20% or more leukemic blasts in the bone marrow or blood [6]. ELN classification was used to categorize divide the patients into three prognostic risk groups; favorable risk, intermediate risk and adverse risk [2]. Complex

karyotype was defined as any karyotype with at least three chromosome aberrations, regardless of their type and the individual chromosomes involved, excluding recurrent cytogenetic abnormalities [7,8]. Conventional cytogenetic analyses were conducted on baseline bone marrow samples of patients at National Accreditation Board for testing and calibration laboratories (NABL). Bone marrow (BM) cells were cultured for 24 hours, then karyotype was analyzed using the standard G-banding technique. The karyogram were constructed, and chromosomal abnormalities were reported in accordance with the International system for human cytogenetic nomenclature (ISCN 2013) [5]. Fms-related tyrosine kinase 3 internal tandem duplication (FLT3-ITD) and nucleophosmin-1 (NPM1) mutation were performed using reverse transcriptase polymerase chain reaction (RT-PCR) from RNA extracted from BM/PB sample obtained at diagnosis from patients [9,10].

CR was defined as bone marrow blast <5%, absolute neutrophil count >1000/uL, platelet count >100000/uL, no residual evidence of extramedullary disease and the patient child independent of transfusion [11]. EFS were measured from the date of diagnosis until relapse or death. Relapse following CR is defined as reappearance of leukemic blast in peripheral blood or the finding of >5% blasts in the bone marrow, not attributable to another cause [11].

Statistical analysis: Differences between groups were assessed using Student *t* test for continuous variables and Pearson chi-square test for categorical variables. Kaplan-Meier curves were obtained for survival analysis for event free survival (EFS) and overall survival (OS) and the log rank test was used for comparison. OS was measured as the time from the date of diagnosis until death or last follow-up. The censoring date of the study was January 31, 2017. $P<0.05$ was considered to be statistically significant. Data were analyzed using the statistical software STATA 11.1 version (Texas; USA).

RESULTS

A total of 617 patients were registered during the study period; 145 patients were excluded from the study (16 had incomplete data, 31 were acute promyelocytic leukemia (APML), cytogenetic assessment was not done for 61 patients and cytogenetic assessment had failed in 37 patients). 472 (non M3, *de novo* AML) patients (320 boys) were eligible for the detailed analysis. The median (range) age was 10 (0.3, 18) years. Of these, 265 (56.1%) patients were in the intermediate risk group and 162 (34.3%) patients in the favorable risk group. There was no significant difference in baseline hemoglobin, platelet- and leucocyte count between the three risk groups. Gum hypertrophy was observed in 124 (26.2%) patients; most of these patients (66.9%) belonged to the intermediate risk group. Chloroma was present in 100 (21.1%) patients, and 54% of these belonged to the favorable risk category. Gum hypertrophy and chloroma were significantly different among the cytogenetic risk groups (both $P<0.01$). Rate of complete remission ($P<0.01$), EFS ($P<0.01$) and OS ($P<0.01$) were significantly different among three cytogenetic risk group (***Table* I**).

The most common cytogenetic abnormality was the loss of Y chromosome observed in 61 (12.9%) patients. In the cohort of 472 patients, trisomy 8 was most frequent gain; while among the losses, the loss of Y chromosome was most commonly observed ($n=61$) (***Web Fig*. 1**). Among the chromosomal gains, trisomy 8 ($P<0.01$) and trisomy 21 ($P<0.01$) were found to be significantly different between all these groups. On analyzing the chromosomal losses, monosomy 5 ($P<0.01$), monosomy 7 ($P<0.01$), monosomy 9 ($P=0.03$), loss of X chromosome ($P<0.01$) and loss of Y chromosome ($P<0.01$) were significantly different in the three cytogenetic risk groups (***Table* II**).

TABLE I BASELINE PATIENT CHARACTERISTICS AND OUTCOMES AMONG DIFFERENT AML CYTOGENETIC RISK GROUPS

Parameter	*Favourable risk (n=162)*	*Intermediate risk (n=265)*	*Adverse risk (n=45)*	*P value*
Hemoglobin (g/dL), mean (SD)	7.7 (2.5)	7.7 (2.3)	7.3 (2.5)	0.54
Platelet (×1000/µL), mean (SD)	53.4 (61.5)	68.6 (117.6)	57.4 (81.9)	0.70
WBC (×1000/µL), mean (SD)	27.2 (38.5)	50.5 (71.0)	46.4 (69.4)	0.23
Gum hypertrophy	28 (17.3%)	83 (31.3%)	13 (26.3%)	<0.01
Chloroma	54 (33.9%)	38 (14.3%)	7 (15.6%)	<0.01
Rate of complete remission	155 (95.7%)	206 (77.7%)	34 (75.5%)	<0.01
EFS (mo), median (IQR)	15.4 (8.8-Not achieved)	11.2 (5.4-27.8)	8.3 (3.6-91)	<0.01
OS (mo), median (IQR)	35.4 (12-Not achieved)	16.9 (7.9-Not achieved)	9.3 (5.5-Not achieved)	<0.01

WBC: White blood cell; SD: Standard deviation; AML: Acute myeloid leukemia; EFS: Event free survival; OS: Overall survival.

TABLE II VARIOUS CYTOGENETIC ABNORMALITIES ACROSS ELN GROUPS

Parameter	*n (%)**	*Favourable risk (n=162)*	*Intermediate risk (n=265)*	*Adverse risk (n=45)*	*P value*
Any Monosomy	29 (6.1%)	8 (4.9%)	1 (0.4%)	20 (44.4%)	<0.01
Monosomy 5	5 (1.0%)	1 (0.6%)	0 (0%)	4 (9.1%)	<0.01
Monosomy 7	17 (3.6%)	3 (1.8%)	0 (0%)	14 (31.1%)	<0.01
Monosomy 9	7 (1.5%)	4 (2.5%)	1 (0.3%)	2 (4.5%)	0.03
Any Trisomy	57 (12.1%)	10 (6.2%)	34 (12.7%)	13 (29.5%)	<0.01
Trisomy 4	16 (3.4%)	8 (4.9%)	6 (2.2%)	2 (4.5%)	0.49
Trisomy 8	39 (8.3%)	3 (1.9%)	26 (9.7%)	10 (22.7%)	<0.01
Trisomy 21	17 (3.6%)	2 (1.2%)	7 (2.6%)	8 (18.1%)	<0.01
Loss of sex chromosome	78 (16.5%)	66 (40.9%)	10 (3.7%)	2 (4.5%)	<0.01
X chromosome	17 (3.6%)	13 (8.1%)	4 (1.5%)	0 (0%)	<0.01
Y chromosome	61 (12.9%)	53 (33.9%)	6 (2.2%)	2 (4.5%)	<0.01
Other abnormalities	425 (90.0%)	161 (100%)	231 (86.5%)	33 (75%)	<0.01

**Chromosomal abnormalities are redundant and may not add up to (100%).*

TABLE III BASELINE PARAMETERS, OUTCOMES AND OTHER CYTOGENETIC ABNORMALITIES WITH AND WITHOUT t (8; 21)

Parameter	*t (8;21) Negative (n=331)*	*t (8;21) Positive (n=141)*	*P value*
Hemoglobin (g/dL), mean (SD)	7.6 (2.3)	7.7 (2.5)	0.55
Platelet (×1000/µL), mean (SD)	65.5 (118.5)	54.9 (63.7)	0.78
WBC (×1000/µL), mean (SD)	50.3 (70.3)	22.9 (30.2)	0.01
Gum hypertrophy	101 (30.5%)	23 (16.3%)	<0.01
Chloroma	46 (13.8%)	54 (38.2%)	<0.01
CR Status	263 (79.4%)	134 (95%)	<0.01
EFS (mo), median (IQR)	11.6 (5.8-39.4)	12.6 (8.6-37.7)	0.15
OS (mo), median (IQR)	16.9 (8.2 - Not achieved)	31.7 (10.9 - Not achieved)	0.04
Trisomy 4, *n* (%)	8 (2.4%)	8 (5.6%)	0.14
Monosomy 7, *n* (%)	14 (4.2%)	3 (2.1%)	0.52
Trisomy 8, *n* (%)	36 (10.8%)	3 (2.1%)	<0.01
Trisomy 21 *n* (%)	15 (4.5%)	1 (0.7%)	0.06
Loss of X chromosome, *n* (%)	4 (1.2%)	13 (9.2%)	<0.01
Loss of Y Chromosome, *n* (%)	8 (2.4%)	53 (37.6%)	<0.01

SD: Standard Deviation; WBC: White Blood cell; CR: Rate of complete remission; EFS: Event free survival; OS: Overall survival.

The information on *t* (8;21) by cytogenetics was available in all 472 patients. Out of these, 141 (29.9%) patients were positive for *t* (8; 21). WBC count (P=0.01), gum hypertrophy (P<0.01) and chloroma (P<0.01) were significantly different between patients with and without *t* (8;21). Choloromas were more frequently noted in *t* (8; 21) positive patients (P<0.01) (***Table* III**). Significant difference was observed for trisomy 8 (P<0.01), loss of X chromosome (P<0.01) and loss of Y chromosome (P<0.01) status between the two groups with or without *t* (8;21). There was no significant difference in the EFS however, significant difference was observed in OS (P=0.04) of the patients with and without *t* (8;21) (***Table* III**).

Survival and relapse information for all the 472 patients included in this study was available (***Table* I**). EFS and OS were statistically significantly different for the three risk groups identified using the ELN criteria (***Fig.*1**).

DISCUSSION

In the current study, cytogenetic abnormalities were detected in about two-thirds of AML cases. Gum

WHAT IS ALREADY KNOWN?

- Data on cytogenetic profile of pediatric acute myeloid leukemia patients is scarce.

WHAT THIS STUDY ADDS?

- Increased frequency of t (8;21) and significant association of t (8;21) with chloromas are seen in Northern Indian children with acute myeloid leukemia.

hypertrophy, chloroma and rate of complete remission were found to be significantly different between ELN cytogenetic risk groups. Translocation t (8; 21), loss of Y chromosome and trisomy 8 were the most common cytogenetic abnormalities. Event-free survival (EFS) and overall survival (OS) were found to be significantly different among the three risk groups identified using the ELN criteria.

Our institute is a major referral center for pediatric AML and caters to a major portion of patients from northern part of India. As this is not a population-based study, the data presented here may not be representative of the Indian population. Our study shows significant difference in overall survival but does not show any significant difference in event free survival of the patients differing by *t* (8;21) status, as we lacked molecular data for all patients. The data on molecular abnormalities is somewhat fragmented because of the retrospective nature of the study.

There are only a few population-based studies on AML patients and most have selection bias (regarding age, treatment protocol *etc*). In general, karyotypic pattern and frequency of specific chromosomal abnormalities were similar to those reported in previous large series except for few remarkable differences [1,12-18]. The median age in our analysis was less than other studies that have included both pediatric and adult patients. Another important finding of this study is an increased frequency (29.9%) of *t* (8; 21) in our population. This compares well with the data published by Amare, *et al*. [18] who had reported a similar frequency among their 567 pediatric patients from a tertiary care cancer center from Western India. Nakase, *et al*. [4] have also reported a higher frequency of *t* (8;21) in the Japanese patients. However, this is in stark contrast to studies from other parts of the world [14]. The 21.2% occurrence of chloromas in our study was significantly higher than the incidence of myeloid sarcoma reported in literature (2-8%) [3]. Out of these, 33.9% had favourable risk cytogenetic. The reason for the association of *t* (8;21) with chloroma is unknown.

Our study has shown an increased frequency of t (8; 21) and its association with chloroma. Further studies using advanced molecular tools like Next generation sequencing (NGS) would pave the way to better understanding of the biology of this disease.

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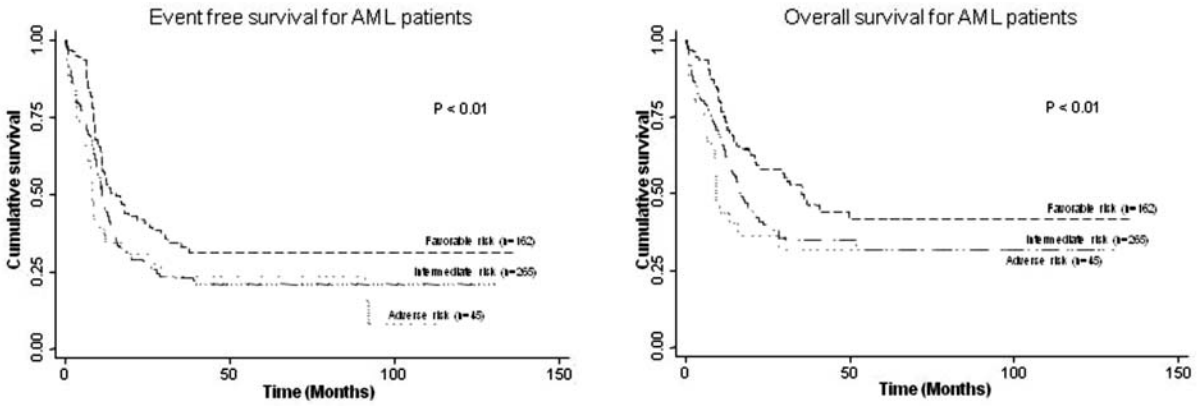

FIG. 1 *Kaplan-Meier survival curves showing Event-free survival and Overall survival (OS) in three cytogenetic risk group patients.*

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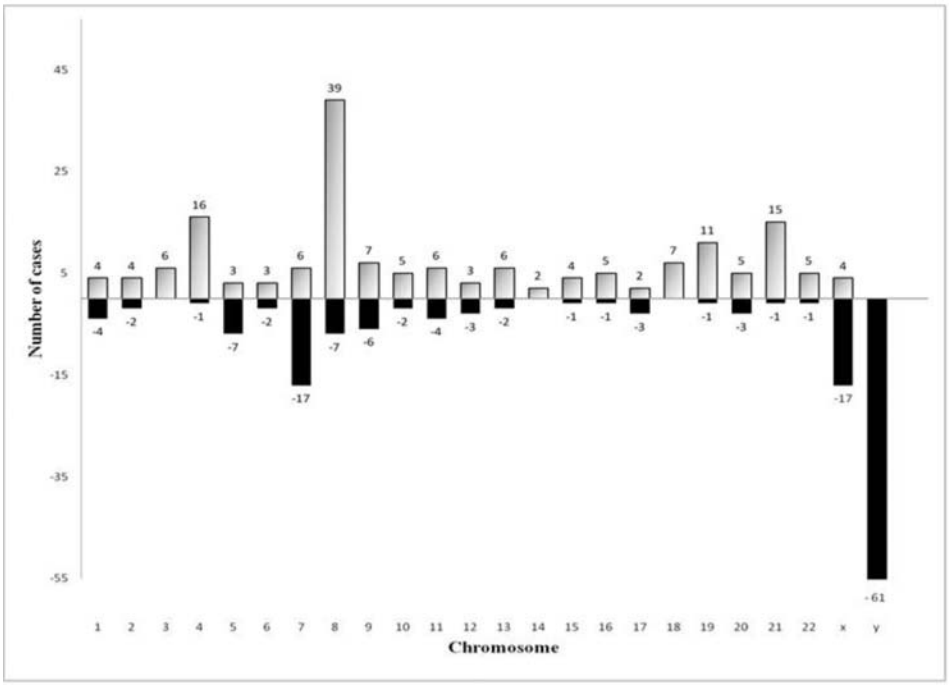

FIG. 1 *Bar graph showing the distribution of numerical cytogenetic abnormalities according to individual chromosome gains and losses (in part or whole of the chromosome).*