Original Article

G-banding and Fluorescence *In Situ* Hybridization in Childhood Acute Myeloid Leukemia from South India

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Abstract

Background: The current WHO classification of hematologic malignancies defines distinct entities of myeloid disorders based on the presence of recurrent cytogenetic abnormalities. Diagnostic clonal chromosomal abnormalities provide important prognostic information and are among the most important factors in predicting initial response to chemotherapy, duration of remission and overall survival.

Methods: This study analyzed chromosomal abnormalities in bone marrow aspirates of 50 children diagnosed with acute myeloid leukemia (AML).

Results: The culture success rate was 94%, clonal chromosomal abnormalities constituted 62% and recurrent chromosomal abnormalities were 56%. In the favorable prognostic category, there were 51.6% of cases with t(8;21)(q22;q22), 16.1% had t(15;17)(q22;q21), and a total of 12.9% had chromosome 16 rearrangement. The adverse risk category showed a low frequency of t(9;11)(p22;q13); t(1;22)(p13;q13); inv(3)(q21q26); add 4(q35) and ring chromosome. According to fluorescent in situ hybridization (FISH) results in 16 cytogenetically normal patients, there were no CBFβ/MYH11 fusion genes observed in chromosome 16 rearrangements.

Discussion: Larger studies of this kind may provide more information about chromosome 16 rearrangements in cytogenetically normal patients. The present analysis suggests that both age and cytogenetics are important strategies for risk stratification (outcome). Additional laboratory parameters should also be considered in childhood AML.

Keywords: Acute Myeloid Leukemia, chromosomal abnormality, FISH, MYH11/CBF G-banding

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Introduction

G-banding is a major cytogenetic technique that identifies acquired genetic alterations in hematologic malignancies. This technique detects a wide variety of genetic abnormalities, however sometimes it is not possible because of the requirement for dividing cells. Hence, normal cytogenetic results may be observed when submicroscopic changes are present. In addition, due to the presence of over condensation and poor chromosomal morphology, it is difficult to detect abnormalities by this technique. Hence, molecular analysis has gained importance in the management of leukemia patients, as it serves in identifying genetic abnormalities undetected by cytogenetic. Fluorescent in situ hybridization (FISH) is one such molecular technique.[1,2]

Acute myeloid leukemia (AML) is a hematological disorder with heterogeneous clinical and biological features. The current WHO classification of hematologic malignancies defines distinct entities of myeloid disorders based on the presence of recurrent cytogenetic abnormalities. This classification is mainly based on adult studies, whereas cytogenetic data is rare in pediatric AML.[3] Diagnostic clonal chromosomal abnormalities provide important prognostic information and are among the most important factors in predicting initial response to chemotherapy, duration of remission and overall survival.[4] In this category, t(8;21) and rearrangement of chromosome 16 result in disruption of genes that encode CBF subunits, α and β, respectively. Similarly, a fusion gene CBFβ/ MYH11 is produced by juxtaposition of bands in the 16(q22) locus of CBFβ and 16(p13) locus of MYH11 that results from inversion or, less frequently, translocation of t(16;16)(p13;q22). Prognosis of these patients is significantly better than patients with other chromosomal abnormalities or a normal karyotype.[5] However, it is difficult to identify del(16)(q22) or inv(16)(p13q22) in average or below average quality metaphases; such patients are placed into normal karyotype category by G-banding. Here we report the results of cytogenetic and FISH in childhood AML patients from Kidwai Memorial Institute of Oncology, a cancer treatment center from South India.

Materials and Methods

From 2009 to 2011, we collected heparinized bone marrow aspirates from 50 childhood AML patients who were between 1 to 14 years of age. Study approved by local ethics committee and informed consents signed. Aspirates were processed for G-banding and FISH analysis. Participants were patients at the Pediatric Oncology Department.

Cytogenetics (G-banding)

Bone marrow samples obtained from the patients were cultured in RPMI 1640 (Gibco, Invitrogen, USA) medium that contained 15% fetal bovine serum for 24 and 48 hours at 37°C. After incubation, the cells were exposed to colcemid (0.10 µg/mL, Gibco) for 30 minutes, followed by hypotonic treatment (0.075 M KCl) for 20 minutes, then were fixed in a 3:1 methanol:acetic acid so-

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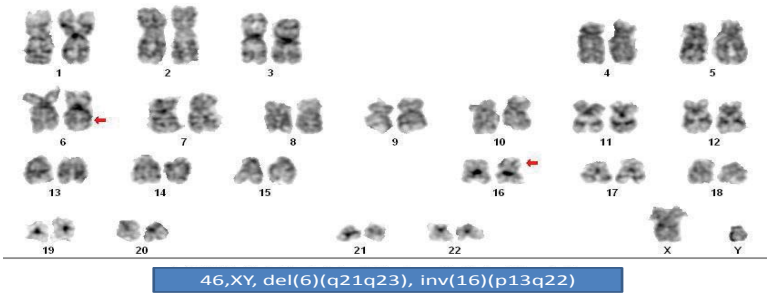

Figure 1. Karyotype of patient no.13.

Table 1. Karyotype details and morphology.

S. No	Cytogenetics findings	No. of patients	Morphology (FAB)
1	46,XX,t(8;21)(q22;q22)	7	AML - M2
2	46,XY, t(8;21)(q22;q22)	1	AML - M2
3	45,X,-X, t(8;21)(q22;q22)	2	AML - M2
4	45,X,-Y, t(8;21)(q22;q22)	4	AML - M2
5	45,XY, t(8;21)(q22;q22),-15	1	AML - M2
6	46,XY, del(6)(q23), t(8;21)(q22;q22)	1	AML - M2
7	46,XX, t(15;17)(q22;q21)	2	AML - M3
8	46,XY, t(15;17)(q22;q21)	2	AML - M3
9	46,XY,add(3)(p25), t(15;17)(q22;q21), i(17)(q10)	1	AML - M3
10	46,XX, t(16;16)(p13;q22)	1	AML - M2/M4
11	46,XX, del(16)(q22)	1	AML - M2/M4
12	45,X,-Y, del(16)(q22)	1	AML - M2/M4
13	46,XY, del(6)(q21q23), inv(16)(p13q22)	1	AML - M2/M4
14	48,XY, +6,+13	1	AML - M1
15	46,XX, der(9)t(9;11)(p22;q13)	1	AML - M2
16	47,XX, add(4)(q35), -13, +21,+mar	1	AML - M5
17	41,XX, t(1;22)(q13;p13), -3,-4,-9,-10,-19	1	AML - M7
18	46,XX, inv(3)(q21q26)	1	AML - M7
19	Ring chromosome	1	AML - M6
20	Normal Karyotype	16	M0, M2, M4
21	No metaphase	3	M0, M1

FAB = the French-American-British classification; AML= acute myeloid leukemia.

lution. The next day, slides were made and maintained at 60°C overnight. Chromosomes were subsequently treated with trypsin and stained with giemsa. Karyotypes were analyzed and interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN).[6]

Fluorescent in situ hybridization (FISH)

According to manufacture construction, the CBFβ/MYH11 Translocation Probe dual fusion (Cytocell aquarius-UK), the CBFβ labeled in red. It covers a 617 kb region of 16(q22) that extends from 298 kb 5′ of CBFβ to 246 kb beyond the 3′ terminus of the gene. The 610 kb region of 16(p13) is devoted to the MYH11 is showed in green. Air dried slides were prepared according to the cytogenetic technique from 23 bone marrow samples that had normal karyotype. Slides were washed in 2 × SSC for 2 minutes at room temperature followed by dehydration in an ethanol series (75%, 85% and 100%), each for 2 minutes, then allowed to dry. A total of 10 µL probe mixture del(16) was spotted on the cell sample. The coverslip was carefully placed on the probe area and the edge sealed with a rubber solution glue. The slides were allowed to dry completely. Slides were kept for denaturation in a hybridization chamber at 75°C for 2 minutes. Then, they were hybridized at 37°C overnight. The next day, the coverslip was removed, then a post-hybridization wash was performed with 0.4 × SSC at 72°C for 2 minutes followed by 2 × SSC and 0.05% Tween-20 at room temperature (pH 7). We applied 10 µL of DAPI antifade on the drained slides, which were covered with a coverslip. Slides were placed in the dark for 10 minutes to allow for color development, then they were viewed by the use of appropriate filters and FISH software. A minimum of 100 cells were scored for signals, whereas available metaphases were also captured with fluorescence microscope with SKY that was supported by Applied Spectral Imaging software (Olympus BX61- Japan).

Results

This study included 50 childhood AML patients, 24 males and

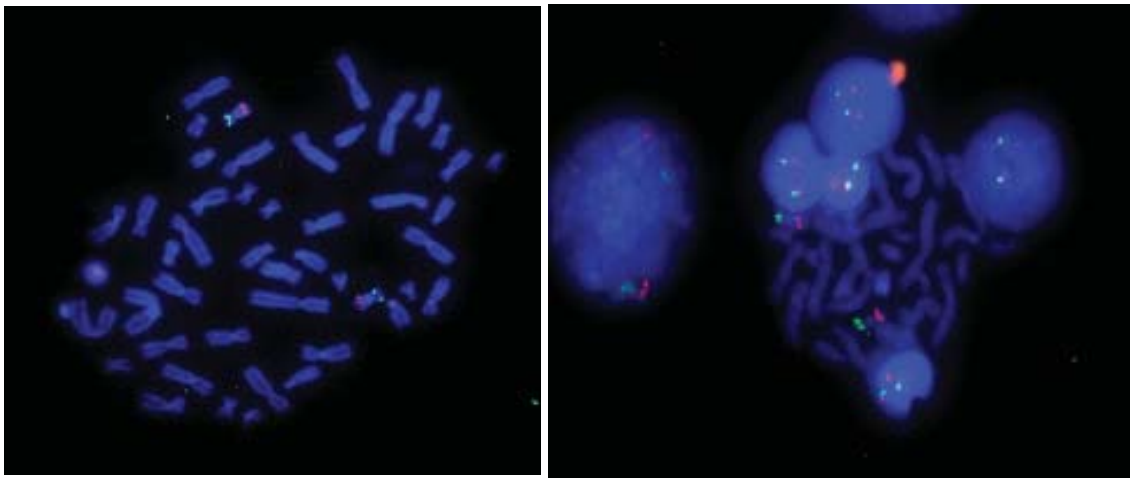

Figure 2. Metaphase of the control with two green and two red signals **(left).** Metaphase and interphase cells with two red and two green signals from an acute myeloid leukemia (AML) patient **(right)**.

26 females, who ranged in age from 1 to 14 years. The median age at diagnosis was 8.18 years. According to the French-American-British (FAB) classification, this study cohort consisted of AML M0, M1, M2, M3, M4, M5, M6, and M7.

Cytogenetics

The culture success rate was 94% with 31 (62%) patients who had abnormal karyotypes, 16 (32%) had a normal karyotype and no metaphase was observed in 3 (6%) patients. Karyotype details and patient morphology are shown in Table 1. Figure 1 shows the karyotype of patient 13 carrying inv(16).

Fluorescent in situ hybridization (FISH)

FISH was performed on 16 cytogenetically normal patients using the CBFβ/MYH11 probe. In all cells, these probes appeared as discrete red and green spots, which resulted in 2G 2R conformation that showed normal results for all 16 patients (Figure 2).

Discussion

The current WHO[7] classification of hematologic malignancies defines distinct entities of myeloid disorder based on the presence of specific cytogenetic abnormalities. The prognostic value of the major cytogenetic subgroups in childhood and adult patients with AML is internationally accepted.[8,9] Cytogenetic analyses are performed in most children with AML. It is reported that an increasing number of rare but recurrent aberrations have a prognostic value.[3] According to WHO[7] the AML category with recurrent genetic abnormalities comprises 60%–65% of all AML cases. The present study has determined that 56% of patients were in this category. More than 70% of pediatric patients show clonal karyotype events at diagnosis, hence the 62% seen in the present study was less than reported in the literature.[10] The incident rate of AML subtypes with recurrent cytogenetic abnormalities that lead to fusion gene formation is approximately 30% and mainly based on adult studies. Analysis of cytogenetic data in pediatric AML cases is rare; however the 52% result seen in the current study is greater than reported in the literature. Favorable karyotypes occur in a higher percentage in children than in adults. Grimwade et al.[11] have established a hierarchical cytogenetic classification as follows for favorable cytogenetics: t(8;21)(q22;q22); t(15;17)(q22;q21); and inv(16)(p13q22). The current study has shown the same results where t(8;21) comprised 51.6% of cases, t(15;17) comprised 16.1% and chromosome 16 abnormalities were seen in 12.9% of cases. In this series there were a higher number of cases in the good prognosis group. Those with poor prognosis had a cytogenetic classification of t(1;22)(q13;p13); inv(3)(q21q26); and t(9;11)(p22;q13), all were at a very low incidence. The prognostic value has yet to be determined in rare chromosomal abnormalities such as ring chromosome and add (4)(q35) with markers and numerical abnormalities such as trisomy 6 and 13.

The rearrangement of chromosome 16 is one of the most common cytogenetic aberrations found in AML-M2/M4, which includes del(16), t(16;16) and inv(16) of M4E0.[11] The original inversion (16)(p13 q22) has been first reported in six cases of AML-M0, M4 by Hogge et al.[12] This AML subgroup generally has a better prognosis in adults and children.[13] In the present study, according to G-banding, this category was identified in 4 (8%) cases which was comparable with a Western series (5.91%).[14] The lowest percentage was from Taiwan (3.91%)[15] and highest (11.6%) from China.[16] According to these results, we proposed that normal karyotype patients might have alterations in the core binding factor (abnormality of chromosome 16). The CBFβ/MYH11 probe by the FISH technique revealed that all 16 patients with normal karyotype were negative for this abnormality. Our results suggested that FISH was less useful for diagnostic studies of AML patients with normal cytogenetics. Larger studies might provide additional information to determine the MYH11/CBFβ gene rearrangement, outcome and better understanding of leukemiogenesis.

Conclusion

Up to knowledge of authors, this paper as the first cytogenetic study from South India suggests that both age and cytogenetics are important strategies for risk stratification (outcome). In addition, other laboratory parameters should also be considered in childhood AML. The high incidence of translocations other than other chromosomal abnormalities indicates that fusion genes are more frequent. Rare aberrations are partly related to the incidence of the different spectrum of genetic changes. Novel genetic changes may contribute to alternative risk stratification, which indicates a need for ongoing studies in this field. The study period was insufficient for survival assessment in this study. The authors strongly

believe that a larger study in routine cytogenetic analysis is required to reach a rational conclusion for prognostic stratification, planning of appropriate management and better outcome.

Larger studies may provide more information in elucidating the MYH11/CBFβ gene rearrangement, outcome and better understanding of leukemiogenesis.

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