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Research Paper

Distribution Pattern of Chromosomal Abnormalities in Pediatric Acute Myeloid Leukemia: A Study on 50 Case from S.india

Medical Science

KEYWORDS :

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ABSTRACT

A total of 50 pediatric Acute myeloid leukemia (AML)patients registered during the year 2009-2011 under 15 years of age were categorised into three groups and subjected for Cytogenetic and Fluorescence In Situ hybridization (FISH)study. 94% culture success rate was achieved. chromosomal abnormalities were detected in 32 patients(64%).The recurrent chromosomal abnormalities Viz., t(8;21)(q22;q22) in16 patients(50%), t(15;17)(q22;q21)in 5(15.6%) including a variant(15;17;22)(22;q21;q12) and del(16)(q22) /t(16;16)(p13;q22)/ inv(16) in in 4(12,5%) were observed. t(9;11), t(1;22), inv(3),which are rare abnormalities formed a small subgroup consists of three patients. The highest frequency of chromosomal abnormalities was observed in the age group of 10-15years. Chromosome 16 rearrangement was not detected by FISH in normal karyotype patients with CBFβ/ MYH11 probe. The frequency and prognostic value of the different karyotypes in the same group of patients were assessed.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease. AML accounts for approximately 25% of childhood cancer and its incidence is expected to increase as the population ages. AML is slightly more common in male, with a male-to-female ratio of 1.3:1. There is some geographic variation in the incidence of AML. Extensive cytogenetic analysis of the leukemias has demonstrated an association of recurrent chromosomal aberrations with the clinical and biological diversity of AML and provided insight into the genetic changes that underline leukogenesis. The strong association of diagnostic karyotype treatment with outcome, demonstrated in some large adult studies, has rendered cytogenetics the most valuable prognostic factor for treatment selection[1-4] The single most important prognostic factor in AML is cytogenetics. Certain chromosomal abnormalities are associated with very good outcomes Viz., t(8;21),t(15;17) and inv(16). Normal karyotype,+8,+21,+22,del(7)(q),del(9)(q) abnormalities of chromosome 11 fall into an intermediate risk group. A number of other chromosomal abnormalities, -5, -7, abnormal 3q, complex karyotype is known to be associated with poor prognosis and a high risk of relapse after treatment. However, for some less frequent, non- random aberrations, the cytogenetic prognostic classification is still inconsistent owing to the small number of patients and the variable treatment modalities used in different study groups[5]. The reported childhood AML series consist of a relatively smaller number of patients and show a different distribution of cytogenetic subsets and sometimes a different response to treatment from adult[6]. Moreover, geographic differences in the frequency of cytogenetic subsets have been noted in adult and childhood AML[7-9]. In view of the above, herewith we are presenting the cytogenetics and FISH data from our Institute.

Materials and methods

A total of 50 pediatric AML patients under 15 years of age presented at Out Patient Department of Pediatric Oncology in our Institution during the year 2009-2011 were selected for this study. Bone marrow aspirate in all these patients was evaluated for morphology, cytochemistry and cytogenetics. Molecular cytogenetics Viz,. Fluorescence In Situ hybridization **(**FISH) was performed on patients with normal karyotype. Other clinical information's were collected from case file.

Cytogenetics

Bone marrow samples from 50 patients were cultured for 24 and 48 hours in RPMI 1640 (Gibco, Invitrogen, USA) medium that contained 15% fetal bovine serum(Gibco) at 37 ° C . After incubation, the cells were exposed to colcemid (0.10 µg/mL, Gibco) for 20 minutes, then fixed in Cornoy`s fixative and refrigerated overnight at 4 ° C. Next day, slides were prepared and maintained at 60 ° C. G- Banding was performed by treating slides with trypsin and stained with Giemsa[10]. Metaphases were captured and analyzed by Image Analysis System (IAS). The karyotypes were interpreted according to International System for Human genetic ISCN (2009)[11].

Fluorescence In Situ hybridization (FISH)

FISH was performed on 15 patients with normal karyotype. Air dried slides were prepared from cytogenetically fixed cells then washed in 2x SSC(Saline-Sodium Citrate)buffer for 2 minutes at room temperature followed by dehydration in an ethanol series and then allowed to dry. 10µl of probe mixture (Cytocell Aquarius- UK) of CBFβ/ MYH11 probe for chromosome 16 rearrangement of AML were spotted on the cell sample. Coverslip was placed on the probe area and sealed with rubber solution glue and allowed to dry completely. The slides were denatured at73 ° C for 5 minutes and hybridized at 37 ° C, for 18hours in hybridization chamber followed by removal of coverslip and post hybridization in 2 x SSC, 0.05% Tween-20. DAPI (10µl) was applied on drained slides and coverslip was placed. Slides were viewed with the use of appropriate filters and FISH software (Zeis Axiophate fluorescence microscope). Minimum 200 cells were scored for signals and captured.

Results

The male- female ratio was 1: 1 (25/25). The median age at diagnosis was 8.18 years (from 1 - <15 years). Diagnosis was confirmed by clinically, morphology, cytochemistry and patients were retrospectively classified in to subgroups. We have categorized patients into three groups A, B and C based on the age. The patients less than 1 to 5 years of age constitutes group A, B from 6-10 years and C from 11 to15years. Group A, B and C con-

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sists of 16, 14 and 20 patients respectively (fig-1).Chromosome abnormalities were more in age group C (Fig.2).

The culture success rate in the present study was 94%. The abnormal karyotype was observed in 32 (64%), normal karyotype in 15 (30%) and no metaphase in 3(6%) patients respectively. The available chromosomal abnormalities were tabulated (Table-1), and their incidental rate has been depicted (Fig. 3).

Among recurrent structural chromosomal abnormalities, translocation and deletion were more commonly seen. The most frequent chromosomal abnormality t(8;21)(q22;q22) was observed in 16 Patients(50%) who were classified as FAB-M2(commonly used French American British classification)subtype with or without Aur rods by morphology.

The second most common abnormality observed was t (15; 17) (q22; q21) in 5 patients (15.6%) of AML-M3 (APML**.** Among five, classic t(15;17) was seen in four, and a variant translocation with involvement of Chromosome 22 in fifth patient (fig-4).

Rearrangement of chromosome 16 of AML-M2/M4 was observed in four patients, which includes del(16)(q22) in two t(16;16) (p13;q22) in one and inv(16) in another. Secondary abnormalities like del(6q) was observed along with inv(16)(p13q22) and t(8;21)(q22;q22).

Among three patients of AML M5 subtype, two (6.2%) were carrying t(9;11)(p22;q23), one with trisomy 9 and another with trisomy 8. The third one revealed a novel chromosomal abnormality with a complex karyotype consisting of add(4)(q35) and del(13)(q14) and trisomy 21. In M7 subtype t(1;22)(p13:q13) in one-year old female and (3)(q21q26) in another patient was observed.

FISH was performed on 15 patients who were cytogenetically normal applying CBF/β MYH11 translocation probe to detect chromosome 16 rearrangement. In all cells, these probes appeared as discrete red (MYH1) and green (CBF_{β}) spots, resulting in 2G 2R conformation showing normal result in all 15 patients as well as control(Fig.%). Other laboratory parameters were collected from case file.

Discussion

The current WHO classification of hematological malignancies defines distinct entities of myeloid disorder based on the presence of specific cytogenetic abnormalities (WHO 2008). The prognostic value of the major cytogenetic subgroups in childhood and adult AML patient is internationally accepted. A male preponderance in AML patients was observed in the previous study. However, equal number of male and female was observed in this study, although this study accepts that the sample size is small. Majority of the patients were seen in group C and the number of chromosome abnormalities also seen more in this group compared to the other groups. This suggests that the disease incidence increase with age. It may be due to exposure to environmental mutagens and accumulation of genetic alterations, which attract the attention of a clinician.

According to WHO, the category of AML with recurrent genetic abnormalities comprises 60- 65 % of all AML. Chromosomal abnormalities (60% recurrent and 4% novel) in this study are consistent with those reported in other pediatric studies[12]. The incidence rate of AML subtypes with recurrent cytogenetic abnormalities that lead to fusion gene formation is about 30%, mainly based on adult studies whereas analyses of cytogenetic data in pediatric AML are rare. The translocation leading to fusion gene is 52% of present study found to be higher than literature. Favourable karyotype occurred in a higher percentage in children than in adults[13,14].

The t(8;21)(q22;q22) (ETO-RUNX1) is morphologically associated with FAB-M2 in approximately 90% of patients and far less commonly in FAB-M1 (6%)[15,16].In the present study, t(8;21)(q22;q22) FAB-M2 subtype (32%)were the most frequent and higher rate of incidence when compared to UK, USA and Nordic series [6,13]. Data from ethnic Omani population shows that AML-M2 subtype with t(8;21)(q22;q22) is 18%[17], from Pakistan 26.6%[18], from Turkey 25.7% [19] and from Israel 23%[13]. Loss of sex chromosome and secondary abnormalities are not influencing the prognostic value of t(8;21)(q22;q22). Grimwade[3] reported from analyzing 421 patients that additional cytogenetic abnormalities did not adversely affect the outcome in t(8;21)(q22;22) CBF leukemia in contrast to previous reports that suggested a negative impact for del(9q)[20], complex karyotype[21]or loss of Y chromosome in male subjects[22]. In present study, loss of sex chromosome, loss of chromosome 15 and del(6)(q23) as an additional abnormality did not influence the prognosis negatively, because these patients' performance was on par with patients of t(8;21)(q22;q22) as sole abnormality.

The t(15;17)(q22;q21)(PML-RARA) is specific to FAB-M320. The lowest frequency of t(15;17) (3%) was reported from Nordic series[6] and a higher one (17.1%) from Italy[8] when compared to Israel 8-11%[13] and the present study(10%). The t(11;17)(q23;q21), t(11;17) (q13,q21) and t(5;17)(q23;q21) are known as variants of t(15;17)(q22;q21). Patients with variant t(15;17) (q22;q21) initially have been reported as having APL morphologically, however, morphological differences exist which is clinically important, t(5;17)(q23;q21) seems to respond to ATRA, while APL variant with t(11;17) does not[23]. Interestingly, in the present study a variant translocation with third chromosome involvement t(15;17;22)(q22;q21;q) was observed in morphologically AML-M3 (hypergranular variant type) in one-year-old female. The clinical significance of this variant was not clear and the patient is under follow up.

Around 7-10% of AML show inv(16)(p13q22) or t(16;16)(p13;q22), most of the patients are associated with the typical morphology of FAB subtype of M4e0 with good prognosis. At the molecular level, inv(16) is characterized by a reciprocal rearrangement of CBF_{β} gene on 16(q22) and MYH11 on 16(p13)[24,25]. The del(16)(q22) also reported in literature and its molecular changes remain unclear[26]. In the present study, two patients with del(16)(q22) were observed in AML-M4 type. The incidental rate of chromosome 16 rearrangement in the present study(8%) is almost similar to other studies of from USA[13], UK and Nordic childhood series that is 5-8%.

These are tiny abnormalities and difficult to identify cytogenetically in poor quality and over condensation of chromosomes ,hence, remains undetected. Using CBF_{β} / MYH11 probe by FISH technique revealed that all 15 patients with normal karyotype were negative for this abnormality. Stack[13] also reported the same by FISH technique in patients with normal or miscellaneous aberrations was found negative for inv(16). Mazloumi[28] explained that despite the possible effect of methodological difference in the incidence determination of normal karyotype, no chromosomal

abnormality was detected by a comprehensive FISH panel in 55 de novo AML adult patients with a normal karyotype, suggesting a true normal karyotype group. Other internal molecular rearrangements cannot be ruled out such as FLT3 and MPM mutation. However, larger studies may provide more information to know the CBF_{β} / MYH11gene rearrangement outcome and better understanding of leukemiogenesis in this category.

The effect of additional abnormalities in the prognosis of chromosome 16 rearrangements is not yet known. In the present study patients with interstitial del(6)(q23q25) along with chromosome 16 rearrangement are doing well, and both are good prognostic indicators.

Grimwade[3] established a hierarchy cytogenetic classification; favourable cytogenetic abnormalities include t(8;21)(q22;q22), t(15;17)(q22;q21) and inv/t(16;16)(q22;p13). In the present study also the same hierarchy maintained with 32%, 10% and 8% respectively, indicating that good prognostic group is more in this geographical area. The backbone of the hierarchy considers translocation more important than deletion.

The t(9;11)(p22;q23) which leads to the MLLT3- MLL gene fusion, and it is now recognized as a distinct disease entity in the WHO classification[29], was found to have a relatively favourable outcome in accordance with the majority of studies[22,30]. The overall outcome for patients with t(9;11)(p22;q23) was significantly worse than those with normal karyotype. The prognostic significance of MLL rearrangement in childhood AML is currently controversial: the outcome of patients with t(9;11)(p22q23) was favourable in the Nordic society for pediatric hematology and oncology studies**,** and it is an intermediate- risk group from BPM98 trials[12]. However, the chromosomal partner of MLL gene was observed to have an important bearing upon prognosis[3]. In the present study two hyperdiploid patients revealed t(9;11)p22;q23), one with trisomy 9 and another with trisomy 8. The influence of additional numerical abnormality on the prognostic value of t(9;11)(p22;q23) is yet to be evaluated.

The close association of trisomy 21 and t(1;22)(p13;q13)(OTT-MAL) with AMKL(acute megakaryoblastic leukemia), both characterized by early onset of the disease, could account for the younger age at diagnosis reported in some series[31]. In present study the occurrence of this abnormality in lower age group and its lower frequency is similar to the literature[31]. The inv(3)(q21q26)) and t(3;3)(q21;q26) (RPN1- EVI1), occurs primarily in adults but rarely reported in children. The inv(3)(q21q26) with an increase in the number of megakaryocytes which are morphologically AML-M7 showing poor prognosis[2,3]. In this study, a 13-year-old female was showing inv(3)(q21q26).

Nevertheless, informed clinical decision making in situations in which cytogenetic analysis shows rare karyotypic abnormalities has been hampered by a lack of consensus regarding the likely outcome of such patients. Apart from the benefit of achieving greater consensus in cytogenetic classification, establishing the outcome associated with rarer cytogenetic abnormalities is important. In the present study, two novel karyotype were observed, one with trisomy 6 and 13 in a 12 year-old male and add(4)(q35), del(13)(q14), trisomy 21 and a marker in a 3 year-old female. Because of their novelty and rarity, the role of them in AML pathogenesis and response to therapy are not clear.

In this study, children with t(9;11), t(1;22), inv(3), rare and novel abnormalities formed a small subgroup. Because of a short study period, their treatment outcome could not be assessed. As in other childhood series, the rare Philadelphia chromosome, t(6;9), high risk cytogenetic category like monosomy 5 and 7, del(5) and del(7) were not detected.

Review of literature revealed that some types of leukemias were more common, in particular, geographical region. The geographical variation in the incidental rate of chromosomal abnormalities also reported. The differential rate of chromosomal abnormalities draws attention of researchers to probe it further. However, such studies should be more comprehensive in a larger series with a higher culture success rate, complete evaluation of normal karyotype and poor morphology by FISH or other molecular techniques from consecutive patients.

Table-1: showing distribution pattern of childhood AML in different age groups.

S.N0	Cytogenetic finding	Total No.	Age group		
			0-5	6-10	11-15
1	t(8;21)(q22;q22)	16	**6**	**7**	**3**
2	t(15;17)(q22;q21)	5	-	**1**	4
3	del(16)(q22)	3	-	**1**	**2**
4	t(16;16)(p13;q22)	1	-	-	**1**
5	inv(16)(p13q22)	1	-	-	**1**
6	48,XY,+6,+13	1	-	-	**1**
7	t(9;11)(p22;q13)	1	-	1	-
8	Add(4)(q35)	1	**1**	-	-
9	t(1;22)(p13;q13)	1	**1**	-	-
10	inv(3)(q21q26)	1	-	-	**1**

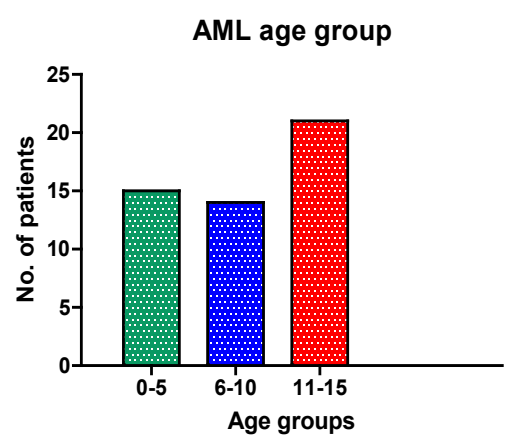

Figure-1: Graph showing distribution of patient of AML in different age groups.

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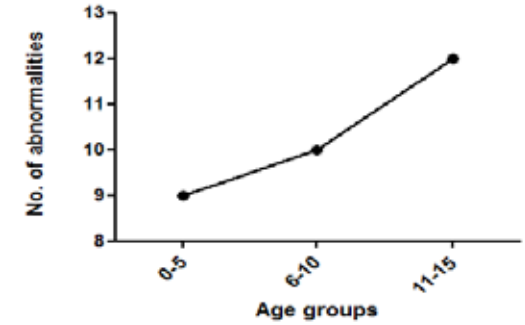

Figure-2: Graph showing distribution of kinds of chromosomal abnormality in different age different age group.

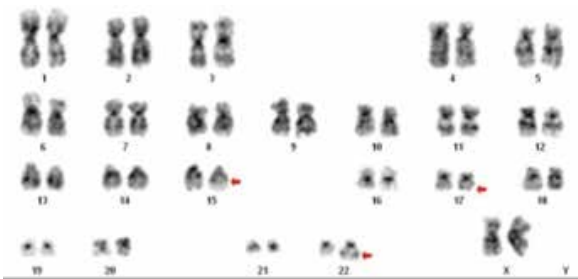

Figure-3:Karyotype: 46,XX,t(15;17;22)(q22;q21;q11)

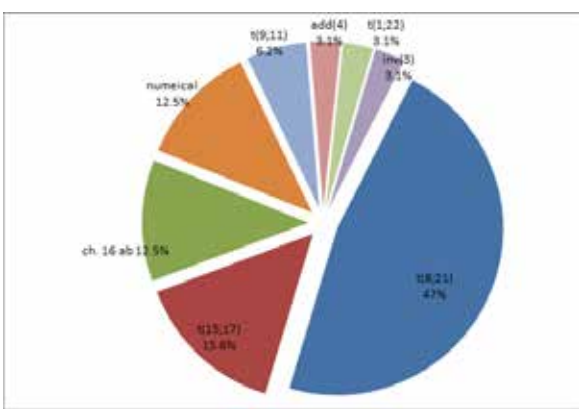

Figure-4: Graph showing incidental rate and distribution pattern of chromosomal abnormalities in AM

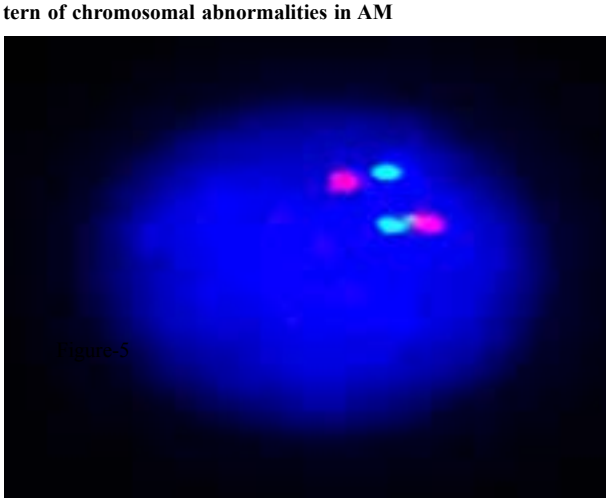

Figure-5

Figure-5: FISH result of CBF-MYH11, interphase cell showing 2 red (CBF/β) and 2 green (MYH11) gene.

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