Study on Acute Leukemia and their Karyotypic Abnormalities in Children

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Abstract

Malignant proliferative diseases affecting the white cell population results in acute or chronic leukemias and lymphomas. There are several categories of acute leukemias based on the cell of origin and are broadly divided into lymphoid leukemias and myeloid leukemias. Acute lymphoid leukemias are very common in children while acute myeloid leukemias are more often seen in older children and young adults. To analyse the morphologic subtypes of acute leukemias in children using cytochemistry. To determine the type of acute leukemia and associated chromosomal abnormality, to assess the chemotherapeutic response of acute leukemias occurring in paediatric age group, to assess the prognostic significance of age, sex and leucocyte count in acute leukemias in children. Out of 45 cases of acute leukemias there were 33 cases of ALL and 12 cases of AML. Acute myeloid leukemias showed a wide range of chromosomal abnormality, interestingly the acute myeloid leukemias was found to be on rise in the present study.

Keywords: Acute Leukemias; Acute Lymphoid Leukemias; Acute Myeloid Leukemias.

Introduction

The nature of abnormal (ph') chromosome is clarified in 1977 by Rowley who reported that Philadelphia chromosome is a translocation between chromosome 9 and 22. In the present, there is vast literature on chromosomal abnormalities in association with leukemias. Several international workshops on chromosomes in leukemias were conducted in 1981, 1983, 1984.

In 1973, Roulay first described a balanced translocation between chromosomes 8 and 21 t (8;21). This abnormality has been found to be the most frequent abnormality in children with AML being reported in 10 (17%) of 60 karyotypically abnormal cases. This abnormality can be seen in AML - M2 and AML - M4. Of the cases reviewed at fourth international workshop 70% of males with t (8;21) were also showing loss of Y chromosomes and 60% of females had loss of one X chromosome. This association is noteworthy because sex chromosomal abnormalities are otherwise rarely observed in AML. AML - M2 subtype with the t (8;21) has a favourable prognosis in adults. The median age of these patients is approximately 25-30 years. In contrast a recent survey suggests that the long term prognosis for children with t (8;21) is
very bleak. Arthus and bloom elddescribed ve
cases(withAML-M2and2withAML-M)in
bone marrow contained an increased number of
eosinophils; all five patients were reported to have
delated chromosome 16, del (16). Among AML -
M4 cases in the university of Chicago series (23%)
have an inv(16) or t (16:16). The median survival
of all 32 patients was longer than 66 weeks and
the median survival of those 25 patients who had
a complete remission was longer than 104 weeks.
In 1980 Berger and Bernhiem first reported higher
than expected frequency of abnormalities of the
long arm of chromosome 11 (11q) in 10 patients
with AML - M5. At the fourth international
workshop on chromosomes in leukemia (1984), the
association between 11q abnormalities in AML -
M5 and young age were confirmed. One common
translocation in infants, the t (4:11) usually had a
lymphoblastic phenotype, although the leukemic
cells may express some myeloid surface makers in
some cases variable number of monocytoid blast cell
have been identified. In 1973, Sakurai and Sandberg
demonstrated the correlation between karyotypic
abnormalities with the patient survival. They
showed that patients with only normal metaphase
cells had a longer survival (11.5 months) than did
patients who had a mixture of normal and abnormal
metaphase cells (AN, 10.3 months) or those who had
only abnormal metaphase cells (AA, 3.2 months).
the fourth international workshop demonstrated
that specific chromosomal abnormalities are
independent predicates of response to therapy.
In 1988, Samuel correlated the specific chromosomal
abnormalities observed in 149 patients with AML
who were treated with modern intensive induction
chemotherapy with drug susceptibility in vivo. Patients with t (8:21), inv (16)/t (16:10) or 11q
abnormalities had high rates of complete remission
periods. Those patients with normal karyotypes
had an intermediate pattern of response.

Materials and Methods

The present study of acute leukemias is taken
up in Niloufer Hospital, Hyderabad, India. Which
is a 350 beded paediatric center for medical and
surgical diseases. The study undertaken during Oct
1999 to Oct 2000. A total number of 52 cases were
diagnosed on preliminary examination as having
acute leukemia.

Every child having splenomegaly, petechial
haemorrhages, lymphadenopathy and pallor
which are associated with fever and malaise
are screened using the basic parameters such
as haemogram, Bleeding time, Clotting time,
Prothrombin time, Platelet count etc. Only children
who are provisionally diagnosed as having acute
leukemias are subjected to bone marrow aspiration,
cytochemistry and cytogenetic analysis. Site for
bone marrow aspiration was either iliac crest or
sternum, based on the age of the child. In some of
the acute leukemia cases due to hypercellularity
of marrow, he aspiration result was a “dry trap”.
In such cases a bone marrow biopsy under short
general anesthesia is performed. Imprints of
these biopsies were immediately subjected to
cytochemical staining as per the routine procedure.
The detailed clinical history, family history, details
pertaining to consanguinitu etc. are recorded in the
given format. Haemogram sample are collected
using EDTA and the peripheral blood smears are
stained by Leishman stain. For differentiation of
acuteleukemias cytochemical stain such as Periodic
Acid-Schiff, Sudan Black B are used routinely (Acute
Lymphoblastic Leukemia -Sum types L1, L2, L3 &
Acute Myeloid Leukemia -Sub typts M0, M1, M2,
M3, M4 & M5 can be detected using Periodic Acid-
Schiff, Sudan Black B and Non Specific Eterase). Non
-Specific Estraestain is done selectively wherever
the clinical picture and the Haemogram findings
were in favour of AML-M4 or M5 as the patients
attending the Niloufer Hospital, Hyderabad, India
are usually Low Middle Class and could and could
not afford the facility of Cytochemistry.

Cases of suspected congenital anomalie such as
Fanconi’s anemia associated with leukemia, the
X-rays are also taken. The clinical photographs of
children presenting with various types of acute
leukemias are also included in the present study
for sake of completion. The diagnosis of various
types of acute leukemias is based on the results
of haemogram and cytochemistry using FAB
classification. For cytogenetic analysis in all these
cases the peripheral blood sample only is used.

Results

The present study was carried out in Niloufer
Hospital, Hyderabad, India. Which is a center for
managing medical & surgical diseases in children.
All the 45 clinically suspected Acute Leukemia
cases were further investigated for confirmation.
Most of the patients (>90%) presented with fever,
pallor and petechiae. A total of 45 acute leukemias
were diagnosed on preliminary investigation.

The 45 patients out of 52 cases of Acute
Leukemias who were hospitalized at Niloufer
Hospital, Hyderabad, India were found to have the
following incidence of Acute Lymphoid Leukemia and Acute Myeloid Leukemia.

A single case of ALL-L2 child had CNS involvement at the time of admission. Organomegaly was more often seen in ALL than in AML. Gum bleeding was seen in AML-M3. Only one case of AML-M4 was noted to have soft tissue involvement in which patient presented with proptosis, as shown in Table 1.

Acute Lymphoid Leukemias was found to be the common type in childhood. Out of 45 cases, 33 cases (73.33%) were diagnosed as ALL, as presented in Table 2.

On the basis of FAB classification criteria, ALL’s were further sub classified in the present study as L1, L2, L3 subtypes. L1 sub types constituted 15 cases (45.45%) and L2 constituted 18 cases (54.55%). L3 was not found in the study. Acute Myeloid Leukemia constituted 12 cases (26.64%) of childhood acute leukemias. Most common subtype noticed in the study was AML-M3 (5 cases) followed by AML-M2 (3 cases), AML-M4 (2 cases) and AML-M1 (2 cases). Other variants were not

### Table 2: Showing types of Acute Leukemias & their incidence.

<table>
<thead>
<tr>
<th>Type of Acute Leukemia According to FAB Classification</th>
<th>No. of Cases</th>
<th>Age of the Patient (Age Range in Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL-L1</td>
<td>15</td>
<td>3-12 yrs</td>
</tr>
<tr>
<td>ALL-L2</td>
<td>18</td>
<td>10 months - 11 yrs</td>
</tr>
<tr>
<td>AML-M1</td>
<td>02</td>
<td>7-9 yrs</td>
</tr>
<tr>
<td>AML-M2</td>
<td>03</td>
<td>6-10 yrs</td>
</tr>
<tr>
<td>AML-M3</td>
<td>05</td>
<td>3-7 yrs</td>
</tr>
<tr>
<td>AML-M4</td>
<td>02</td>
<td>3-5 yrs</td>
</tr>
</tbody>
</table>

The Presenting Symptoms were different in ALL and AML in Table 1:

**Type of Acute Leukemia**

<table>
<thead>
<tr>
<th>Type of Acute Leukemia</th>
<th>ALL (%)</th>
<th>AML (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Tenderness</td>
<td>25 (75.75%)</td>
<td>02 (16.66%)</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>21 (63.63%)</td>
<td>03 (25.00%)</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>20 (60.60%)</td>
<td>07 (58.33%)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>16 (48.48%)</td>
<td>04 (33.33%)</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>01 (3.33%)</td>
<td>00 (0.00%)</td>
</tr>
<tr>
<td>Soft tissue involvement</td>
<td>00 (0.00%)</td>
<td>01 (8.33%)</td>
</tr>
</tbody>
</table>

The Presenting Symptoms were different in ALL and AML in Table 1:
encountered in our study. One case of Fanconi’s anemia in a child of 6 years was found to have pancytopenia on peripheral smear study. The bone marrow aspiration was a dry tap. The bone marrow biopsy revealed hypocellular marrow with increased fat spaces. Followup of this case revealed the child developing acute myeloid leukemia (AML-M2) which was confirmed by repeat bone marrow aspiration and cytochemistry. Cytogenetic study was not undertaken as the patient was on treatment with steroids. Child expired of haemorrhagic diathesis weeks after the hospitalization.

Following are the abnormalities noted. As good banding pattern is difficult to achieve in Acute Leukemias, the Translocation could not be assessed in the present study and also FISH technique was not available for banding of chromosomes in this study. Chromosomal abnormalities found in ALL’s, mentioned in table 3.

Out of 33 cases 20 cases showed normal study, 8 cases showed pseudodiploidy, 2 cases hyperdiploidy and 3 cases hyperdiploidy. One case of ALL L₁ showed Monosomy 3, 22 & with total absence of chromosomes 6ⁿ pair. Another case of ALL L₂ showed monosomy 22. A case of ALL L₁ showed monosomy 9.

Acute myeloid leukemia & associated chromosomal abnormalities

In our study out of 45 cases of acute leukemias, 12 cases were AML. Cytogenetic analysis showed 2 cases of AML M1 with -5q del. 1 case of AML M2 showed monosomy 5, 6, 7, 9, 16, 17, -18 and -Y. 2 cases of AML M3 showed random chromosomal structural abnormalities i.e. 1 case showed -3, -6, -8 and -Sex chromosome and showed monosomy 4, 5, 9, 10, 12, 17, 18, 21, 22 as printed in table 4.

The other case of AML M3 showed monosomy 3,13, and loss of Sex Chromosomes.

Discussion

The present study pertains to 45 cases though 52 acute leukemia cases were diagnosed in the pathology laboratory of Niloufer Hospital. As mentioned earlier, out of the 52 cases four patients absconded from the hospital after the initial diagnosis of acute leukemia on the haemogram.
3 children in whom the diagnosis was acute leukemia on hemogram, expired during the first few hours after admission secondary to bleeding diathesis. Amongst the three children who expired one was ALL-L2 and the other two were AML-M2 and M3 respectively.

For these above mentioned reason 45 cases were further analysed for which the hemogram, bone marrow aspiration and cytochemistry along with all the relevant clinical data are available. Out of 45 cases, 33 cases had been diagnosed as acute lymphoid leukemia. The subtype noticed were ALL L1 and L2. ALL L3 was not seen.

Chromosomal abnormalities in relation to ALL in present study were 33 cases out of which 20 were normal 2 were hyperdiploid, 8 were pseudodiploid and 3 were hypodiploid. In present study the hyperdiploid is seen in case of trisomy 21 with ALL-L1. ALLs were associated with monosomy 3 in one case, monosomy 9 in one case and monosomy 22 with total absence of chromosome 6th pair in one case. There was also a case of hypodiploidy with 26 number of chromosomes in a ALL-L2 patient. Another interesting feature was 2 case of ALL L2 having hyperdiploidy with 54 chromosomes showed very early remission on chemotherapy on par with ALL L1 (trisomy 9 & 10).

Acute lymphoid leukemias were also found to be associated with other chromosomal abnormalities such as deletions, translocations and other structural abnormalities which were not seen in this study.

In the present study chromosomal ploidy in cases of acute lymphoid leukemias showed hyperdiploidy, pseudodiploidy, which had good prognosis when compared to hyperdiploidy. In this study 2 cases were hyperdiploidy, 8 cases were pseudodiploidy and 3 cases were hypodiploidy.

In review of literature James A. Whitlock and Pan C.H mentioned similar outcome with ploidy study. Out of 45 cases, 12 cases had been diagnosed as acute Myeloid Leukemia. The various subtypes noticed in the study were AML M1, M2, M3 and M4. Other sub-types were not noticed.

According to Heim and Mitelman 1986 almost 1/5th of AML patients with identifiable abnormalities had a simple numerical aberration as their only cytogenetic abnormality and none of the numerical rearrangement was restricted to any particular FAB group. The mos common primary numerical arrangements were +4, -5,-7,+8,+21 & -Y.

Children with AML only rarely had the -7/7q- and -5/5q- changes seen so frequently in secondary AML, indicating that pathogenetic factors other than exposure to mutagenic agents may be of decisive importance in childhood leukemias.

According to Hiem & Mitelman 1986 main conclusion to emerge from a recent quantitative review of the secondary chromosome aberration in acute leukemias was that chromosome 1, 7, 8, 9, 21, X & Y were preferentially involved.

Of the 12 cases of AML in present study 2 cases were of AML-M1 subtype, during followup one case had remission, the other case expired. 3 cases were encountered in AML-M2 category, there were two deaths reported, one being Fanconi’s anemia which progressed to AML-M2 and the other case is on consolidation therapy.

Among the AML-M3 subtype recorded highest number of cases and also carried worse prognosis in children below 5years of age. 3 deaths were being reported during our study with one on remission and another on treatment. Most of these deaths occurred secondary to heamorrhagic diathesis (DIC).

In AML-M1 category there were two cases both of which had bad prognosis. One had soft tissue involvement and other case showed hyperleucocytosis and CNS manifestation.

In present study, chromosomal studies of acute myeloid leukemia, two cases of AML-M1 with structural abnormalities of - 5q del and 1 with monosomy 5, 6, 7, 9, 16, 17, 22, -18 and -Y. 2 cases of AML-M3 showed monosomy 4, 5, 9, 10, 12, 17, 18, 21, 22, -3, -6, -8 and -sex chromosomes, another case showed monosomy 3, 13, -sex chromosomes.

Summary

Heamatologic and karyotypic study was done on 45 cases. 52 cases of acute leukemia were diagnosed on heamatologic workup but for various reasons explained the detailed karyotypic study with relation to heamatologic findings was possible in 45 cases only. Children in the age group of 10 months to 11 years were diagnosed as having acute leukemia. The peak age for ALL was 2-6 years whereas equal incidence were seen for AML in age range of 2-8 years. In both the types of acute leukemias (ALL & AML) there was male predominance. In all the cases at the time of diagnosis there was full blown leukemia picture (with >30% blasts) in peripheral blood was used for karyotyping. It was found that leucocyte burden (>12000) per cu.mm was associated with poor prognosis in both AML and ALL. Organomegaly
and lymphadenopathy were a common feature (more than 50% cases) of ALL. Out of 45 cases of acute leukemias, there were 33 cases of ALL and 12 cases of AML (M1, M2, M3, M4 subtypes). A single case of fanconi’s anemia was diagnosed who on followup developed AML M2 and expired. Among the karyotypic abnormalities acute lymphoid leukemias showed 2 cases of hyperdiploidy, 8 cases with pseudodiploidy ans 3 cases of hypodiploidy. One case of ALL showed monosomy 3 and 22 with complete loss of 6th chromosome. Acute myeloid leukemias showed a wide range of chromosomal abnormalities not related to FAB classification of AML. The procedure for karyotyping, permitted good cell cultures prior to chemotherapy and not in post chemotherapy cases. Hence follow up of cases in remission and relapse was done based on hemogram and cytochemistry only and not on karyotyping. Interestingly the acute myeloid leukemia were found to be on rise in the present study(AML M1-2 cases, M2-3 cases, M3-5 cases, M4-2 cases).

References

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