IJPRP

Indian Journal of Pathology: Research and Practice
Volume 7 Number 9, September 2018
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.7918.9

Original Research Article

Study on Acute Leukemia and their Karyotypic Abnormalities in Children

Mohd Irshad Ahmed[1], C.R.H.T. Pavani[2]

[1]Associate Professor, Department of Pathology, Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga, Kalaburagi, Karnataka 585104, India. [2]Professor, Department of Pathology, Osmania Medical College, Hyderabad, Telangana 500095, India.

Corresponding Author:
Mohd Irshad Ahmed, Associate Professor, Department of pathology, Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga, Kalaburagi, Karnataka 585104, India.
E-mail: mohdirshad1156@gmail.com
Received on 05.09.2018,
Accepted on 25.09.2018

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 field described five cases (3 with AML - M2 and 2 with AML - M4) in bone marrow contained an increased number of eosinophils; all five patients were reported to have a deleted 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 predicators 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, lymphoadenopathy and pallor which are associated with fever and malaise are screened using the basic parameters such as heamogram, 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 typs M0, M1, M2, M3, M4 & M5 can be detected using Periodic Acid–Schiff, Sudan Black B and Non Specific Eterase). Non –Specific Estrase stain 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.

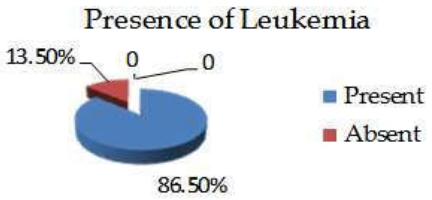
The 45 patients out of 52 cases of Acute Leukemias who were hospitalized at Niloufer Hospital, Hyderabad, India were found to have the

following incidence of Acute Lymphoid Leukemia and Acute Myeloid Leukemia.

A single case of ALL – L_2 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 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 this 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

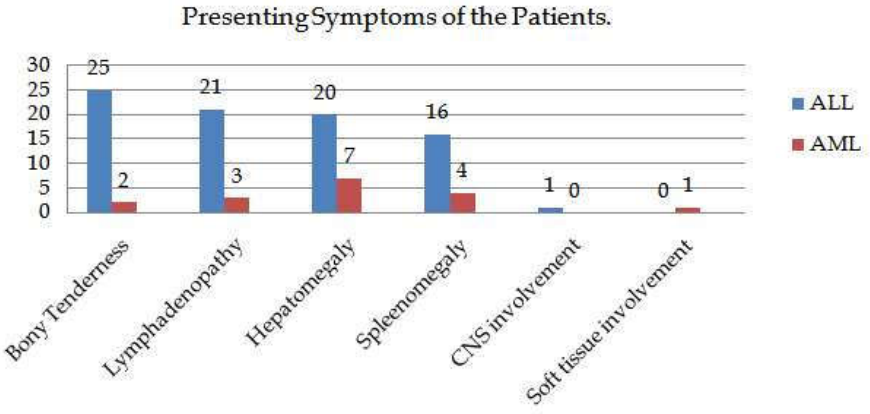


The Presenting Symptoms were different in ALL and AML in Table 1:

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

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

Type of Acute Leukemia According to Fab Classification	No. of Cases	Age of the Patient (Age Range in Years)
ALL-L1	15	3-12 yrs
ALL-L2	18	10 months - 11 yrs
Aml-M1	02	7-9 yrs
AML-M2	03	6-10 yrs
AML-M3	05	3-7 yrs
AML-M4	02	3-5 yrs



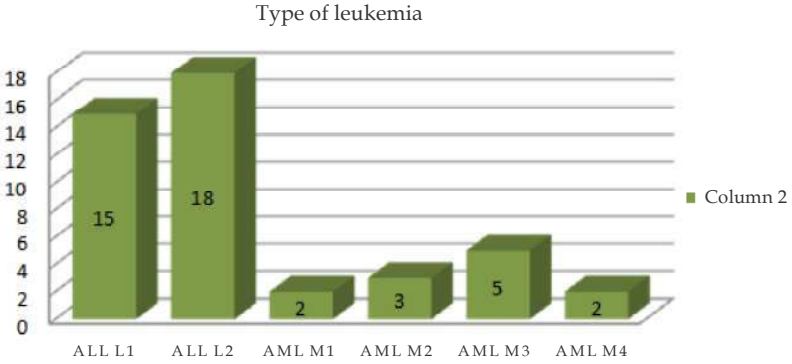


Table 3: showing chromosomal abnormalities in ALL.

Type of Leukemia	Chromosomal Abnormalites				
	Normal	Hyperdiploidy	Pseudodiploidy	Hypodiploidy	Marker Chromosomes
L1	12	2	-	1	6
L2	8	-	8	2	3

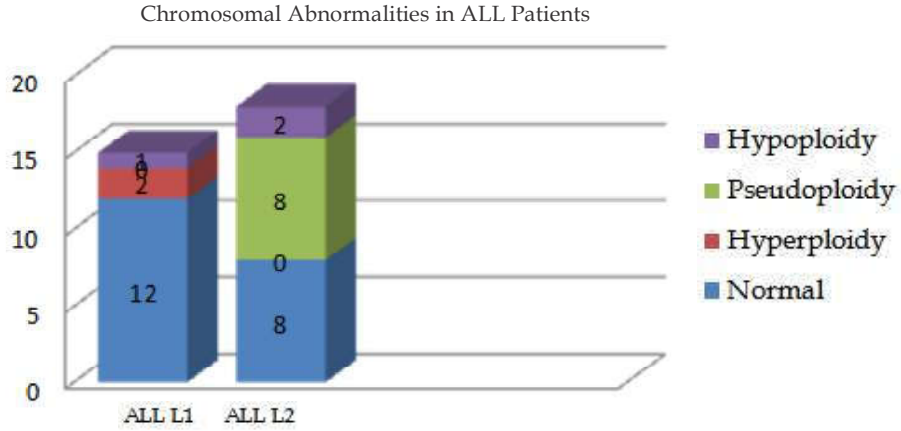


Table 4: showing chromsomal abnormalites in AML

Type of Leukemia	Chromosomal Abnormalities	Marker Chromosome
M1	-5q (2 cases)	-----
M2	Monosomy 5,6,7,9,16,17,22,-18,-Y	10
M3	Monosomy 4,5,9,10,12,17,18,21,22 & -3,-6,-8,-Sex Choromosomes	16
	Monosomy 3,13,-Sex Chromosomes	2
M4	------	-----

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 hypoplastic 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 6 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 pseudodiplody, 2 cases hyperdiploidy and 3 cases hyperdiploidy. One case of ALL L_2 showed Monosomy 3, 22 & with total absence of chromosomes 6thpair. Another case of ALL L_2 showed monosomy 22. A case of ALL L_1 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 heamogram.

3 children in whom the diagnosis was acute leukemia on heamogram, 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 heamogram, 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 6^{th} 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, ppseudodiploidy, 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 $1/5^{th}$ of AML patients with identifiable abnormalities had a simple numerical aberration as their ony 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 leukamias.

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 heamorhagic 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 heamotologic 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 psedodiploidy ans 3 cases of hypodiploidy. One case of ALL showed monosomy 3 and 22 with complete loss of 6^{th} 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

1. Rowley et.al. a consistent chromosomal change in acute promyelocytic leukemia lancet 1977;1:549-50.
2. Third international workshop on chromosomes in leukemia. Cancer Genet cytogenet 1981;4;95-142.
3. Third international workshop on chromosomes in leukemia chromosomal abnormalities and their clinical significance in acute lymphoid leukemia. Cancer Res. 1983;43:868.
4. Fourth international workshop on chromosome in leukemia cancer genet cytogenet 1984;7:249-360.
5. Rowley J.D. identification of a translocation with quinacrine flourscense in patient with acute leukemia Ann,. Genet 1973;16:109-12.
6. Arthur DC, Bloom field CD. Partial deletion of the long arm of chromosome 16 and bone marrow eosinophilia in acute non lymphocytic leukemia.
7. Berger.R. and Bernheim A. Acute monocytic leukemia chromosome studies, Leuk Res 1982;6: 17-26.
8. Sukurai.M and Sandberg. Prognosis in acute myeloblastic leukemia chromosomal correlation, Blood 1973;41;93-104.
9. Samuel BL. Specific chromosomal abnormalities in AML correlate with drug susceptibility in vivo capital Leukemia 1988;2:79-83.
10. James A. Whitlock, Wintrobes text book of haematology edi. 10^{th} yr. 1998.
11. Pui C-H et.al. Hypodiploidy is associated with a poor prognosis in childhood ALL. Blood 1987;70; 247-53.
12. Heim S and Mitelman F. Numerical chromosome aberrations in human neoplasia cancer genet. Cytogenet, 1986;22:99-108.
13. Heim S and Mitelman F. Secondary chromosome aberrations in acute leukemia cancer genet. Cytogenet, 1986;22:331-38.