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Original research article

Cytogenetic pattern profiling in cases of Acute Lymphoblastic Leukemia in pediatric age group

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ABSTRACT

Introduction: Acute lymphoblastic leukemia (ALL) comprises about 70–80% of childhood leukemia. The present work was undertaken to study the spectrum of chromosomal abnormalities in North Indian population in haematologically confirmed pediatric ALL patients using bone marrow aspirates.
Methods: Bone marrow aspirates (0.6 ml) after adding 15 ml RPMI medium were divided into three parts for immediate culture, 24 h culture and 48 h culture method, were incubated according to their respective time duration and karyotyping was done.
Results: Out of 20 cases results were obtained in 14 cases. Out of these 9 cases (64.2%) in present study belonged to hypodiploid group. Trisomy was found in 3 (21.42%) cases and polyploidy in 1 (7.1%) case. Three year old male patient showed translocation t (21; 4) with deletion of long arm of chromosome 5 and absence of 7, 11, 12 and Y chromosomes. 4 Year old male patient showed translocation involving chromosome 13 with absence of chromosomes 7, 10, 11 and 12.5 year old male patient showed one dicenteric 5 chromosome with additional copies of chromosomes 6, 8, 9, 21 and 22.
Discussion: Numerical and structural chromosomal abnormalities found in Acute Lymphoblastic Leukemia have prognostic significance. Review of world literature shows that there is geographical variation in ploidy pattern of ALL. Our findings will help to play a key role in risk stratification and treatment protocols considering the genetic diversity of pediatric ALL in North Indian population.

1. Introduction

Acute lymphoblastic leukemia (ALL) comprises about 70–80% of childhood leukemias.[1] ALL has a striking peak incidence at 2–3 years of age and occurs more in boys than in girls at all ages.[2] In India, ALL accounts for one fourth of all childhood cancers and three fourth of all childhood leukemias.[3]

ALL is associated with a spectrum of structural and numerical chromosomal abnormalities. Various studies have been conducted in various populations to study geographical and ethnic variations in cytogenetic patterns of acute lymphoblastic leukemia and the data thus obtained shows significant geographical differences thus indicating strong gene environment interactions. ALL in Indian patients has been shown to have phenotypic and genotypic differences from west. The studies conducted in India have addressed this gene environment interaction but are few in the North Indian population.

The present study was undertaken to study the spectrum of chromosomal abnormalities in North Indian population in haematologically confirmed pediatric ALL patients using bone marrow aspirates. This will help in categorizing children as per cytogenetic classification, who need more intensive treatment owing to presence of specific cytogenetic findings which have poor prognosis in the North Indian population.

2. Material and methods

The study was conducted from November 2011 to April 2013 after obtaining ethical clearance from institutional ethics

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committee at Maulana Azad Medical College and associated Lok Nayak hospital, Delhi.

2.1. Sample collection

Bone marrow aspirates taken from iliac crest were collected from 20 haematologically confirmed cases of acute lymphoblastic leukemia in pediatric age group after obtaining informed consent. Samples were collected in heparinised vacutainer and were transported immediately for culture. All patients were in the pediatric age group and were recruited from the pediatrics ward of LokNayak hospital.

2.2. Culture and Harvesting

Bone marrow aspirates (0.6 ml) after adding 15 ml RPMI medium were divided into three parts for immediate culture, 24 h culture and 48 h culture method and incubated according to their respective time duration. 0.05 ml of colchicine was added. Centrifugation was done at 1000 rpm. Supernatant was discarded. 5 ml of isotonic solution was added to the pellet and incubated for 45 min. Centrifugation was done at 1000 rpm for 10 min. 5 ml of chilled fixative was added. (Methanol and glacial acetic acid in ratio 3:1).Centrifugation was done at 1000 rpm. These steps were repeated till pellet turned white.

2.3. Preparation of Slides and banding

The cell suspension was dropped from a height of about 20 cms on a chilled glass slide. Slides were air dried, labelled and Giemsa banding was done.

2.4. Screening for metaphase spreads

Each slide was screened for well banded metaphase spreads using a bright field binocular microscope and the position of metaphase spreads was recorded. Metaphase spreads were captured using a satellite capture station using BX61, motorized, upright microscope incorporating infinity corrected optics attached with DP71 colour digital camera and the image was transferred to an image analyser. After analysing the chromosomes the prints were taken and cut and pasted on the recording sheet. Human cytogenetic nomenclature used in reporting was according to ISCN 2009.

3. Results

In the present study, Well spread metaphase plates were obtained in 14/20 cases for analysis. It was observed on the basis of modal chromosome number that most of the cases had numerical abnormalities and majority (9 cases) belonged to hypodiploidy (64.2%) (Table 1). Trisomy was found in 3 (21.42%) cases (Fig. 1A, D and F). Polyploidy was seen in 1 (7.1%) case. Diploidy was seen in 4 (28.5%) cases (Table 1).

In the present study it was observed that all the cases had multiple cell lines signifying the clonal nature of the disease (Table 3).

Among structural abnormalities, three year old male patient showed translocation t (21; 4) with deletion of long arm of chromosome 5 and absence of chromosomes 7, 11,12 and Y. (Fig. 1B) 4 Year old male patient showed translocation involving chromosome 13 with absence of chromosomes 7,10,11 and 12. (Fig. 1C) 5 year old male patient showed one dicenteric 5 chromosome with additional copies of chromosomes 6, 8, 9, 21 and 22 (Fig. 1A) (Table 2).

4. Discussion

Both structural and numerical abnormalities are detected in acute lymphoblastic leukemia and have strong prognostic importance in risk stratification and decisive role in guiding the treatment. Found in 25–30% of cases hyperdiploidy with greater than 50 chromosomes in the leukemic clones is one of the most powerful means of identifying patients with very good prognosis.[4] In contrast hypodiploidy is associated with bad prognosis as hypodiploid cases have a high rate of chromosomal translocations. [5]

There is a regional variation in the ploidal pattern as can be seen that hyperdiploidy(25%) is more common in ALL in America[6] In Europe, hyperdiploidy is seen in the range of 63% in B cell ALL and no clonal abnormality detected in T cell ALL,[7] Studies from Asia (Pakistan, China and Taiwan) also is suggestive of hyperdiploidy as the most common abnormality associated with ALL in children.[8–10] In Africa most common numerical abnormality detected was hypodiploidy (38%) followed by hyperdiploidy (18%)[11] (Fig. 3).

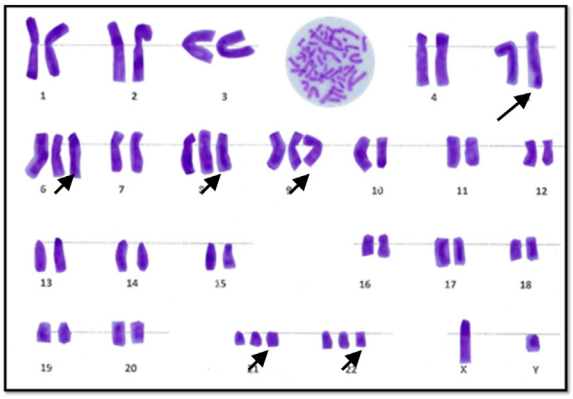
Indian scenario shows that children with ALL from north India show chromosomal abnormalities in 49% of cases and 21% have hyperdiploidy, while studies from western part of the country show hypodiploidy (38.4%) as the most common ploidy.[12–14] Eastern part of the country reflects hypodiploidy seen in 51%–63% of cases, [15,16] while hyperdiploidy is observed to be 14.2% in south India,[17] which speaks of the regional variations (Fig. 3).

In the present study, among the 20 cases taken up for the study, in 14 cases analysis and recording of karyotyping was possible and it was observed that most of the cases had numerical abnormalities and majority belonged to hypodiploidy (64.2%) (Tables 1 and 3). Trisomy was seen in 5 year old male patient (51,XY dic(5)+(6)+(8), +(9)(,+(21),+(22,) (Fig. 1A) 5 year old male (49,XY,+(6),+(7),+(9) (Fig. 1D) and 12 year old female (47,XX,+(9) (Fig. 1F). It is evident from the present study that there is a regional variation in ploidy pattern of ALL (as our findings which showed hypodiploidy in 64.2% cases from North India are similar those seen from eastern part of country (hypodiploidy 51–63%) and dissimilar from other parts of India (Fig. 3).

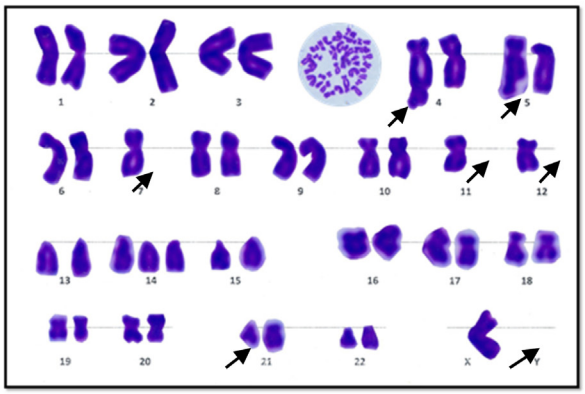
It is observed that ALL is more common in males than females across all the populations and in all the geographical conditions (Table 4). In our study, male to female ratio was 1.2:1 (Table 4). In the present study, it was observed that the male to female ratio was

Table 1
Numerical abnormalities found in all cases of ALL.

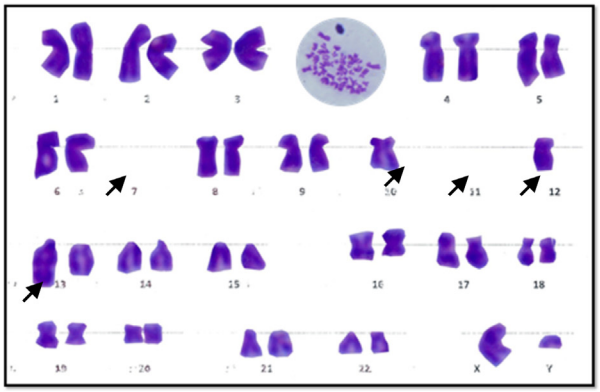
Numerical changes	*No of pts*	*%age*	*Age Range*	*Chromosome gain or loss*
Diploid (2n = 46)	4	28.5	3-12	–
Hypodiploid (2n = 31–39)	1	7.1	4	–
Hypodiploid (2n = 40–45)	8	57.14	2-8	−7,−10,−11,−12
Polyploid (2n = 92)	1	7.1	3	–



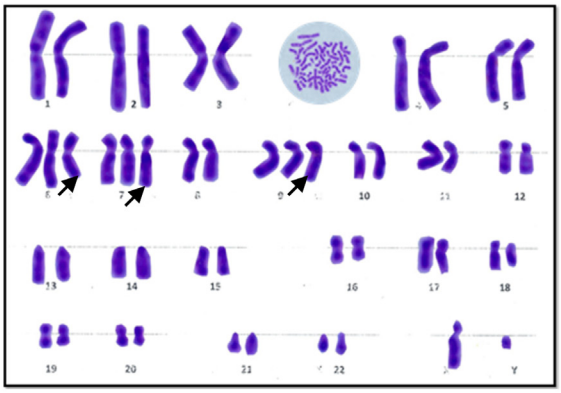
A: Karyotype of 5 year old male patient showing additional copies of chromosomes 6, 8, 9, 21 and 22 and dicentric 5 chromosome.(51XY)



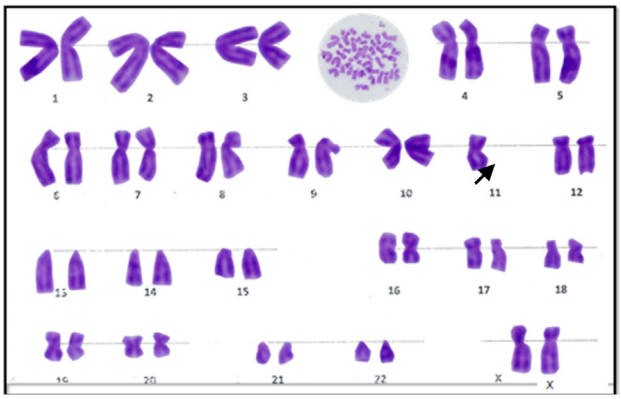
B: Karyotye of 3 year old male patient showing translocation between chromosomes 21 and 4,deletion in long arm of 5 and absence of chromosomes 7,11,12,Y(43X)



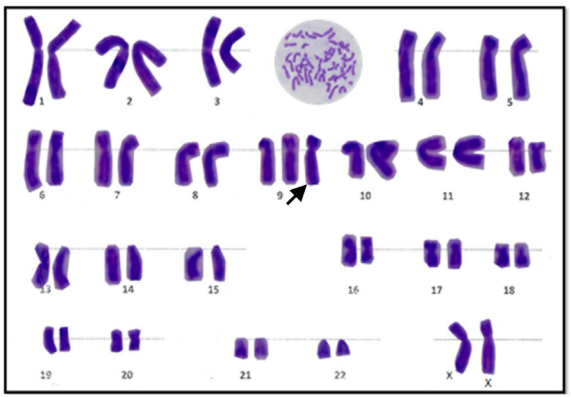
C: Karyotype of 4 Year old male patient translocation involving chromosome 13 and absence of chromosomes 7,10,11 and 12(40XY)



D: Karyotype of 5 year old male patient showing additional copies of chromosomes 6, 7 and 9.(49XY)



E: Karyotype of 12 Year old Female patient showing absence of chromosome 11 .(45XX)



F: Karyotype of 12 year old female patient showing additional copy of chromosome 9.(47 XX)

Fig. 1. Karyotype of patients showing numerical and structural abnormalities.

slightly different from other parts of the country where male to female ratio is 2:1 (Fig. 2).

Structural chromosome aberrations with breakage at characteristic bands have been related to the existence of fragile sites within the genome. The breakpoints for the deletions are variable, but a common chromosome region, the so-called critical region, is almost always for chromosome no. 5, the critical region is 5q31.42. Deletions of various parts of the short arm of chromosomes no. 12 and 17 and of the long arm of chromosome no. 20, and loss of a whole chromosome no. 18 as well as gain of a whole chromosome

Table 2
Structural abnormalities found in all cases of ALL.

Structural abnormalities	*No of cases*	*%age*
1) No identifiable abnormalities	10	71.4
2) a.) Primary chromosomal translocations		
1) t(21;4)	1	7.1
2) t(13)	1	7.1
b.) Secondary/additional aberration		
1.) dic(5)	1	7.1
2.) del 5q	1	7.1

Table 3
Types of metaphase spreads obtained in 14 cases of ALL.

Types of metaphase spreads	*No. of cases*	*%age*
Abnormal and normal	11	11/14*100
Abnormal only	3	3/14*100
Normal/Near normal	–	–

Table 4
Sex ratio of children affected with acute lymphoblastic leukemia.

Region	Male: Female Ratio
America	2.20: 1
Europe	1.17: 1
Africa	2.1: 1
India – North	1.5–2.7: 1
India – South	2: 1
India – West	2:1
India – East	2: 1
Present study	1.2: 1

no. 8 or of the long arm of chromosome 1, have also been observed. The four major chromosomal translocations observed in pre B-ALL in children include the t(12;21) (p13;q22), t(1;19) (q23;p13), t (9;22) (q34;q11), t(4;11) (q21;q23). These translocations define the clinico-pathological entities that have also been used in risk stratification of ALL, at least in USA and Europe.[18]

Christine Harrison (2011) recorded that those structural abnormalities with the most significant impact for risk stratification for treatment are t(9:22) (q34:q11)/BCR- ABL and rearrangement of MLL gene. The detection of these two abnormalities provides the basic criteria for classification of poor risk groups, which is applicable to all treatment protocols.[19]

Among structural abnormalities, Raimondi et al. (America) reported t(1;19)(6.5%), t(9;22)(6%), t(4;11)(2.6%), t(7;9)(2.6%). t (1;19) is found to be commonest translocation in western population.[20] Forestier et all in their analysis in European population showed t(9;22)(2.2%), t(4;11)(2%), t(11;19)(1.4%)t (1;19)(1.3%) and t(8;14)(1%).[21] Study in African population showed structural abnormalities to be t(4;11)(4.9%), t(1;19)(2.4%), t(9;22) (2.4%), t(8;14)(2.4%) and t(11;14)(2.4%) while in Asia TEL/AML1 t (12;21) was found in 17.9% of cases.[22,23]

Translocations reported from north India were t(9;22) in 2.8% and t(1;19) in 5.7% cases.[24] In their study of west indian population Siraj et al. reported t(12;21) and t(1;19) in equal frequencies(7%) followed by t(9;22)(5%)[25] Data from eastern india revealed t(9;22) as the commonest translocation seen in 12.88% cases followed by t (4;11)(9.6%) and t(1;19)(6.4%)[16] Syed Hashem et al. in their study of 50 ALL patients in south india reported t(9;22) in2-5%, t(4;11) in 4% and t(1;19) in 6% cases.[17] In the present study, three year old female patient showed translocation t(21;4) with deletion 5q and absence of 7, 11,12 and Y (Fig. 1B). 4 Year old male patient showed translocation involving chromosome 13 with absence of chromosomes 7, 10, 11 and 12. (Fig. 1C). 5 year old male patient showed one dicenteric 5 chromosome with additional copies of chromosomes 6, 8, 9, 21 and 22 (Fig. 1A) (Fig. 4).

In the observations recorded in this study all the cases have numerical abnormalities but did not show any apparent structural abnormality but all the cases which have structural abnormality showed some numerical abnormality.

5. Conclusion

The present study was done to study cytogenetic analysis in North Indian pediatric population. World literature proves that there is a regional variation in numerical and structural cytogenetic findings in various populations. Our study demonstrated hypodiploidy (64.2%) as the most common ploidy .Trisomy was seen in 3 cases. Polyploidy was seen in 1 case. Among structural abnormalities, t(21;4),del 5q and t(13) each in one case. Such studies will play a key role in risk stratification and treatment protocols considering the heterogeneity of the pediatric ALL which is compounded by the diverse genetic profile of Indian population. A sequential cytogenetic follow-up should be done in these

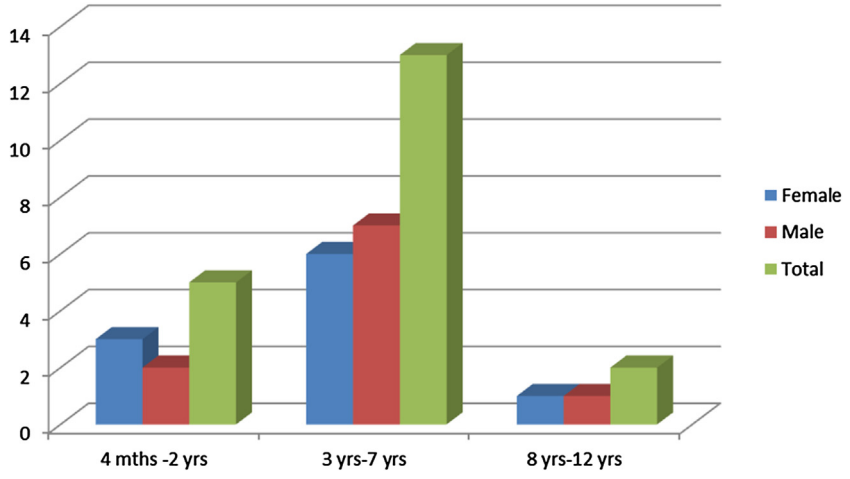


Fig. 2. Age and sex distribution of 20 cases of pediatric acute lymphoblastic leukemia.

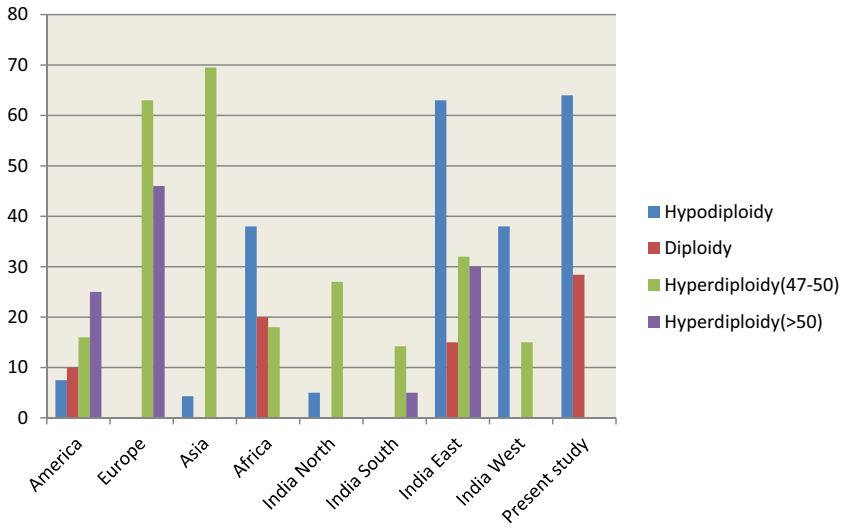


Fig. 3. Comparison of numerical findings seen in cases of ALL worldwide.

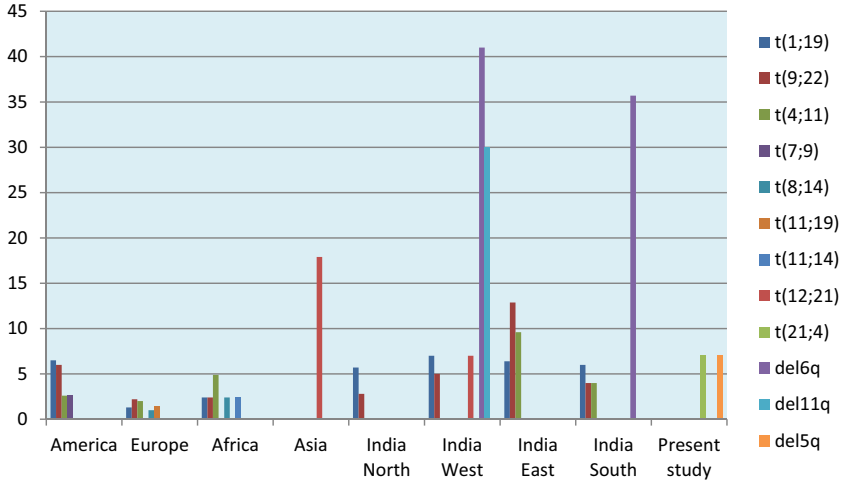


Fig. 4. Structural abnormalities seen in cases of ALL worldwide.

patients for identification of minimal residual disease and in remissions.

Conflict of interest

The authors have none to declare.

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