Journal of the Anatomical Society of India 66 (2017) 48–53

Contents lists available at ScienceDirect

Journal of the Anatomical Society of India

journal homepage: www.elsevier.com/locate/jasi

Original research article

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

Shweta Jha[a,b], Dinesh Kumar[b,*], J.M. Kaul[c,b], T. Singh[d,e], A.P. Dubey[f]

[a] Department of Anatomy, University College of Medical Science, Delhi, India
[b] Department of Anatomy, Maulana Azad Medical College, Delhi, India
[c] Department of Anatomy, Baba Saheb Ambedkar Medical College, Delhi, India
[d] Oncquest Lab, Delhi, India
[e] Department of Pathology, Maulana Azad Medical College, Delhi, India
[f] Department of Pediatrics, Maulana Azad Medical College, Delhi, India

ARTICLE INFO

Article history:
Received 12 September 2016
Accepted 22 March 2017
Available online 31 March 2017

Keywords:
Acute Lymphoblastic Leukemia
Cytogenetics
Karyotyping
Bone marrow culture

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

* Corresponding author at: University College Of Medical Science, Dilshad Garden, Delhi, 110095, India.
E-mail address: jha350@gmail.com (S. Jha).

http://dx.doi.org/10.1016/j.jasi.2017.03.001
0003-2778/

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	–