RESEARCH ARTICLE

Cytogenetic Profile of De Novo B lineage Acute Lymphoblastic Leukemia: Determination of Frequency, Distribution Pattern and Identification of Rare and Novel Chromosomal Aberrations in Indian Patients

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Abstract

Background: Chromosomal aberrations identified in acute lymphoblastic leukemia (ALL) have an important role in disease diagnosis, prognosis and management. Information on karyotype and associated clinical parameters are essential to physicians for planning cancer control interventions in different geographical regions. Materials and Methods: In this study, we present the overall frequency and distribution patterns of chromosomal aberrations in both children and adult *de novo* B lineage ALL Indian patients using conventional cytogenetics, interphase FISH and multiplex RT-PCR. Results: Among the 215 subjects, cytogenetic results were achieved in 172 (80%) patients; normal karyotype represented 37.2% and abnormal 62.8% with a distribution as follows: 15.3% hypodiploidy; 10.3% hyperdiploidy; 15.8% t(9;22); 9.8% t(1;19); 3.7% t(12;21); 2.8% t(4;11); 2.8% complex karyotypes. Apart from these, we observed several novel, rare and common chromosomal rearrangements. Also, FISH studies using LSI extra-signal dual-color probes revealed additional structural or numerical changes. Conclusions: These results demonstrate cytogenetic heterogeneity of ALL and confirm that the incidence of chromosomal abnormalities varies considerably. To the best of our knowledge, this is one of the largest reported series of cytogenetic investigations in Indian B-lineage ALL cases. In addition, ongoing cytogenetic studies are warranted in larger groups of B-lineage ALL cases to identify newly acquired chromosomal abnormalities that may contribute to disease diagnosis and management.

Keywords: Acute lymphoblastic leukemia - cytogenetic analysis - fluorescence in situ hybridization

Introduction

Around 80 % of all childhood acute leukemia is ALL, especially B-cell-precursor acute lymphoblastic leukemia (BCP-ALL) (80-85 % of ALL), which is the leading cause of cancer-related death in children and young adults. In India, this proportion varies between 60-85% (Arora et al., 2009) and with the current population rates, it is likely to increase. Over the years, there has been a significant improvement in the outcome of ALL patients with a 5-year EFS >80% in developed countries (Hunger et al., 2012) and the future is encouraging.

Most ALL patients are genetically heterogeneous, harboring different chromosomal (numerical and/or structural) and/or submicroscopic molecular abnormalities crucial in diagnosis, prognosis, chemo-therapeutic response and outcome (Iacobucci et al., 2012). In turn, their identification contributes towards understanding disease pathogenesis. Their relevance is likely to increase

as targeted therapies are introduced, as exemplified by the success of tyrosine kinase inhibitor in the treatment of Philadelphia positive ALL (Pui and Evans, 2006). Current genetic abnormalities of significance in ALL management include: t(9;22)(q34;q11) (BCR-ABL1), t(4;11)(q21;q23) (MLL-AFF1 alias MLL-AF4) and near haploid/low hypodiploidy, all of which are poor prognostic markers; t(1;19)(q23;p13) (TCF3-PBX1 alias E2A-PBX1) as an intermediate prognostic marker; while t(12;21) (p13;q22) (ETV6-RUNX1 alias TEL-AML1) and high hyperdiploidy, are both favorable prognostic markers (Harrison et al., 2010). The presence of recurrent genetic markers represents subtypes of the disease which may have different etiologies.

The frequencies of genetic aberrations differ distinctly in childhood and adult ALL. In childhood ALL, the impact of cytogenetic factors like specific translocations and DNA ploidy is well defined, while the prognostic significance of karyotype in adult ALL is less clear, partially as

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the disease is less frequent. Furthermore, the relative distribution of molecular subtypes in ALL is not uniform in different geographic regions. Thus, we examined the cytogenetic profile of newly diagnosed B-lineage ALL cases to determine the frequency and subtypes of cytogenetic aberrations from an Indian perspective, and their correlation with clinical parameters. An attempt was also made to compare the major aberrations (enlisted by WHO) in childhood and adult Indian ALL patients with Asian and global reports.

Materials and Methods

Patients

The present study included 215 *de novo* B-lineage ALL subjects referred from July 2011-October 2013 and were subjected to cytogenetic and/or molecular analysis of chromosomal rearrangements conducted at SRL Ltd., Mumbai, India. The study was in accordance with the declaration of Helsinki and approved by the Institutional ethics committee. Written consent was obtained from all the subjects.

Cytogenetic analysis

We karyotyped bone marrow aspirate or whole blood cells withdrawn at diagnosis as per standard unstimulated direct (0 h, 3 h) and short term (24 h, 48 h) cell culture technique previously described (Bhandari et al., 2014). In brief, cells were cultured in RPMI (Sigma, Schnelldorf, Germany) medium supplemented with 20% FBS (Gibco, Grand Island, NY) at 37°C. After incubation at respective time intervals, the cells were harvested i.e. cell-growth was arrested by exposure to colchicine (HiMedia, 4mg/ml) for 45 minutes, then hypotonised by 0.075 M KCl for 20 minutes, then fixed with chilled Carnoy's fixative (methanol: glacial acetic acid 3:1). Fixed cells were dropped on chilled frosted slides, then aged overnight at 60°C and stained for GTG banding at resolution of about 400-band level. At least 20 metaphase plates were screened, and 4-5 well spread metaphases were photographed and karyotyped using Ikaros Software (MetaSystems GmbH, Germany). The karyotype was interpreted according to ISCN 2013 (ISCN 2013) guidelines.

Fluorescence In Situ Hybridization (FISH) Analysis

FISH was performed on selected cases to refine complex or ill-defined rearrangements identified by chromosome analysis and to confirm recurrent /cryptic chromosomal aberrations such as BCR-ABL, ETV6-RUNX1, and MLL rearrangements, using LSI Vysis Probes (Abbott Molecular, Des Plaines, IL). The slides were hybridized overnight according to Vysis protocol and analyzed using Isis Software (MetaSystems GmbH, Germany).

Molecular Analysis for Detection of Gene Fusion Transcripts

To confirm /reveal recurrent or cryptic chromosomal rearrangements such as t(9;22)(q34;q11), t(1;19)(q23;p13), t(4;11)(q21;q23) and t(12;21)(p13;q22) in cases with apparently normal karyotype or with insufficient metaphases, multiplex nested reverse transcriptase PCR with minor modification (Pallisgaard et al., 1998) was performed. Briefly, fifty nanograms of total RNA extracted using QIAamp RNA blood mini kit (Qiagen, Hilden, Germany) used to prepare cDNA using random hexamer primer (Fermentas, Hanover, MD) and Sensiscript RT Kit (Qiagen). This was followed by two rounds of nested RT-PCR for detection of the fusion genes. Appropriate internal control was also analysed to check the integrity of RNA.

Statistical analysis

The χ^2 test and Fisher's exact test were used to compare the categorical data between adult and pediatric ALL patients. Z-test was performed to compare the frequencies of different abnormalities with available published reports of different countries. Two-side P-values <0.05 were considered statistically significant.

Results

Total 215 newly diagnosed B-lineage ALL patients, comprising of 130 males and 85 females (M:F ratio=1.5:1), with age ranging from <1-75 years were analysed (Table 1). The study population consisted of 143 children (aged ≤14 years; M:F ratio=1.6:1) and 72 adults (aged ≥15 years; M:F ratio=1.4:1). Of the 215 subjects, 172 patients (80%) showed successful cultures. Total 135 patients (62.8%) showed chromosomal aberrations, while 80 (37.2%) displayed normal karyotype.

Table 1. Clinical Characteristics of Indian B-lineage ALL Patients

Demographic Parameters	Total Cases	Pediatric* Cases	Adult† Cases
Total, n (%)	215 (100)	143 (66.5)	72 (33.5)
Gender			
Male, n (%)	130 (60.5)	87 (61)	43 (60)
Female, n (%)	85 (39.5)	56 (39)	29 (40)
Age (years), Median (range)	8 (0.01-75)	4 (0.01-14)	26.5 (15-75)
Hb level (gm/dl) median (range)	7.5 (1.6-15)	7.4 (1.6-15)	7.7 (2.5-15)
Platelets count (x 10^9/L) median (range)	40 (0.7-470)	40 (3-470)	37.8 (0.7-154)
WBC count (x 10^9/L) median (range)	17.5 (0.2-536)	21 (0.9-536)	10.8 (0.2-451)
<50 (x 10^9/L), n (%)	154 (71.6)	102 (71.4)	52 (72)
≥50 (x 10^9/L), n (%)	61 (28.4)	41 (28.6)	20 (28)

Hb: Hemoglobin; WBC: white blood cell; *age range: <15years; †age range: ≥ 15 years

Figure 1. Partial karyotypes of Novel & Rare Structural Chromosomal Rearrangements Showing only the Chromosomes Involved. [i] t(1;12)(p34.1;q24.33), [ii] t(2;17)(p23;q25), [iii] t(6;12)(q13;q13), [iv] t(10;15)(q22;q22), [v] t(11;14)(p15;q24), [vi] inv(4)(p14p16), [vii] del(X)(q11.1), [viii] t(7;21)(?q32;q22.1), [ix] t(12;22)(p11.2;p11.2), [x] t(X;?17)(p22.3;?q11.2), [xi] inv(17)(p11.2p13.1), [xii] i(1)(p10), and [xiii] t(3;5)(q21;q31), [xiv] t(7;9)(p14;p24), [xv] inv(14)(q11.2q32), [xvi] dup(1)(q21q42), [xvii] i(8)(q10), [xviii] del(8)(p23), [xix] del(5)(p11), del(5)(q33q35), [xx] del(17)(p11.2), del(17)(q21.1), del(17)(q11.2), [xxi] del(22)(q12), [xxii] t(2;14)(?p13-16;q32), [xxiii] t(4;8)(q31;q24.3), [xxiv] t(5;21)(q13;q22), [xxv] t(6;7)(q21;q22), [xxvi] t(17;19)(q21;q13.3), [xxvii] t(9;12)(p13;q13), [xxviii] del(7)(p15.3)

Figure 2. FISH Analysis Using Vysis LSI Probes on Interphase Cells [i] in BCR-ABL positive patients allowed detection of additional alterations like [A] cryptic deletions at ABL breakpoint on chromosome 9(q) (indicated via reduction of 2 red to 1 red signal or by absence of native red signal), [B] multiplication involving the 22q11 BCR locus (indicated by presence of >2 green signals) and [C] presence of masked double/ multiple Philadelphia chromosome (indicated by presence of >1 fusion signal); and in [ii] cases suspected of ETV6-RUNX1 it allowed detection of [D] deletions of non-translocated ETV6 allele from 12p13 (indicated via absence of native green signal) [E] deletions of residual RUNX1 allele on chromosome 21 (indicated via reduction of 2 red to 1 red signal) , and [F] multiplication involving the 21q22 RUNX1 locus (indicated by presence of >2 red signals)

Distribution and frequencies of chromosomal rearrangements

Table 2 shows relative frequencies and distribution of B-ALL specific chromosomal rearrangements and their associated clinical characteristics. Amongst 215 patients, the major alterations included hyperdiploidy (10.3%), hypodiploidy (15.3%), t(9;22) (15.8%), t(1;19) (9.8%), t(12;21) (3.7%), t(4;11) (2.8%), other structural changes (28%) and complex karyotypes (2.8%). Further amongst our pediatric and adult patients, chromosomal abnormalities were more frequent in the adult patients (75% vs 56.6%) (P<0.05).

Numerical chromosomal changes

Elucidating modal chromosome number at diagnosis is essential to stratify hypo/ hyperdiploid patients into appropriate risk group for treatment. Table 3 shows Age-Sex-Modal number distribution observed:- 37.2% Normal diploid (2n=46 chromosomes); Hypodiploidy (2n<46 chromosomes) with sub-groups: 1% Near-haploidy (2n=25-29), 6% Low-hypodiploidy (2n=30-39), 8.4% Moderate-hypodiploidy (2n=40-45); and Hyperdiploidy (2n>46 chromosomes) with sub-groups: 4.2% Moderate-hyperdiploidy (2n=47-50), 3.7% High-hyperdiploidy (2n=51-65), 1% Near-triploidy (2n=66-80) and 1.4% Near-tetraploidy (2n= ≥81). Ploidy distribution amongst pediatric and adult groups was mostly alike with few exceptions. Like near-triploidy and near-tetraploidy were seen exclusively only in adults aged >20 years. Also, both moderate (5.6% vs 1.4%) and high hyperdiploidy (4.9% vs 1.4%) were more frequent in pediatric than adult patients, but these differences were not significant (all P>0.1).
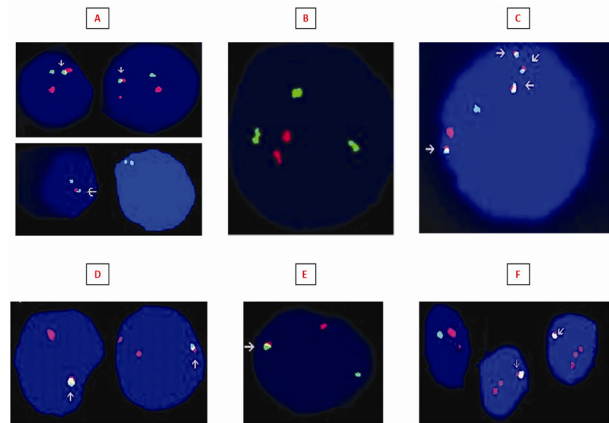
Structural chromosomal changes

A higher frequency of structural abnormalities was observed as compared to numerical aberrations in both pediatric (53% vs 25%) and adult groups (75% vs 25%) (all P<0.0001). As seen in Table 2 amongst the two groups, adults showed a higher frequency of t(9;22) (29.2% vs 9.1%, P<0.001). In contrast, while 5.6% pediatric cases showed t(12;21), it was absent in the adult patients. Slightly elevated t(4;11) was observed in pediatric than adult patients (3.5% vs 1.4%), while almost equal pediatric (9.1%) and adult (11.1%) cases showed t(1;19) but these differences were not significant (all P>0.1).

Partial karyotypes of rare and novel structural chromosomal rearrangements observed are shown in Figure 1. In our study, cytogenetic analysis revealed several novel abnormalities like t(1;12)(p34.1;q24.33), t(2;17)(p23;q25), t(6;12)(q13;q13), t(10;15)(q22;q22), t(11;14)(p15;q24), paracentric inv(4)(p14p16) and del(X)(q11.1) in childhood ALL. While we observed t(7;21)(?q32;q22.1), t(12;22)(p11.2;p11.2) , t(X;?17)(p22.3;?q11.2), paracentric inv(17)(p11.2p13.1) and i(1)(p10) among adults. In addition, we observed several rare structural chromosomal rearrangements that were differently distributed among the pediatric and adult patients like t(3;5)(q21;q31), t(7;9)(p14;p24), pericentric inv(14)(q11.2q32), dup(1)(q21q42), i(8)(q10), del(8)(p23), del(5)(p11), del(5)(q33q35), del(17)(p11.2), del(17)(q21.1) and del(22)(q12) were exclusively observed in childhood cases. While t(2;14)(?p13-16;q32), t(4;8)(q31;q24.3), t(5;21)(q13;q22), t(6;7)(q21;q22), t(9;12)(p13;q13), t(17;19)(q21;q13.3), del(7)(p15.3) and del(17q11.2) were observed only in adult patients.

Table 2. Distributions of Cytogenetic & Molecular Chromosomal Rearrangements in Indian B Lineage ALL Patients

Cytogenetic/ Molecular subgroup	Total Cases		Pediatric Cases						Adult Cases					
	N (%)	Sex, M/F	N (%)	Sex, M/F	Age (years) median (range)	Hb (gm.%) median (range)	Platelets (x 10⁹/L) median (range)	WBC (x 10⁹/L) median (range)	N (%)	Sex, M/F	Age (years) median (range)	Hb (gm.%) median (range)	Platelets (x 10⁹/L) median (range)	WBC (x 10⁹/L) median (range)
Total	215 (100)	130/85	143 (100)	87/56	4 (0.01-14)	7.4 (1.6-15)	40 (3-470)	21 (0.9-536)	72 (100)	43/29	26.5 (15-75)	7.7 (2.5-15)	37.8 (0.7-154)	10.8 (0.2-451)
Normal [a]	80 (37.2)	50/30	62 (43.4)	40/22	4 (0.01-13)	7.5 (1.6-14)	46 (5-470)	10 (0.9-180)	18 (25)	10/8	23.5 (15-75)	8.2 (2.5-15)	36.25 (7-154)	9.5 (1.2-343)
Cytogenetic/ Molecular Abnormal Rearrangements*	135 (62.8)	80/55	81 (56.6)	47/34	4 (0.06-14)	7.2 (2-15)	40 (3-200)	28 (1.2-536)	54 (75)	33/21	30.5 (15-69)	7.5 (3.9-13)	39.5 (0.7-150)	11.4 (0.2-451)
Hyperdiploidy (>46 chromosomes)	22 (10.3)	12/10	15 (10.5)	8/7	4 (2.06-14)	8 (3.1-12)	20 (10-200)	32.5 (4.3-536)	7 (9.7)	4/3	37 (19-69)	9.5 (7.3-13)	67 (3.8-143)	3.6 (2.3-15)
Moderate Hyperdiploidy (47~50 chromosomes)	9 (4.2)	3/6	8 (5.6)	3/5	6 (3-14)	8.75 (3.1-12)	32.5 (10-200)	34.33 (4.3-536)	1 (1.4)	0/1	19	9.9	67	2.3
High Hyperdiploidy (51~65 chromosomes)	8 (3.7)	6/2	7 (4.9)	5/2	3 (2-4)	7.5 (5.5-12)	20 (15-150)	32.5 (9.5-56)	1 (1.4)	1/0	66	13	143	10.7
Near Triploidy (68~80 chromosomes)	2 (1)	1/1	-	-	-	-	-	-	2 (2.8)	1/1	55 (22-55)	8.95 (7.8-10.1)	21.9 (3.8-40)	7.55 (3.1-12)
Near Tetraploidy (>80 chromosomes)	3 (1.4)	2/1	-	-	-	-	-	-	3 (4.1)	2/1	37 (21-54)	8.5 (7.3-9.5)	70 (39-90)	3.6 (2.1-15)
Hypodiploidy (<46 chromosomes)	33 (15.3)	24/9	22 (15.4)	14/8	3 (0.11-14)	6.5 (2.6-14)	39 (3-150)	45.8 (4.3-451)	11 (15.2)	10/1	37 (17-69)	7.2 (3.9-12.2)	23 (8-114)	8 (0.8-416)
Near Haploidy (<30 chromosomes)	2 (1)	2/0	1 (0.7)	1/0	8	7	40	42.5	1 (1.4)	1/0	23	7.3	17	7.34
Other Hypodiploidy (30~45 chromosomes)	31 (14.4)	22/9	21 (14.7)	13/8	3 (0.11-14)	6 (2.6-14)	39 (3-150)	49 (4.3-451)	10 (13.8)	9/1	48 (17-69)	6.6 (3.9-12.2)	24 (8-114)	8.3 (0.8-416)
t(9;22)(q34;q11) / BCR-ABL [a]	34 (15.8)	17/17	13 (9.1)	7/6	10 (0.06-14)	6.4 (2-10.8)	39 (5-101)	15 (1.2-468)	21 (29.2)	10/11	34 (15-69)	6.9 (4-13)	29 (0.7-150)	30 (0.2-320)

Cytogenetic/ Molecular subgroup	Total Cases		Pediatric Cases						Adult Cases					
	N (%)	Sex, M/F	N (%)	Sex, M/F	Age (years) median (range)	Hb (gm.%) median (range)	Platelets (x 10⁹/L) median (range)	WBC (x 10⁹/L) median (range)	N (%)	Sex, M/F	Age (years) median (range)	Hb (gm.%) median (range)	Platelets (x 10⁹/L) median (range)	WBC (x 10⁹/L) median (range)
t(1;19)(q23;p13) / TCF3-PBX1	21 (9.8)	13/8	13 (9.1)	9/4	5 (1-13)	8.9 (4.9-13)	54 (5-150)	40 (6-160)	8 (11.1)	4/4	22.5 (15-50)	7.7 (5-9.9)	37.5 (8-60)	39.1 (1.4-200)
t(4;11)(q21;q23) / MLL–AFF1	6 (2.8)	3/3	5 (3.5)	3/2	3 (2-10)	5.9 (4.8-8.9)	50 (6-79)	50 (16-153)	1 (1.4)	0/1	17	5.9	99	451
t(12;21)(p13;q22) / ETV6-RUNX1	8 (3.7)	5/3	8 (5.6)	5/3	4 (1.05-7)	9.9 (5-15)	55.5 (10-200)	12.1 (2-92)	-	-			-	-
Other structural changes														
t(1;12)(p34.1;q24.33)	1 (0.5)	0/1	1 (0.7)	0/1	6	13.5	150	2	-	-	-	-	-	-
t(10;15)(q22;q22)†	1 (0.5)	1/0	1 (0.7)	1/0	6	4.5	13	7.1	-	-	-	-	-	-
t(11;14)(p15;q24)	1 (0.5)	0/1	1 (0.7)	0/1	12	8.6	20	4.3	-	-	-	-	-	-
t(2;17)(p23;q25)	1 (0.5)	0/1	1 (0.7)	0/1	5	7.8	154	67.9	-	-	-	-	-	-
t(6;12)(q13;q13)	1 (0.5)	1/0	1 (0.7)	1/0	3	11	35	45	-	-	-	-	-	-
t(X;?17)(p22.3;?q11.2)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	19	5.5	25	2.1
t(7;21)(?q32;q22.1)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	17	7.3	65	6.8
t(12;22)(p11.2;p11.2)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	22	7.3	17	10.7
inv(4)(p14p16)	1 (0.5)	0/1	1 (0.7)	0/1	2	9.9	89	2	-	-	-	-	-	-
inv(17)(p11.2p13.1)	1 (0.5)	0/1	-	-	-	-	-	-	1 (1.4)	0/1	24	8.7	25	17.5
i(1)(p10)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	60	6	50	60
del(X)(q11.1)	1 (0.5)	0/1	1 (0.7)	0/1	12	8.6	20	4.3	-	-	-	-	-	-
t(3;5)(q21;q31)	1 (0.5)	1/0	1 (0.7)	1/0	5	7.2	33	23.4	-	-	-	-	-	-
t(7;9)(p14;p24)	1 (0.5)	0/1	1 (0.7)	0/1	1	11.9	139	49	-	-	-	-	-	-
t(2;14)(?p13-16;q32)	1 (0.5)	0/1	-	-	-	-	-	-	1 (1.4)	0/1	56	6	25	320.76
t(4;8)(q31;q24.3)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	19	5.5	25	2.1
t(5;21)(q13;q22)	1 (0.5)	0/1	-	-	-	-	-	-	1 (1.4)	0/1	24	9	19	65
t(6;7)(q21;q22)	1 (0.5)	0/1	-	-	-	-	-	-	1 (1.4)	0/1	24	9	19	65

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t(17;19)(q21;q13.3)	1 (0.5)	0/1	-	-	-	-	-	-	1 (1.4)	0/1	24	9	19	65
inv(14)(q11.2q32)	1 (0.5)	0/1	1 (0.7)	0/1	2	9.9	89	2	-	-	-	-	-	-
dup(1)(q21q42)	1 (0.5)	1/0	1 (0.7)	1/0	4	10	20	15	-	-	-	-	-	-
i(8)(q10)	1 (0.5)	0/1	1 (0.7)	0/1	10	6.4	40	10.1	-	-	-	-	-	-
del(8)(p23)	1 (0.5)	0/1	1 (0.7)	0/1	1	11.9	139	49	-	-	-	-	-	-
del(5)(p11/q33q35)	2 (1)	1/1	2 (1.4)	1/1	8 (4-12)	7.05 (3.1-11)	20 (10-30)	324.5 (113-536)	-	-	-	-	-	-
del(7)(p15.3)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	19	5.5	25	2.1
del(17)(p11.2/q11.2/q21.1)	3 (1.4)	2/1	2 (1.4)	1/1	7.53 (1.05-14)	7.8 (5.1-0.5)	48 (35-61)	10.5 (5-16)	1 (1.4)	1/0	19	5.5	25	2.1
del(22)(q12)	2 (1)	2/0	2 (1.4)	2/0	3.5 (3-4)	4.95 (3-6.9)	35 (30-40)	17.95 (7.9-28)	-	-	-	-	-	-
i(7)(q10)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	15	8.6	30	30.7
i(9)(q10/q12)	3 (1.4)	2/1	3 (2.1)	2/1	10 (4-12)	9.9 (6.4-11)	40 (10-99)	113 (101-186)	-	-	-	-	-	-
i(17)(q10)	1 (0.5)	1/0	-	-	-	-	-	-	1 (1.4)	1/0	33	9	114	8
del(3)(p24-25)	3 (1.4)	2/1	2 (1.4)	1/1	0.55 (0.11-1)	7.65 (3.4-1.9)	79.5 (20-139)	82.3 (49-115.6)	1 (1.4)	1/0	59	9.9	145	3
del(6)(q15/q13q21)	2 (1)	1/1	1 (0.7)	0/1	5	14.5	150	7.5	1 (1.4)	1/0	19	5.5	25	2.1
del(9)(p13/p22/q32q34)	3 (1.4)	3/0	2 (1.4)	2/0	15 (13-17)	7.3	65	6.8	1 (1.4)	1/0	17	7.3	65	6.8
del(11)(q23)	7 (3.2)	5/2	4 (2.8)	2/2	3.5 (1-10)	6.1 (5.2-8.9)	32 (4-70)	31.25 (6-60)	3 (4.1)	3/0	46 (18-50.1)	9.6 (8-9.9)	119 (40-143)	8.6 (2.4-49)
del(12)(p11.2-13)	7 (3.2)	5/2	5 (3.5)	4/1	5 (3-14)	10.2 (8-14.5)	20 (10-150)	7.5 (6.1-113)	2 (2.8)	1/1	19.5 (17-22)	10 (7.8-12.2)	29.5 (19-40)	209.6 (3.1-416)
del(21)(q22)	1 (0.5)	1/0	1 (0.7)	1/0	4	10.2	20	6.1	-	-	-	-	-	-
Complex‡	6 (2.8)	4/2	3 (2.1)	2/1	1.05 (1-12)	11 (10.5-12)	61 (10-139)	49 (16-113)	3 (4.1)	2/1	19 (17-24)	7.3 (5.5-9)	25 (19-65)	6.8 (2.1-65)

* molecular abnormalities detected includes t(9;22),t(1;19), t(4;11) and t(12;21) rearrangements; † t(10;15) reported earlier (Bhandari et al., 2014); ‡ complex karyotype: defined as presence of ≥3 structural &/ numerical chromosomal aberrations (other than any one of the main established chromosomal abnormalities); ᵃ Significant difference between adult and pediatric cases (P<0.05)

Table 3. Age-Sex-Modal Number Distribution Pattern in Various Indian B-lineage ALL Patients

Type of Ploidy change (Modal No.)	Pediatric Patients				Adult Patients				Total Patients				Males	Females
	(T=143)	Sex		Age Range	(T=72)	Sex		Age Range	(T=215)	Sex		Age Range	(T=130)	(T=85)
	N (%)	M	F		N (%)	M	F		N (%)	M	F		N (%)	N (%)
Hypo (2n≤23-29)	1 (0.7)	1	0	8	1 (1.4)	1	0	23	2 (1)	2	0	8-23	2 (1.5)	0 (0)
Hypo (2n=30-39)	10 (7)	8	2	0.11-8	3 (4.2)	3	0	17-48	13 (6)	11	2	0.11-48	11 (8.5)	2 (2.35)
Hypo (2n=40-45)	11 (7.7)	5	6	1-14	6 (9.7)	5	1	33-69	18 (8.4)	11	7	1-69	11 (8.5)	7 (8.2)
Normal (2n=46)	62 (43)	40	22	0.01-13	18 (25)	10	8	15-75	80 (37.2)	50	30	0.01-75	50 (38.5)	30 (35.3)
Hyper (2n=47-50)	8 (5.6)	3	5	3-14	1 (1.4)	0	1	19	9 (4.2)	3	6	3-19	3 (2.3)	6 (7)
Hyper (2n=51-65)	7 (4.9)	5	2	2-4	1 (1.4)	1	0	66	8 (3.7)	6	2	2-66	6 (4)	2 (2.35)
Hyper (2n=66-80)	0 (0)	0	0	0	2 (2.8)	1	1	22-55	2 (1)	1	1	22-55	1 (0.77)	1 (1.17)
Hyper (2n ≥81)	0 (0)	0	0	0	2 (4.2)	2	0	21-54	3 (1.4)	2	1	21-54	2 (1.5)	1 (1.17)

Table 4. Comparison of Chromosomal Aberrations between Indian and Western Pediatric ALL Patients

Study	Country	Total cases, n	Hyperdiploidy, n (%)	Hypodiploidy, n (%)	t(9;22)/ BCR–ABL, n (%)	t(1;19)/ TCF3–PBX1, n (%)	t(4;11)/ MLL–AFF1, n (%)	t(12;21)/ ETV6–RUNX1, n (%)
Present Study, 2015	India	143	15 (10.5)	22 (15.4)	13 (9.1)	13 (9.1)	5 (3.5)	8 (5.6)
Fauzdar et al. 2010	India	77	21 (27) [a]	4 (5) [a]	9 (10)	—	—	9/21 (42) [a]
Hill et al. 2005	India	42	—	—	—	0	0	2 (4.8)
Sazawal et al. 2004	India	35	—	—	1 (2.8)	2 (5.7)	0	0
Siraj et al. 2003	India	259	—	—	14 (5)	18 (7)	0	18 (7)
Safaei et al. 2013	Iran	88	28 (31.8) [a,b]	4 (4.5) [a]	1 (1.1) [a]	1 (1.1) [a]	—	0
Chen et al. 2012	China	726	77 (10.6) [b]	—	106 (14.6)	39 (5.3)	20 (2.7)	83/541 (15.3) [a]
Alonso et al. 2012	Argentina	326	63 (20)[a]	6 (1.8) [a]	6 (1.8) [a]	19 (5.8)	38 (11.6) [a]	49 (15) [a]
DeBraekeleer et al. 2010	France	93	37 (39) [a,b]	4 (4.3) [a]	4 (4.3)	6 (6.5)	2 (2.1)	13 (14) [a]
Moorman et al. 2010	Britain	1725	562 (38) [a,b]	18 (1) [a]	43 (3) [a]	50 (4) [a]	17 (0.98) [a]	368 (25) [a]
Mesquita et al. 2009	Brazil	88	11 (12.5)	4 (4.5) [a]	(3.03)	(9.68)	0	(21.21) [a]
Ceppi et al. 2009	Nicaragua	64	16 (25) [a,b]	—	1 (1.5) [a]	—	2 (3.1)	9 (14) [a]
Santamaria-Quesada et al. 2009	Costa Rica	65	15 (23) [a]	1 (1.5) [a]	3(4.6)	2 (3)	1 (1.5)	12 (18.4) [a]
Hann et al. 2001	Britain	1658	566 (34) [a]	109 (6.5) [a]	25 (1.5) [a]	47 (2.8) [a]	15 (0.90) [a]	128/659 (19.4) [a]
Gaynon et al. 2000	USA	1946	494 (25.4) [a,b]	114 (5.8) [a]	44 (2.3) [a]	67 (3.4) [a]	42 (2.2)	95/504 (18.8) [a]

[a]Significant difference on comparing with our cohort (P<0.05); [b] (HeH) High Hyperdiploidy (51~65 chromosomes) only

Besides, common alterations observed varied between 0.5-5% cases while 6 patients showed complex karyotypes (Table 2).

Additionally as shown in Figure 2, in the current study FISH analysis helped reveal additional structural or numerical changes. These included cryptic deletions at ABL breakpoint on chromosome 9(q) (n=7), multiplication involving the 22q11.2 BCR locus (n=1), presence of masked double/ multiple Philadelphia chromosome (n=4) in BCR-ABL positive patients; and deletions of non-

Table 5. Comparison of Chromosomal Aberrations between Indian and Western Adult ALL Patients

Study	Country	Total cases, n	Hyperdiploidy, n (%)	Hypodiploidy, n (%)	t(9;22)/ BCR–ABL, n (%)	t(1;19)/ TCF3–PBX1, n (%)	t(4;11)/ MLL–AFF1, n (%)	t(12;21)/ ETV6–RUNX1, n (%)
Present Study, 2015	India	72	7 (9.7)	11 (15.2)	21 (29.2)	8 (11.1)	1 (1.4)	0
Sazawal et al. 2004	India	34	—	—	5 (14.7)	2 (5.8)	0	0
Safaei et al. 2013	Iran	40	3 (7.5) [b]	1 (2.5) [a]	7 (17.5)	1 (2.5)	—	0
Chen et al. 2012	China	296	7 (2.3) [a b]	—	106 (35.8)	2 (0.6) [a]	21 (7)	2/241 (0.8)
Moorman et al. 2010	Britain	240	17 (7) [b]	7 (3) [a,c]	32/208 (15.3) [a]	(<10) [a]	(<10)	—
DeBraekeleer et al. 2010	France	84	9 (10.7) [b]	13 (15.47)	28 (33.3)	1 (1.2) [a]	6 (7.1)	0
Mancini et al. 2005	Italy	266	18 (6.77) [b]	—	101 (37.9)	7 (2.6) [a]	24 (9) [a]	1/162 (0.6)
Secker et al. 1997	Britain	204	24 (12) [b]	8 (4) [a]	33 (16) [a]	7 (3) [a]	3 (1)	—
GFCH, 1996	France	339	30 (8.8) [b]	34 (10)	127 (37.4)	11 (3.2) [a]	16 (4.7)	—
Walters et al. 1990	USA	103	(12)	(2) [a]	(12.6) [a]	—	—	—

[a] Significant difference on comparing with our cohort (P<0.05); [b] (HeH)-High Hyperdiploidy (51~65 chromosomes) only; [c] Low hyperdiploidy (30-39 chromosomes) / Near triploidy (60-78 chromosomes).

translocated ETV6 allele from 12p13 (n=2), deletions of residual RUNX1 allele on chromosome 21 (n=2) and multiplication involving the 21 RUNX1 locus (n=1) in cases suspected of ETV6-RUNX1. The novel abnormality of t(10;15)(q22;q22) has previously been reported by our group (Bhandari et al., 2014). Further, in the present study molecular [FISH and RT-PCR] techniques helped overcome hurdles of detection of cryptic rearrangements and lack of metaphase cells allowing identification of t(9;22) (n=24), t(12;21) (n=8), t(1;19) (n=16) and 11q23 rearrangements (n=5). In the present series, there was a patient in which two different fusion oncogenes t(9;22)/ BCR-ABL and t(1;19)/TCF3-PBX1 were confirmed by RT-PCR analysis although its karyotype was unsuccessful.

Discussion

The current study bears similarities with several ALL reports in certain aspects- viz. preponderance of ALL in males (60.5% observed *vs*. previously reported 60-85%) (Sazawal et al., 2004; Fauzdar et al., 2010; Chen et al., 2012); higher frequency of ALL in children (66.5% observed *vs*. previously reported 70%) (Chen et al., 2012; Safaei et al., 2013); preponderance of WBC counts less than 50,000/mm³ (71.6% observed *vs*. previously reported 72-77%) (Siraj et al., 2003; Chen et al., 2012); karyotypic culture success rate (80% observed *vs*. previously reported 83%) (Alonso et al., 2012) and higher frequency of chromosomal abnormalities (62.8% observed *vs*. previously reported 61.8%) (Santamaria-Quesada et al., 2009).

Although several molecular cytogenetic techniques

allow detection of chromosomal aberrations, conventional karyotyping still is the gold standard as it provides a global overview of the genome and a baseline to trace the evolution of the disease. To the best of our knowledge, this is one of the largest reported series of cytogenetic investigation in Indian B-lineage ALL cases presenting prevalence of each cytogenetic subtype listed in WHO classification.

Distributions of chromosomal rearrangements

In the current study, we compared frequencies of major cytogenetic subtypes between different groups and our series for pediatric and adult ALL, respectively (Tables 4 and 5). Considering the potential ethnic background difference between Western and Indian populations, such kind of investigation is important for Indian ALL patients.

As seen in Tables 4 and 5, Hypodiploidy was the most common abnormality with a high frequency in our pediatric (15.4% vs 1-6.5%, all P<0.05) and adult (15.2% vs 1.9-3.9%, all P<0.05) series. Several reports from France (15.4%, 10%) show concordance with our adult results (GFCH, 1996; Braekeleer et al., 2010) (Table 5). Interestingly, Amare et al. From India reported further enhanced incidences that were two fold higher or even greater (44.4% in adults and 38.4% in pediatrics) (Amare et al., 1999). These findings specify the fact of relatively high frequency of hypodiploidy in India suggesting that geographical and ethnic factors cannot be excluded. The scientific significance of hypodiploidy in ALL is its association with various clinical outcomes. A progressively worse outcome is reported with decreasing chromosome number, with an approximate 6-year EFS

of 65%, 40% and 25% for patients with 45, 33-44 and <28 chromosomes respectively (Heerema et al., 1999). Notably, near-haploidy and low-hypodiploidy are rare subgroups, associated with very poor outcome. Often they go underdiagnosed when masked by a co-existing good-prognostic hyperdiploid (>50 chromosomes) line (Stark et al., 2001). In the present study, two male patients revealed near-haploid karyotypes with various levels of ploidy ranging from near-haploidy (21 chromosomes) to hypodiploidy (45 chromosomes) to near-triploidy (60 chromosomes) to near-tetraploidy (90 chromosomes).

Interestingly a higher frequency of t(1;19) was observed in both our pediatric (9.1% vs 1-3.4%, all P<0.05) and adult (11.1% vs 0.6-3.2%, all P<0.05) groups (Tables 4 and 5). Notably, similar results in childhood-B-ALL were reported from Brazil (9.68%) (Mesquita et al., 2009) and Mexico (11.5%) (Jimenez-Morales et al., 2008). While t(1;19) is associated with nearly good outcome with modern intensive protocols, t(9;22) cases are associated with extremely poor prognosis and high relapse rates; hence, early detection is utterly crucial to initiate more aggressive therapy. In our adult ALL series, t(9;22) frequency was in agreement with most reports (29.2% vs 33-37%) and higher than few countries (29.2% vs 12.6-19%, all P<0.05) (Table 5). Likewise high frequency of t(9;22) was observed in pediatric cases (9.1% vs 1.1-3%, all P<0.05) (Table 4). Strikingly, several Asian studies showed a similar trend for t(9;22) in childhood-ALL (Pakistan-44%; India-12%,15%) (Awan et al., 2012; Mazloumi et al., 2012; Pandita et al., 2015) and adult-ALL (India-25%; Pakistan-20%) (Bhatia et al., 2012; Sabir et al., 2012).

The t(12;21) subtype was absent in our adult series, in agreement with previous reports (Table 5). In our pediatric series, t(12;21) was lower than most reports (5.6% *vs.* 14.2-42%, all P<0.05) (Table 4), yet similar to previous Indian studies (4.3-7%) (Siraj et al., 2003; Hill et al., 2005; Mazloumi et al., 2012). Likewise, t(4;11) was less frequent in our study, in agreement with previous reports for this sub-group (Table 4 and 5). Exceptions to our pediatric and adult results (3.5%, 1.4%) included lower frequencies from UK (0.98%, 0.9%) (Hann et al., 2001; Moorman et al., 2010a) and higher frequencies from Argentina (11.6%) (Alonso et al., 2012), Italy (9%) (Mancini et al., 2005) (all P<0.05) (Table 4 and 5). Previous Asian studies have associated t(12;21) patients with favorable clinical outcome and t(4;11) patients with poor outcome; hence, identifying these prognostic markers aids in determining the risk and further treatment (Amare et al., 1999; Sabir et al., 2012).

In contrast, hyperdiploidy in our pediatric cases was significantly lower than few Asian (Fauzdar et al., 2010; Safaei et al., 2013) and most western countries (10.5% vs 25-39%, all P<0.05) (Table 4). Strikingly, similar reports of lower hyperdiploidy in childhood-ALL are observed in India (5%; 14.8%; 15%) (Amare et al., 1999; Mazloumi et al., 2012; Pandita et al., 2015), China (10.6%) (Chen et al., 2012), Brazil (12.5%) (Mesquita et al., 2009) and Pakistan (13.4%) (Shaikh et al., 2014). Also, gain of chromosomes 2, 5, 6, 8, 9, 10, 21, 22 (n=2, 9.1%) and 1, 3, 7, 11, 12, 14, 15, 16 (n=1, 4.5%) were observed in pediatric hyperdiploid

cases. Hyperdiploidy in our adult series (9.7%) was similar to most reports, yet exceptionally higher than 2.3% of China (Chen et al., 2012) (P<0.05) (Table 5). Response to treatment rates varies among chromosome ploidy groups. Hyperdiploid-cases are associated with favorable outcome and less intense therapy (5-year EFS >84%), probably owing to increased accumulation of anti-leukemic drugs (methotrexate) in hyperdiploid blast-cells (Moorman et al., 2010a). Near-triploidy occurs rarely in childhood ALL (1%), and represents only 3-5% of the adult cases (GFCH, 1996; Secker-Walker et al., 1997). In present study, similar results were observed as triploidy and tetraploidy were absent in the pediatric cases and represented 3% adult cases. Thus in contrast to western reports, our study highlights ploidy variability such as scarcity of hyperdiploids and preponderance of hypodiploids.

Conventional cytogenetics, interphase FISH and multiplex RT-PCR measure chromosomal abnormalities at different biological levels. Cytogenetics involves analysis of metaphases and hence results are based only on the dividing leukemic cells. Whereas FISH and RT-PCR can determine rearrangements independent of cell division. Thus failure to identify chromosomal rearrangements cytogenetically may be attributed (a) to the presence of low levels of non-dividing leukemic cells (containing the chromosomal rearrangements) in the sample (Campbell et al., 1999; Watanabe et al., 2003); and (b) to the more sensitive molecular techniques like RT-PCR (detection limit of one abnormal cell from 10^4-10^6cells) and FISH (detection limit of 10^3cells) (Polampalli et al., 2011).

The contribution of novel chromosomal rearrangements in B-Lineage ALL is less clear and few have been mapped in detail in the Indian sub-continent. All such abnormalities detected in the present study were checked against database of chromosomal aberration [http://atlasgeneticsoncology.org/]. Although the exact consequences of such rare, novel aberrations remain to be elucidated, they could represent an important event in the tumorigenesis of hematological malignancies.

Certain common changes observed included del(12)(p11.2-13) (3.2%), del(11q23) (3.2%), del(6)(q15/q13q21) (1%), del(3)(p24-25) (1.4%), del(9)(p13/p22/q32q34) (1.4%), i(7)(q10) (0.5%), i(9)(q10/q12) (1.4%) and i(17)(q10) (0.5%). In childhood ALL, all 12p cytogenetic abnormalities do not involve ETV6 (12p13.2) and reports suggest that native ETV6 deletions in ETV6-RUNX1+ childhood ALL are associated with a favorable treatment outcome. Recent data indicates no difference in overall outcome between childhood ALL cases with *vs.* without 12p abnormalities (Heerema, 2000). In this study, we observed terminal deletions in 12p at 3 different breakpoints (p11.2, p12 and p13) to co-exist with hyperdiploidy, hypodiploidy, del(21)(q22), del(6)(q15), +16, +21, del(5)(q33q35), +8, and i(9)(q12).

Chromosomal rearrangements of MLL (11q23) gene are associated with high-risk acute leukemias (Pui et al., 2003). In the present study, del(11q23) occurred in cases with t(1;19)(q23;p13), hypodiploidy and XYY karyotype. In ALL, del(6q) occurs as a sole anomaly (30% of cases) or in association with specific abnormalities like del(12p), del(9p), t(4;11), t(1;19), t(9;22), t(12;21) or with random

chromosomal changes (Brigaudeau C and Bilhou-Nabera C, 1998). In the present study, we observed terminal deletion at 6q15 along with del(12)(p11.2); and interstitial deletion at 6q13q21as part of a complex karyotype.

Deletion of chromosome arm 3p25/p26 and 9p/q is frequent in hematologic malignances (Johansson et al., 1997). Furthermore, 9p abnormalities may hold significant negative impact on survival in adult BCP-ALL patients (Nahi et al., 2008). In this study, we observed deletion at 3p25-26 in a hypodiploid infant, in a complex karyotype and as a sole abnormality; while deletion at 9p/q occurred in a complex karyotype, with XXY karyotype and as a sole anomaly. Isochromosomes are relatively unusual and those readily identified in 1-4% childhood ALL include i(6p), i(7q), i(9q), and i(17q). Both i(9q) and i(7) occur in ALL either as a sole anomaly or in combination with non-random translocations like t(1;19), t(9;22) and (4;11) (Pui et al., 1992).

In conclusion, this study reveals that chromosomal aberrations are frequent in *de novo* Indian B-lineage ALL patients. Furthermore their frequency and distribution differs between childhood and adult patients, and also differs between Indian and Western settings. Hence the potential impact of these differences on clinical behavior and treatment outcome merits further investigation. Our study bears important epidemiological data and confirms previous reports demonstrating the identity of unusual complex clonal chromosome aberrations in B-lineage ALL, suggesting that it is heterogeneous in its pathogenesis. Similarities and dissimilarities of the present findings with those of other researchers may be attributed to the influence of differential environmental exposure to unknown carcinogenic agents, differences in geographical distribution and ethnic origins.

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