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Cytogenetic Profile in 7209 Indian Patients with *de novo* Acute Leukemia: A Single Centre Study from India

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Abstract

Background: Cytogenetics is one of the most important diagnostic parameters in the classification of acute leukemia. Recurrent chromosomal aberrations in acute leukemia have provided insights into the molecular mechanism of leukemogenesis. The variable frequencies of recurrent cytogenetic markers due to ethical/racial differences have been reported from Western and some Asian countries. Objective: We report cytogenetic data of largest cohort of 7209 adult and pediatric patients with *de novo* acute leukemia (AL) to determine the prevalence of various cytogenetic sub groups and compare with the Western and Asian population. Material & Methods: The AL patients included 2609 AML (adult: 2042, pediatric: 567), 3708 B-cell-precursor (BCP)-ALL (adult: 1300, pediatric: 2408) and 892 cases of T-ALL (adult: 480, pediatric: 412). Cytogenetic studies included conventional karyotyping and FISH using panel of probes. Results: The incidence of t(8;21) was high, comparable to other Asian countries. In comparison to our series and Western population, t(15;17) was more prevalent in Chinese population. Cytogenetic profiling of BCP-ALL revealed low prevalence of *ETV6/RUNX1* in ours as well as other Asian population. The *MLL* aberrations in BCP-ALL and *TLX1* & *TLX3* aberrations in T-ALL occurred less frequently in our series as compared with Western population. Conclusion: The present study with a large cohort showed the heterogeneity of AL that involved various factors, such as age, gender and prevalence of distinct cytogenetic subgroups. Our data in comparison with other population based studies revealed differential distribution of some cytogenetic sub-groups indicating geographic heterogeneity due to differential environmental exposure which probably influenced underlying genetic susceptibility.

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Keywords

Cytogenetics, Acute Leukemia, Incidence, Asian Population, Geographic Heterogeneity

1. Introduction

Acute leukemia (AL) that includes genetically heterogeneous acute myeloid and acute lymphoblastic leukemias accounts for 25% of all leukemias in adults [1]. Cytogenetics is one of the most important diagnostic parameter in the classification of acute myeloid leukemia (AML) [2] [3]. In addition to its importance in diagnosis, prognostic significance of cytogenetic subtypes has also been identified by various cooperative groups such as SWOG, MRC and CALGB. This has further led to establishment of ELN and NCCN guidelines that include cytogenetic risk groups: 1) favorable which includes t(8;21), inv(16) and t(15;17) with overall survival (OS) rates of 55% - 65%; 2) intermediate with normal karyotype and; 3) adverse that frequently shows MDS-related abn(5), abn(7), inv(3), -17/abn(17p) and monosomal and/or complex karyotype with OS rates of 5% - 14% [2] [4]-[10].

As in AML, recurrent chromosomal aberrations observed in acute lymphoblastic leukemia (ALL) have provided insights into the molecular mechanism of leukemogenesis [11] [12]. Around 80% of childhood and adult B-cell precursor-ALL (BCP-ALL) reveal recurring numerical and structural alterations such as translocations, deletions and copy number changes [13]-[18].

Over the past three decades, cytogenetic alterations have been an integral part of diagnosis and prognostication of disease in ALL. High hyperdiploidy: 51 - 65 chromosomes, t(12;21): *ETV6*/*RUNX1* and t(1;19): *TCF*3/*PBX*1 are associated with favorable outcome, whereas t(9;22) and 11q23 (*MLL*) translocations, intrachromosomal amplification of chromosome 21 (iAMP 21) and hypodiploidy are associated with poor prognosis [13] [14] [17] [19]-[21].

Recurring chromosomal abnormalities with variable prognosis have also been identified in 70% of T-ALL which includes hypodiploidy, 9p deletion, translocations involving T-cell receptor genes *TCR*-α and -β, transcription factor genes viz., *TAL*1, *LYL*1, *TLX1*/*HOX* 11 and *TLX*3/*HOX11L*2 [15] [22]-[24]. Fluorescence *in situ* hybridization (FISH) and conventional cytogenetics are the main strategies for cytogenetic workup of acute leukemia in disease management [13] [25]-[27]. Independent FISH analysis is used in several laboratories due to high sensitivity for detection of various cryptic structural and submicroscopic abnormalities, and also copy number changes in AL within short turnaround time [25]-[30].

The variable frequencies of recurrent cytogenetic markers due to ethical/racial differences have been reported in some published studies from Western and some Asian countries [31]-[36]. In the current study, we present cytogenetic data of a largest cohort of 7209 patients with *de novo* acute leukemia from a single centre. The aim of the study is to analyze the cytogenetic pattern with prevalence of various recurrent cytogenetic markers and to compare it with reported literature.

2. Material and Methods

The study was conducted at the Department of Medical Oncology, Tata Memorial Hospital, Mumbai, India. It is the largest cancer diagnostic, treatment and research centre in India, with 40,000 - 50,000 new cancer cases being diagnosed every year. The present study includes 7209 untreated patients that were newly diagnosed with AL between January, 2008 and December, 2015. Of these, 4600 cases were of ALL and 2609 cases were of AML that included patients diagnosed with acute promyelocytic leukemia (APL). Among the 2609 AML cases, 567 were pediatric and 2042 were adult cases, whereas the 4600 cases of ALL consisted of 2408 cases of pediatric BCP-ALL, 1300 cases of adult BCP-ALL, 480 cases of adult T-ALL and 412 cases of pediatric T-ALL.

The initial diagnostic criteria included standard clinical and laboratory findings including immunophenotyping. On the other hand, cytogenetic workup by FISH and conventional cytogenetics is an integral part of routine classification, risk stratification with appropriate treatment decision and monitoring of the disease. Cytogenetic studies were performed using standard protocols preferably in bone marrow aspirate and in peripheral blood in around 10% of cases, and occasionally on bone marrow morphology smears. At least 15 - 20 GTG-banded metaphase cells were karyotyped and analysed as per ISCN 2009 and 2013 [37] [38]. In ALL, ploidy analysis was

done by counting chromosome number in 30 - 40 metaphase cells. Ploidy was classified into various subgroups: Low and high hyperdiploidy, low and high hypodiploidy, near haploidy, diploidy and near triploidy/tetraploidy.

In addition to conventional cytogenetics, FISH was performed in every specimen by using a panel of probes-LSI dual fusion *RUNX1*/*RUNX1T*1, *PML*/*RARA*, break apart *CBF-B*, *EVI*1, *MLL*, *RARA*, LSI 5q31, 5q33/5p15, 7q22, 7q36/SE7, *TP53*/CEP 17 in AML; dual fusion *BCR*/*ABL*1, *ETV6*/*RUNX*1 *ES*, *TCF*3/*PBX*1, break apart *MLL*, *TCF*3, CEP 4, 6, 10, 17 in BCP-ALL; and break apart probe *TCR-A*, *TCR-B*, *TLX*1, *TLX*3, *MLL* and LSI 9p/CEP 9 in T-ALL. *MLL* translocations were characterized using dual fusion *MLL*/*MLLT*3 for t(9;11), *MLL*/*MLLT*2 for t(4;11), *MLL*/*MLLT*1 for t(11;19) and *MLL*/*MLLT*4 for t(6;11) (Abbott Molecular, Zytovision, Kreatech Diagnostic and Cytocell, UK). Minimum 100 - 200 interphase cells and 5 - 10 metaphase cells were analyzed in each specimen. The cut off threshold for trisomy and dual fusion was 2% and for break apart probe and LSI deletion probe was 5%. The cut off threshold for CEP and LSI on BMA smear was 5% and 10%, respectively.

Age-related prevalence and variable ploidy association of recurrent chromosomal alterations were analyzed by the Pearson's chi-square test for determining the statistical significance (SPSS version 20).

3. Results

3.1. Incidence of Recurrent Cytogenetic Abnormalities in AML Patients

Among the 2042 adult AML patients, 1240 were males and 802 were females (M/F ratio of 1.5) in the age range of 16 - 86 years (median age 38 years). Among the 567 pediatric AML patients, 394 were males and 173 were females (M/F ratio of 2.2) in the age range of 5 months - 15 years (median age 8 years). Of these 567 pediatric AML cases, 42 were in the age range of 5 months - 1 year.

3.2. Adult AML

Among the 2042 AML patients, 9% (179 cases) showed the translocation, t(15;17), whereas 1.4% (3 cases) revealed *RARA* variant translocation, t(11;17)(q13;q21), t(11;17)(q23;q21) and t(v;17). The detection rate of *PML/RARA* by FISH was 98% (220/225) (**Table 1**).

Among 1906 patients with successful results, frequency of t(8;21), inv(16)/t(16;16) and *MLL* translocations was 15% (280 cases), 4.4% (85 cases) and 5% (103 cases), respectively. In the t(8;21) positive group, 98% patients were below 50 years. The complex variants of t(8;21) were detected in 11% (30/280) cases, whereas two

Table 1. Incidence of recurrent cytogenetic aberrations in present series, adult AML (n = 2042), pediatric AML (n = 567) and comparison with other populations.

Cytogenetic Markers	Adult AML				Pediatric AML			
	Frequency				Frequency			
	Present Study	Western population	Asian population		Present Study	Western population	Asian Population	
			China	Japan			China	Japan
t(8;21)	15%	6% - 8%	8% - 15%	16%	26%	7% - 16%	9%	25%
t(15;17)	9%	7% - 13%	14% - 18%	11%	7%	5% - 10%	16% - 18%	7%
inv(16)/t(16;16)	4%	4% - 7%	3% - 4%	5%	5%	5% - 8%	11%	4%
MLL translocations	5%	2% - 5%	2% - 4%	5%	8%	7% - 15%	-	9%
inv(3)/t(3;3)	1.5%	1% - 3%	1%	-	0.6%	< 1%	1%	-
−5/del(5q)	3%	3% - 10%	1%	6%	2%	1% - 2%	1%	1% - 2%
−7/del(7q)	6%	4% - 7%	1%	7%	9%	2% - 7%	1%	1% - 2%
+8	8%	4% - 10%	4%	7%	9%	5% - 10%	3%	1% - 2%
−17/abn(17p)	2%	2% - 4%	-	-	-	-	-	-

Western population: 3, 6, 8, 39, 40, 42, 44, 45, 46, 48, 49, 50; Asian population: 31, 32, 35, 43, 47.

cases (0.1%) revealed (iAMP21). Overall frequency of t(8;21), inv(16) and t(15;17) was 62% - 70% in children and young adults (1 - 38 years). Of 1906 patients, 103 patients with *MLL* translocation comprised of 36 cases (2%) with t(9;11)(p22;q23), 13 cases (0.7%) with t(6;11)(q27;q23), 3 cases (0.16%) with t(11;19)(p13;q23), 2 cases (0.1%) with t(11;17)(q21;q23) and 45 cases (2%) with t(v;11). The *MLL* amplification was detected in two cases (0.1%) above 50 years. Trisomy 8 (8.4%: 16/1906) was another common abnormality (**Table 1** and **Figures 1(a)-(e)**).

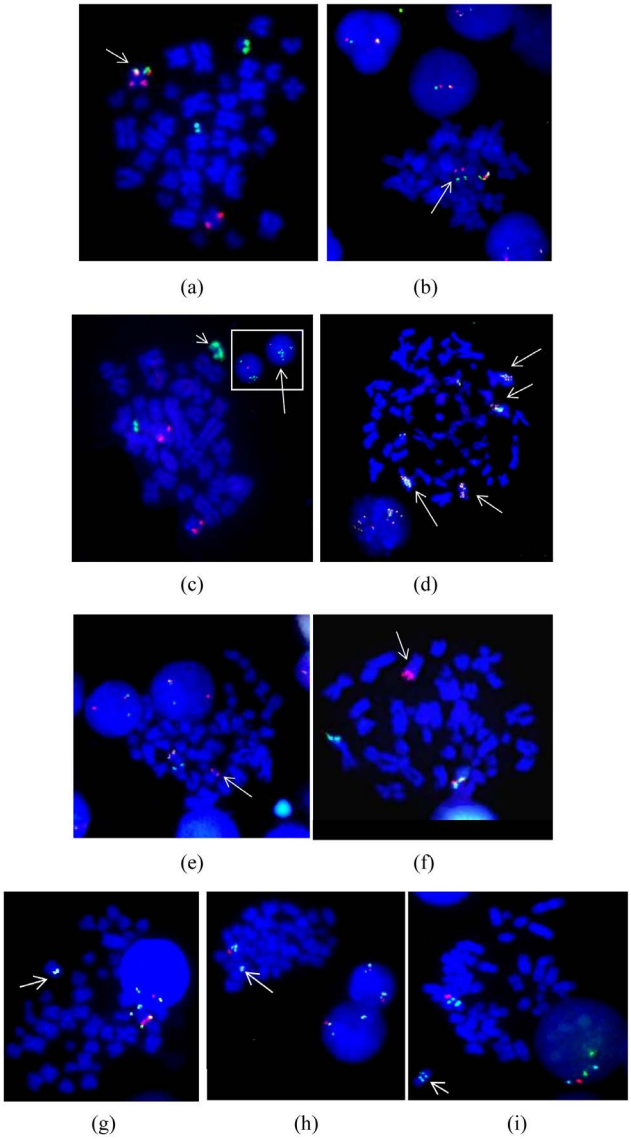

Figure 1. (a) *LSI RUNX*1/*RUNX*1*T*1 dual fusion probe on metaphase cell shows der(8) with *RUNX*1/*RUNX*1*T*1 (Yellow signal) and residual *RUNX*1*T*1 (Red signal) indicating variant of t(8;21); (b) LSI dual colour *CBF-β* break apart probe on metaphase and interphase shows 1R1G1Y signal pattern indicative of inv(16); (c) LSI dual fusion *RUNX*1/*RUNX*1*T*1 probe on metaphase and interphase cell shows *RUNX*1 amplification on der(21) (iAMP21); (d) LSI dual colour *MLL* break apart probe on metaphase and interphase cell shows *MLL* amplification; (e) LSI dual colour *MLL* break apart probe on metaphase and interphase cell shows der(9) with residual *MLL* (Red signal) indicating t(9;11); (f) LSI dual colour *EVI*1 break apart probe on metaphase cell shows 1R1G1Y signal pattern with residual *EVI*1 (Red signal) on another chromosome indicating *EVI*1 translocation; (g) Triple colour LSI 5q31,5q33/*hTERT* 5p15 probe on metaphase and interphase cell shows absence of Red & Green signal on der(5) indicating del(5)(q31q33); (h) Triple colour LSI 7q22, 7q36/SE7 on metaphase and interphase cell shows absence of Red & Green signal on der(7) indicating del(7)(q22); (i) Triple colour LSI 7q22, 7q36/SE7 on metaphase and interphase cell shows absence of Red signal on der(7) indicating del(7)(q36).

In the adverse cytogenetic group, frequency of *EVI1* aberrations inv(3)/t(3;3) was 1.5% (14/919 cases). Among the 3.4% (47/1387 cases) of abn(5) positive patients and 6.3% (87/1387 cases) of abn(7) positive patients, 5q deletion (96%) and 7q deletion (58%) were most common, respectively. Both these MDS-related abnormalities were frequently detected (80% - 83%) in patients above 30 years. The abn(17) [two cases of -17 and 13 cases of del(17p)] occurred in 1.6% (15/919) cases, especially in those above 38 years (**Table 1**) (**Figures 1(f)-(i)**).

3.3. Pediatric AML

The incidence of t(8;21), t(15;17), *MLL* translocations and inv(16)/t(16;16) in pediatric cases was 26% (148/567), 7.2% (41/567), 8% (45/567) and 5% (18/342) respectively. Frequency of complex variant of t(8;21) was 3.5% (20/567). Of 567 cases, 45 patients with MLL translocations comprised 20 cases (3.5%) with t(9;11), 4 cases (0.7%) with t(6;11), 3 cases (0.5%) with t(11;19), 1 case (0.2%) with t(4;11) and 7 cases (1.2%) with variant MLL translocation. The incidence of abn(5) and abn(7) was 2% (6/295) and 8.8% (26/295), respectively. Among these, 5q deletion (100%) and 7q deletion (62%) were most common (**Table 1**).

3.4. Incidence of Recurrent Cytogenetic Abnormalities in BCP- ALL Patients

Among the 2408 patients with pediatric Pre-B ALL, 1592 were males and 816 were females (M/F ratio of 2) in the age range of 2 months - 15 years (median age 5 years) and 137 cases were ≥1 years. Among the 1300 adult patients, 930 were males and 370 were females (M/F ratio of 2.5) in the age range of 16 - 77 years (median age 28 years).

3.5. Pediatric BCP-ALL

Ploidy analysis was performed in 2016 patients (84%) due to failure of cultures and unavailability of BM aspirate. The ploidy analysis revealed diploidy in 923 cases (46%), high hyperdiploidy in 540 cases (27%), low hyperdiploidy in 339 cases (17%), low hypodiploidy in 45 cases (2.2%), high hypodiploidy 128 cases (6.3%), haploidy in 7 cases (0.3%) and near triploidy/tetraploidy in 33 cases (1.6%) (**Table 2**). Trisomy 4, 6, 10, 17 were detected either as a sole or in combination in 41% (407/986) cases (**Table 2**).

Table 2. Incidence of recurrent cytogenetic aberrations in present series, adult BCP-ALL (n = 1300), pediatric BCP-ALL (n = 2408) and comparison with other populations.

Cytogenetic Markers	Adult BCP-ALL				Pediatric BCP-ALL			
	Frequency				Frequency			
	Present Study	Western population	Asian population		Present Study	Western population	Asian population	
			China	Japan			China	Japan
Hyperdiploid	24%	7% - 10%	3%	-	44%	25% - 30%	12%	
High Hyperdiploidy	9%	-	-	-	27%	-	-	-
Low Hyperdiploidy	15%	-	-	-	17%	-	-	
Tri-tetraploidy	2.5%	1%	-	-	2%	1%	-	-
Hypodiploidy	13%	1% - 3%	2%	-	8%	2% - 5%	3%	
Low Hypodiploidy	4%	-	-	-	2%	-	-	-
High Hypodiploidy	9%	-	-	-	6%	-	-	
Haploidy	0.5%	1>%	-	-	0.3%	1>%	-	-
+4, +10, +17	19%	-	-	-	41%	40% - 65%	-	-
t(9;22)	27%	25% - 30%	28%	7%	6%	2% - 5%	6%	3%
t(12;21)	0.2%	2% - 3%	0%	5%	12.2%	20% - 25%	13% - 19%	13%
(iAMP21)	0.4%	-	-	3%	1%	1% - 3%	-	2%
t(1;19)	4%	3%	1%	5%	7%	5% - 6%	3%	6%
t(17;19)	-	-	-	-	0.6%	-	-	-
MLL translocations	2.4%	5% - 10%	2%	3%	3%	5% - 8%	1.5%	1.6%

Western population: 12, 13, 14, 16, 19, 21, 51, 52, 53, 59, 60, 61, 62, 63, 64; Asian population: 36, 55, 56.

Among 2408 patients, *BCR*/*ABL*1 and *MLL* translocations were detected in 154 cases (6.3%) and 74 cases (3.1%), respectively. *MLL* translocations were characterized as t(4;11) in 28 cases (1.2%), t(11;19) in 8 cases (0.3%) and *MLL* variants in 21 cases (0.9%). Among the 28 cases of t(4;11), 22 cases (79%) were in ≥10 years age group, of which 11 cases (39%) were infants (2 months - 1 year). Translocation (1;19) was observed in 7.2% cases (169/2362).

Translocation t(12;21): *ETV*6/*RUNX*1 was detected in 12.2% cases (291/2982), of which loss of *ETV*6 allele in 162 cases (55%) and trisomy 21 in 80 cases (27%) were the most common additional abnormalities. The *ETV*6 allelic loss and trisomy 21 were also detected in 5% cases (102/2101) and 37% cases (783/2101), respectively in case of *ETV*6/*RUNX*1 negative group, whereas (iAMP21) was found in 23 cases(1%) (**Table 2** and **Figures 2(a)-(e)**).

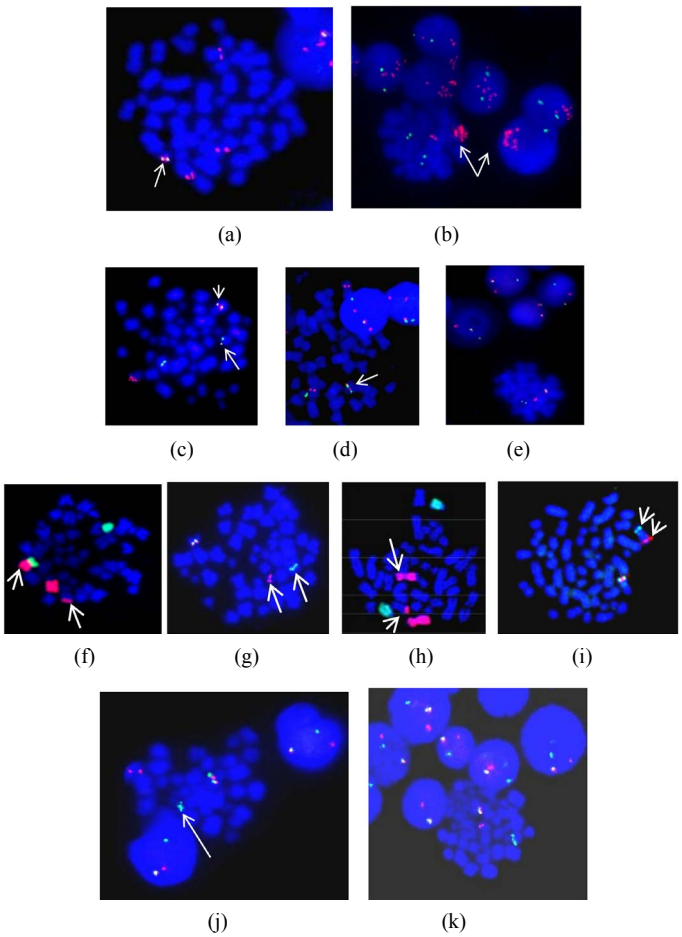

Figure 2. (a) LSI dual colour *ETV*6/*RUNX*1 ES translocation probe on metaphase and interphase cell shows *ETV*6/*RUNX*1 (Yellow signal) on der(21) with additional copy of *RUNX*1 and absence of *ETV*6 allele indicating *ETV*6/*RUNX*1 with trisomy 21 and loss of *ETV6* allele; (b) LSI dual colour *ETV*6/*RUNX*1 ES translocation probe on metaphase and interphase cell shows *RUNX1* amplification on der(21) (iAMP21); (c) LSI *MLL*/*MLLT*2 dual fusion probe on metaphase cell shows reciprocal *MLL*/*MLLT*2 (Yellow signal) on der(4) and der(11): t(4;11); (d) LSI *TCF*3/*PBX*1 translocation probe on metaphase and interphase cell shows *TCF*3/*PBX*1 (Yellow signal): t(1;19); (e) LSI dual colour *TCF*3 break apart probe on interphase and metaphase cell shows 1R1G1Y signal pattern in a specimen with no evidence of *TCF*3/*PBX*1 indicating variant t(17;19); (f) WCP 7 (Red) and WCP 14 (Green) on metaphase cell shows t(?;7;14)(?;p12;q11.2); (g) LSI dual colour *TCR-α* break apart probe on metaphase cell shows residual *TCR-α* (Green signal) on der(11) indicating t(11;14)(p15;q11.2); (h) WCP 7 (Red) and WCP 14 (Green) on metaphase cell shows residual 7q36 region (Red signal) on 10q24 indicating t(7;10)(q34;q24); (i) LSI dual colour *TCR-β* break apart probe on metaphase cell shows residual *TCR-β* (Green signal) on 7p indicating t(7;7) (q34;q15)/inv(7)(p15q34); (j) LSI dual colour *TLX*1 break apart probe on metaphase and interphase cell shows green signal on der(14) indicating t(10;14)(q24;q11.2); (k) LSI dual colour *TLX*3 break apart probe on metaphase and interphase cell shows 1R1G1Y signal pattern indicating *TLX*3 translocation.

3.6. Age and Ploidy Distribution of Cytogenetic Subgroups in Pediatric Pre-B ALL

Hyperdiploidy ($p = 0.0005$), trisomy 4, 6, 10, 17 ($p = 0.0005$), *MLL* translocations ($p = 0.025$) and *ETV*6/*RUNX*1 ($p = 0.0005$) were associated with lower age group (1 - 10 years), whereas *BCR/ABL1* ($p = 0.0005$) was associated with higher age group (11 - 15 years) (**Table 3(a)**). The ploidy distribution revealed association of *BCR/ABL*1, *ETV*6/*RUNX*1 and *TCF*3-*PBX*1 with diploidy ($p = 0.0005$) and of trisomy 4, 6, 10, 17 with hyperdiploidy ($p = 0.0005$) (**Table 3(b)**).

3.7. Incidence of Recurrent Cytogenetic Abnormalities in Adult BCP-ALL

Ploidy analysis was performed in 1015 of total 1300 cases (78%) due to poor mitotic index. Ploidy analysis revealed diploidy in 617 cases (61%), low hyperdiploidy in 147 cases (15%), high hyperdiploidy in 93 cases (9%), low hypodiploidy in 39 cases (4%), high hypodiploidy in 88 cases (9%), haploidy in 5 cases (0.5%) and near triploidy/tetraploidy in 26 cases (2.5%). The trisomy 4, 6, 10, 17 were detected either alone or in combination in 19% (83/432) cases (**Table 2**).

The incidence of *BCR*/*ABL*1 was observed in 27% (357/1300) cases, *MLL* translocation in 2.4% (31/1300) cases and *TCF*3/*PBX*1 in 4.4% (56/1268) cases. The variant t(17;19) was detected in 0.6% of the 166 cases

Table 3. (a) Cytogenetic markers and age distribution in Pediatric and adult BCP-ALL; (b) Cytogenetic markers and ploidy distribution in Pediatric and adult BCP-ALL.

(a)

Cytogenetic Markers	Pediatric BCP-ALL				p-value	Adult BCP-ALL				p-value
	Age					Age				
	1 - 10 years		11 - 15 years			≤40 years		>40 years		
	Pos	Neg	Pos	Neg		Pos	Neg	Pos	Neg	
+4, +10, +17	368 (90%)	428 (74%)	39 (10%)	151 (26%)	0.0005	64 (77%)	273 (78%)	19 (23%)	77 (22%)	0.826
t(9;22)	90 (58%)	1803 (80%)	65 (42%)	448 (20%)	0.0005	235 (66%)	755 (80%)	122 (34%)	186 (20%)	0.0005
t(12;21)	271 (93%)	1613 (77%)	20 (7%)	488 (23%)	0.0005	-	-	-	-	-
MLL translocations	66 (89%)	1825 (78%)	8 (11%)	505 (22%)	0.025	22 (71%)	967 (77%)	9 (29%)	297 (23%)	0.474
t(1;19)	127 (75%)	1737 (79%)	42 (25%)	456 (21%)	0.213	51 (91%)	922 (76%)	5 (9%)	290 (24%)	0.009

(b)

Cytogenetic Markers	Pediatric BCP-ALL					Adult BCP-ALL				
	Ploidy					Ploidy				
	Diploidy		Hyperdiploidy			Diploidy		Hyperdiploidy		
	Pos	Neg	Pos	Neg	p-value	Pos	Neg	Pos	Neg	p-value
+4, +10, +17	23 (6%)	395 (79%)	374 (94%)	103 (21%)	0.0005	10 (13%)	241 (81%)	65 (87%)	57 (19%)	0.0005
+21	85 (11%)	817 (80%)	677 (89%)	201 (20%)	0.0005	-	-	-	-	-
t(9;22)	66 (80%)	856 (50%)	17 (20%)	864 (50%)	0.0005	167 (78%)	450 (70%)	47 (22%)	192 (30%)	0.007
t(12;21)	152 (65%)	764 (49%)	83 (35%)	797 (51%)	0.0005	-	-	-	-	-
MLL translocations	43 (86%)	879 (50%)	7 (14%)	873 (50%)	0.0005	13 (72%)	603 (72%)	5 (28%)	234 (28%)	0.987
t(1;19)	121 (79%)	792 (49%)	33 (21%)	833 (51%)	0.0005	33 (75%)	573 (72%)	11 (25%)	222 (28%)	0.673
Age 1 - 10 years 11 - 15 years	- 690 (47%) 232 (69%)	-	- 778 (53%) 103 (31%)	-	- 0.0005	≤40 years 477 (72%) >40 years 140 (73%)	-	- 189 (28%) 51 (27%)	-	0.649

(1/166). Thirty one patients with MLL translocation included 19 cases (1.5%) of t(4;11), two cases (0.15%) of t(11;19), one case (0.07%) each of t(9;11) and t(6;11) and eight cases (0.6%) of variant *MLL* translocations (**Table 2**).

*ETV*6/*RUNX*1 was observed in three cases (0.2%) in the age group 21 - 26 years, whereas (iAMP21) was observed in five (0.4%) young adults (16 - 20 years) apart from one, 56 years old.

3.8. Age and Ploidy Distribution of Cytogenetic Subgroups in Adult BCP-ALL

The incidence of t(9;22) and t(1;19) was higher in patients under 40 years ($p = 0.0005$ and $p = 0.009$, respectively). Ploidy distribution revealed association of *BCR*/*ABL*1 with diploidy ($p = 0.007$) and association of trisomy 4, 6, 10, 17 with hyperdiploidy ($p = 0.0005$) (**Table 3**).

3.9. Incidence of Recurrent Cytogenetic Abnormalities and Age and Ploidy Distribution in T-ALL

Among the 480 adult patients with T-ALL, 391 were males and 89 were females (M/F ratio of 4.3) in the age range of 16 - 78 years (median age 24 years). Among the 412 pediatric T-ALL cases, 329 were males and 83 were females (M/F ratio of 4) in the age range of 1 - 15 years (median age 10 years).

3.10. Adult T-ALL

Ploidy analysis was performed in 296 cases due to low mitotic index. The ploidy distribution was as follows: Diploidy in 182 cases (61%), low hyperdiploidy in 36 cases (12%), high hyperdiploidy in five cases (1.7%), low hypodiploidy in 13 cases (4.4%), high hypodiploidy in 45 cases (15%) and triploidy/tetraploidy in 15 cases (5%) (**Table 4**).

Heterozygous/homozygous 9p deletion occurred in 32% (154/480) cases, whereas *MLL* translocations occurred in 1.3% (6/460) cases, of which t(11;19) and MLL variant translocations occurred in 0.2% (1/460)and 1.1% cases(5/460), respectively.

TCR-α and *TCR-β* occurred in 18% cases (85/460), whereas *TLX*1 and *TLX*3 translocations occurred in 3.5% (16/460) and 7.6% (35/460) cases, respectively (**Table 4** and **Figures 2(f)-(k)**).

There was no association of ploidy or variable age groups with cytogenetic subgroups, except for association of 9p deletion with young adults ≤40 years ($p = 0.0005$) and with diploidy ($p = 0.03$) (**Table 5(a)** and **Table 5(b)**).

Table 4. Incidence of recurrent cytogenetic aberrations in present series, adult T-ALL (n = 480), pediatric T-ALL (n = 405) and comparison with other populations.

Cytogenetic Markers	Adult T-ALL		Pediatric T-ALL	
	Frequency		Frequency	
	Present Study	Western population	Present Study	Western population
Hypodiploidy Low Hypodiploidy High Hypodiploidy	20% 5% 15%	-	18% 2% 16%	-
Hyperdiploid Low Hyperdiploidy High Hyperdiploidy	14% 12% 2%	-	11% 10% 1%	-
Tri-tetraploidy	5%	-	5%	-
del(9p)	32%	40% - 50%	44%	50% - 60%
MLL translocations	1.3%	4%	5%	-
TCR*α*, TCR*β*	18%	20% - 30%	21%	20% - 30%
TLX1	4%	15% - 20%	2.4%	5% - 10%
TLX3	8%	10% - 20%	11%	20%

Table 5. (a) Cytogenetic markers and age distribution in Pediatric and Adult T-ALL; (b) Cytogenetic markers and ploidy distribution in Pediatric and Adult T-ALL.

(a)

Cytogenetic Markers	Pediatric T-ALL					Adult T-ALL				
	Age					Age				
	1 - 10 years		11 - 15 years			≤40 years		>40 years		
	Pos	Neg	Pos	Neg	p-value	Pos	Neg	Pos	Neg	p-value
del(9p)	109 (61%)	111 (49%)	70 (39%)	115 (51%)	0.018	147 (95%)	255 (79%)	8 (5%)	66 (21%)	0.0005
MLL translocations	9 (47%)	205 (56%)	10 (53%)	160 (44%)	0.452	5 (83%)	328 (84%)	1 (17%)	62 (16%)	0.959
TCR*α*, TCR*β*, TLX1, TLX3	72 (56%)	130 (52%)	56 (44%)	119 (48%)	0.467	102 (90%)	285 (83%)	12 (10%)	58 (17%)	0.101

(b)

Cytogenetic Markers	Pediatric T-ALL							Adult T-ALL						
	Ploidy							Ploidy						
	Hypodiploidy		Diploidy		Hyperdiploidy			Hypodiploidy		Diploidy		Hyperdiploidy		
	Pos	Neg	Pos	Neg	Pos	Neg	p-value	Pos	Neg	Pos	Neg	Pos	Neg	p-value
del(9p)	20 20%	24 20%	72 67%	86 70%	14 13%	13 10%	0.826	32 27%	26 16%	73 63%	107 66%	12 10%	29 18%	0.03
MLL translocations	2 18%	43 20%	9 82%	142 67%	0 0%	27 13%	0.414	2 33%	51 22%	4 67%	141 62%	0 0%	37 16%	0.519
TCR*α*, TCR*β*, TLX1, TLX3	18 22%	25 19%	54 67%	88 69%	9 11%	15 12%	0.895	21 25%	35 19%	57 67%	116 63%	7 8%	32 18%	0.111
Age	-		-		-		0.447	≤40 years						0.212
1 - 10 years	21 47%	-	91 56%	-	16 59%	-	- -	50 86%	-	161 88%	-	32 78%	-	- -
	-	-	-		-		-	>40 years						-
11 - 15 years	24 53%		70 44%		11 41%		- -	8 14%		21 12%		9 22%		- -

3.11. Pediatric T-ALL

The ploidy analysis of 244 cases revealed diploidy in 161 cases (66%), low hyperdiploidy in 24 cases (10%), high hyperdiploidy in three cases (1.2%), low hypodiploidy in six cases (2.5%), high hypodiploidy in 39 cases (16%) and triploidy/tetraploidy in 11 cases (4.5%).

The 9p deletion occurred in 44% (179/405) cases, whereas *MLL* translocations occurred in 5% (18/386) cases, of which t(9;11) occurred in four cases (1%), t(11;19) and t(6;11) in one case each (0.25%) and variant *MLL* translocations in 12 cases (3.1%). The incidences of *TCR-α* (14q11) and *TCR-β* (7q34) translocations was 21% (79/377 cases), *TLX*1 (10q24) was 2.4% (9/377 cases) and *TLX3* (5q35) translocation was 11% (40/377 cases) (**Table 4**). The 9p deletion was associated with lower age group ≤10 years ($p = 0.018$) (**Table 5(a)** and **Table 5(b)**).

4. Discussion

The comprehensive analysis of heterogeneity of acute leukemia of the large cohort in the present study has demonstrated the involvement of various factors that involves age, incidence pattern, gender and genetic factors. Several studies from various locations have shown the geographic heterogeneity in acute leukemias [6] [20] [27] [30]-[32] [34] [36] [39] [40]. The cytogenetic pattern of acute leukemia in Indian population however is not clear due to lack of large scale studies. Hence, the main approach of the present study was to retrospectively analyze a large cohort to focus upon the epidemiology of various cytogenetic subtypes which may reveal the involvement of various etiological factors, thereby improving our understanding of the biology of the disease

and thus help in better management of the disease.

Our combined approach of conventional cytogenetics and FISH significantly improved the detection rate of submicroscopic cryptic rearrangements within a short turnaround time. We could detect *PML*/*RARA* with *RARA* variants in 98% of APL cases. **Table 1** summarizes the prevalence of recurrent chromosomal abnormalities in pediatrics and adult AML in present study and compares the incidence with different populations from USA, European and Asian countries.

In the present study, the median age of adult AML was lower (38 years) in comparison to Western population (40 - 45 years) and other Asian population [6] [8] [9] [31]-[34] [41]. In adult AML, the incidence of t(8;21) was higher (15%) than that of Western population (6% - 8%) and comparable to that in Chinese (8% - 15%) and Japanese (16%) populations [3] [6] [8] [31] [32] [35] [42]. Similarly, in pediatric AML, the frequency of t(8;21) was higher (26%) than that of Western population and comparable to that in Japanese population (25%) [8] [32] [39] [42]-[46]. However, the frequency of t(15;17) was comparable to that of Western population and was lower than that in Chinese population, both in adult and pediatric groups (14-18% and 16-18% respectively).

The higher incidence of t(15;17) in Chinese population could be due to high prevalence of APL in their population [3] [6] [8] [31] [39] [44] [46] [47]. Similar to literature reports, our data on age distribution of favorable subtypes t(8;21), t(15;17) and inv(16) in adult and pediatric AML also indicated that incidence of favorable markers are most common in children and young adults compared to older adults [3] [6] [8] [39] [44] [46]. Although overall *MLL* frequency in both adult and children were comparable with Western populations, our study showed lower (3%) incidence of t(9;11) in pediatric AML, similar to that in Chinese population [8] [31] [39] [44]-[48]. The translocation (4;11) occurred less frequently in pediatric AML probably due to lower number of infantile cases below one year in our series. The aberrations of chromosome 5, 7 and 8 were comparable with other studies however, these aberrations occurred with higher frequency compared to that in both adult and pediatric Chinese population [6] [31] [39] [40] [47]. The *RUNX*1 and *MLL* amplification frequencies (both 1%) of adult AML in our study have been reported as rare secondary events with dismal outcome [49] [50].

In BCP-ALL, the age and gender distribution was comparable to reports from other geographic areas [14] [15] [17]-[19] [41]. BCP-ALL has various cytogenetic subtypes which have significant impact on risk stratification and hence remain strong independent indicators of disease outcome [13] [14] [16] [17] [19]-[21]. The incidence of high hyperdiploidy in children (27%) and adult ALL (9%) are similar to that of Western population [12] [16] [51]. The overall hyperdiploidy rate observed in our study was probably due to slightly higher incidence of low hyperdiploidy in our patients. As reported in literature, hyperdiploidy in childhood ALL was strongly associated with gain of chromosomes 4, 10, 17 and 21 (**Table 3(b)**) [12] [51]. The hypodiploidy incidence was comparatively higher in both pediatric and adult populations due to elevated frequency of high hypodiploidy. Near triploidy-tetraploidy, which is rare in childhood ALL was observed both in childhood and adult ALL with incidence of 1% - 2% [13] [52]. Near haploidy with chromosome numbers 23 - 29 is a rare hypodiploid group in pre-B-ALL occurring in 0.3% - 0.5% in ALL group in our study falling within the universal frequency of <1% [13] [53].

The *ETV*6-*RUNX*1 is another common favorable subset of pediatric BCP- ALL that occurs with an average frequency of 25%, but showed lower incidence of 12% in our study. This finding is consistent with our previous study [27] and another cohort of 928 patients [Nahar A *et al.* in communication]. Data from various studies from Asian countries also indicated low prevalence (13% - 19%) of *ETV*6/*RUNX*1 [54]-[56]. Studies have reported incidence rates of <10% from other parts of India, however, these were smaller studies with sample size not large enough to focus on true prevalence [57] [58]. The peak incidence of *ETV*6/*RUNX*1 was in lower age group of 1 - 10 years as reported in our previous studies as well as in studies from other Asian countries [27] [52] [55] [59]. Trisomy 21 and *ETV*6 allelic loss were most frequent additional abnormalities in *ETV*6/*RUNX*1 positive group, however these abnormalities were common in overall BCP-ALL patients [27] [59].

The (iAMP21), a distinct high risk cytogenetic subtype was identified with 1% incidence in pediatric BCP-ALL patients predominantly in older children of 3 - 14 years age group which is in accordance with other reported studies [21] [60].

In our study, the t(9;22) incidence in childhood ALL was 6%, whereas it showed variable frequencies of 2% - 6% in Western and other Asian populations [14] [36] [59]. The t(9;22), which is the most common chromosomal abnormality in adult ALL occurred in 27% of the adult patients, falling within the universal range of 25% - 30%. It was more prevalent in older children as well as adult ALL under 40 years. Literature also indicate rising incidences of t(9;22) in older patients [61]-[63]. Geographic heterogeneity of variable frequencies of *BCR/ABL1* has

been reported [14] [62] [63].

The translocation (1;19) is another cytogenetic subgroup of BCP-ALL that occurs more frequently in children than adults. The incidence in both pediatric and adult patients in our study was closer to other reports [61]-[64] except in Chinese population which showed comparatively lower frequency [36].

Translocations involving the *MLL* (11q23) gene occur in up to 5% of childhood and adult BCP-ALL [19] [61]-[63]. The lower incidence of 2% - 3% observed in the present study as well as in studies from other Asian countries indicate that *MLL* rearrangement incidence vary in different populations [36]. Data from previous studies from our lab supports the finding that *MLL* rearrangement incidence is low in Indian population [30]. The t(4;11) that is known for its adverse outcome is the common *MLL* translocation in childhood ALL with higher frequencies (60% - 80%) in infants [61]-[63]. The lower incidence of 38% of t(4;11) in children in the present study is due to the low number of infants in our study. The incidence of t(4;11) was also found to be lower in childhood AML in the present study indicating under representation of infantile leukemia in India. This could be due to poor tendency of referring infantile leukemia cases to tertiary centres. Pattern of age distribution in *MLL* and t(1;19) positive groups as observed in other studies, suggested that these translocations tend to occur in lower age group [19] [30] [52] [61]-[64]. The ploidy distribution revealed association of favorable t(12;21)/*ETV*6-*RUNX*1, t(1;19) as well as unfavorable t(9;22), *MLL* translocation subtypes with diploidy.

T-ALL commonly affects young males than females. This was evident from the data obtained in the present study which revealed 3 - 4 times higher prevalence of T-ALL in young males [13] [15] [65]. Hypodiploidy is common in T-ALL as compared with Pre-B-ALL. It was comparatively higher in the present study in both pediatric and adult cases [13] [61]. On the contrary, *MLL* translocation frequency was lower in adult patients in our study than that of western population [66]. As reported previously, 9p deletion and translocation involving T-cell receptors *TCR-α* (14q11.2) and *TCR-β* (7q34), transcription factor genes *TLX*1 (*HOX*11) (10q24) and *TLX*3 (*HOX*11*L*2) (5q35) occurred in more than 50% of cases [22]-[24] [67] [68]. The incidence of *TCR-α* and *TCR-β* translocations in pediatric and adult T-ALL was similar to previous reports [65] [69]. Among the most common partner genes, the *TAL*1, *LYL*1, *LMO*1, *LMO*2, *TLX*1 and *TLX*3 of *TCR-α* and *TCR-β* translocations, *TLX*1 is associated with better outcome, whereas *TLX*3 has been found to have increased risk of treatment failure [22] [23].

In comparison to previous reports, the incidence of *TLX*1 and *TLX*3 translocations in the present study was lower in both pediatric and adult patients [22] [65] [69]. The study also observed that 9p deletion was common in young age group and associated with diploidy as has been reported previously [15] [22]-[24] [46] [69] [70].

5. Conclusion

The present study with a large cohort showed the heterogeneity of AL that involved various factors, such as age, gender and prevalence of distinct cytogenetic subgroups. Cytogenetic data when compared with data from other geographic areas revealed geographic heterogeneity due to differential environmental exposure which probably influenced the underlying genetic susceptibility.

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