ORIGINAL RESEARCH

Detection of Genetic Aberrationst(12;21)/ETV6-RUNX1, t(1;19)/TCF3-PBX1, t(4;11)/MLL-AF4and Their Prognostic Significance in Pediatric Acute Lymphoblastic Leukemia by Fluorescence In Situ Hybridisation

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ABSTRACT

Purpose: To find the frequency of any of these t(12;21)/ETV6-RUNX1, t(1;19)/TCF3-PBX1, t(4;11)/MLL-AF4 in pediatric Acute Lymphoblastic Leukemia by Fluorescence-In-Situ-Hybridization (FISH) and their prognostic implications. Multiple studies have been performed across the world for its detection through various techniques.But there is a paucity of literature available for detecting this cytogenetic aberration by FISH technique in India and so as to correlate the association of these genetic translocations with clinicohaematological parameters.

Methods: A hospital- based descriptive observational study was performed in forty-five newly diagnosed paediatric ALL cases after bilingual written informed consent and detailed history. 1ml sample(peripheral blood/bone marrow) of the cases was collected in a heparinised vial(before the commencement of induction therapy)and was subjected to FISH analysis.Vysis LSI dual colour dual fusion translocation probe for ETV6-RUNX1,TCF3-PBX1 and Vysis split signal apart probe for MLL rearrangements were used and the signals were interpreted in interphase nuclei.

Result: The study showed that four out of the forty five paediatric ALL cases (8.8%) showed ETV6-RUNX1 fusion signals with classic 2F1R1G pattern. 75% of cases had hepatosplenomegaly, lymphadenopathy (50%),high total leucocyte count and high tumorburden. All the four positive cases were CALLA positive B cell ALLimmunophenotypically and went into remission and was found to be disease free for 6 months follow up in the time periodof study.A single case (2.2%) of MLL split signal detected was also a female child had hepatosplenomegaly and lymphadenopathy. This case had anaemia, thrombocytopenia and blast percentage was more than 50%.Immunophenotypically it was a case of Pro B cell ALL. The typical pattern of 1G1R1F was obtained. This child had succumbed to death and associated with poor prognosis. No comment can be made regarding TCF3-PBX1 fusion as no case was found to be positive.

Conclusion: The study find a lower percentage (8.8%) of ETV6-RUNX1 fusion protein in Indian paediatric leukemic patients compared to Western literature. No statistical significance was established between the translocations and clinicohematological parameters. Thus,bigger sample size should be more representative for a definitive conclusion. The study couldsuggest incorporating FISH as a mandatory diagnostic tool in paediatric ALL workup for prognostic implications and risk stratifications.

Keywords: Genetic Abberations,t(12;21)/ETV6-RUNX1, t(1;19)/TCF3-PBX1, t(4;11)/MLL-AF4, Acute Lymphoblastic Leukemia, Fluorescence In Situ Hybridisation.

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is a neoplastic disease characterized by clonal expansion of leukemic cells in blood, bone marrow, thymus, spleen or lymph nodes. It is most common malignancy of childhood.[1] In the pediatric population, ALL alone accounts for 81% of childhood leukemias. Global incidence of ALL is 4,37,033 cases per year. In India, approximately 42,055 cases per year are reported, accounting for 10% of global burden (IACR 2019).[2] Cytogenetics is essential for prognostication & risk stratification in ALL. Chromosomal analysis still has an important role in the initial cytogenetic workup.[3]Cytogenetic aberrations are seen in upto 75% cases of pediatric ALL. These not only initiate leukemogenesis but are also used to predict prognosis.[4] The WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues 2017 classifies cytogenetic abnormalities further into low risk t(12;21), intermediate risk t(1;19) , and high risk t(4;11), t(9;22) to emphasize their impact on response to treatment and survival.[5] The cytogenetic abnormalities can be assessed by using techniques such as karyotyping, reverse transcriptase-polymerase chain reaction (RT-PCR) and fluorescence-in-situ-hybridization (FISH).[6] FISH is a sensitive and specific technique for accurate detection of all clinically relevant translocations. The detection of translocations is essential for risk stratification and in planning the treatment protocols in children.[7] The risk stratification according to the National Cancer Institute (NCI) criterion is[8]

A. Standard Risk (SR)

- Age >1 and <10 years
- Non-T-cell
- Prednisolone Good Responder
- No high-risk cytogenetics
- WBC <50,000/mm3
- MRD <10-4 after induction
- Complete Remission after induction
- No CNS disease

B. Intermediate Risk (IR)

- Good risk features but age ≥10 years
- Good risk features but WBC ≥50,000/mm3
- Good risk features but bulky lymph nodes (≥5 cm in peripheral region and in chest >5 cm onCT scan or occupying ≥1/3 diameter on chest x-ray) and/or bulky liver/spleen reaching beyond midway to umbilicus and/or presence of testicular disease
- MRD is less than 0.01%
- No High-Risk criteria

C. High Risk (HR)

- All prednisolone poor responders, irrespective of age and presenting WBC count
- High risk cytogenetics
- CNS disease
- MRD ≥10-4 after induction

D. High Risk (HR)

- All prednisolone poor responders, irrespective of age and presenting WBC count
- High risk cytogenetics
- CNS disease
- MRD ≥10-4 after induction

The study included 45 newly diagnosed paediatric ALL patients in which t(12;21), t(1;19), t(4;11) fusion gene analysis was done by FISH.

MATERIALS AND METHODS

Inclusion and exclusion criteria: This is ahospital based descriptive observational study was conducted on all newly diagnosed cases of paediatric ALL below18 years of age presenting to theKalawati Saran Children's Hospital (KSCH), over a period of 1 year 4 months (Nov 2018- March 2020) at Department of Pathology in Lady Hardinge Medical College and Kalawati Saran Children's Hospital (KSCH), New Delhi. The diagnosis of ALL was made on bone marrow morphology and flow cytometry.All cases of ALL already on chemotherapy and patients with relapse of ALL were excluded from the study.

Methodology: The cases at the time of diagnosis (day 0) were subjected to complete hemogram with peripheral smear, bone marrow aspiration, cytochemistry, immunophenotyping followed by FISH.As a part of FISH analysis, 1mlheparinised blood/bone marrow sample was taken and converted to a pellet which was preserved in Carnoy' s fixative. Once completed with the pellet wash, it was subjected to pre-treatment denaturation, hybridisation with probe (Vysis LSI dual colour dual translocation probe and Vysis break apart probe) followed by washing and interpretation of signals. Slides viewed under Nikon 80i fluorescence microscope equipped with DAPI, fluorescein isothiocyanate (FITC) and tetramethy lrhodamine B is othiocyanate (TRITC) fluorescence filters. 200 interphase nuclei were observed. If no fusion had occurred, the signal pattern obtained is 2R2G (R-red,G- green) for t(12;21), t(1;19) and 2F for MLL aberration. If t(12;21) , t(1;19) fusion and MLL aberrationwere present, then the signal pattern obtained was 1R1G2F& 1F1R1G (R-red,G-green,F-fusion)typically. Atypical patterns like 1R1G1F,1F2R1G,1F1R2G and 1F2R2G were also seen.

Statistical Analysis: Data was entered and analysed in SPSS version 20.Qualitative data was expressed in proportions. For quantitative data mean, range and standard deviation were calculated. Chi-square test was applied. P value of <0.05 were considered as significant.

RESULTS

The presence of ETV6-RUNX1 fusion gene was studied in relation to all the clinico-hematological parameters. However, only one parameter i.e BMA blast percentage was statistically significant parameter (<0.006). (Table 1) The presence of MLL split signals gene was studied in relation to all the clinico hematological parameters. However, only immunophenotyping parameters were only statistically significant ($p < 0.048$). (Table 2) There was no positive case of t (1;19)/ TCF3-PBX1 fusion detected in our study. So, no clinic-hematological correlation was done for TCF3-PBX1 fusion gene.On Day 0, FISH for ETV6-RUNX1 was put up in 45 newly diagnosed cases of ALL and signals were analysed. The fusion gene was detected in 4/45 cases (8.8%). All the cases were co-related to the clinic-haematological parameters. (Table 3) Though, there was no significant correlation between clinico-hematologial parameters and the detection of fusion gene. Among the 04 cases, there were 3 female and 1 male patient. 02 cases were of more than 10 years of age. 03 cases showed features of hepatosplenomegaly and 02 cases had lymphadenopathy. Only 01 case had TLC > 50,000/microliter. All the 04 cases had thrombocytopenia. All were CALLA+ B cell ALL. All were negative for minimal residual disease on day 35. All patients are alive and in remission. At Day 0, FISH for MLL aberrations was put up in 45 newly diagnosed cases of ALL and signals were analysed. The fusion gene was detected in 1/45 cases (2.2%%). (Table 4) Though, there was no significant correlation between clinico-hematologial parameters and the detection of fusion gene. 01 case of MLL split signal positive was a 4-month female child, had hepatosplenomegaly and lymphadenopathy. Patients had no genetic predisposition like ataxia telangiectasia and Down syndrome.TLC count was < 50,000/ microliter. Patient had thrombocytopenia. On flow cytometry, it was ProB cell ALL and negative for minimal residual disease at day 35. Child succumbed to death after threemonths of initial diagnosis.

Table 1: Association between patient characteristic and presence of t(12:21)

Characteristics	t(12;21)		Total n (%)	Chi square	p value
	Present n (%)	Absent n (%)			
Age					
0-5	1(25.0)	18(43.9)	19(42.2)	3.119	0.210
5-10	1(25.0)	17(41.5)	18(40.0)		
10-15	2(50.0)	6(14.6)	8(17.8)		
Gender					
Male	1(25.0)	29(70.7)	30(66.7)	3.43	0.101
Female	3(75.0)	12 (29.3)	15(33.3)		
Clinical features					
Abdominal distension	0(0.0)	06(14.6)	06 (13.5)	1.752	0.816
Fever	02 (50)	22(53.6)	24(53.3)		
Bone pain	0(0.0)	02(4.8)	02(4.4)		
Pallor	03(75)	04(9.7)	07(15.6)		
Petechiae	0 (0.0)	03(7.3)	03(6.6)		
Weight loss	0(0.0)	03(7.3)	03(6.6)		
HSM					
Present	3(75.0)	36(87.8)	39(86.7)	0.472	0.448
Absent	1(25.0)	5(12.2)	6(13.3)		
LN					
Present	2(50.0)	29(70.7)	31(68.9)	0.393	0.578
Absent	2(50.0)	12(29.3)	14(31.1)		
TLC					
<4000	0(0.0)	3(7.3)	3(6.7)	1.564	0.815
4000-11000	0(0.0)	4(9.7)	4(8.8)		
11000-50000	3(75.0)	20(48.8)	23(51.1)		
50000-100000	1(25.0)	10(24.4)	11(24.4)		
>100000	0(0.0)	4(9.7)	4(8.9)		
Hb					
<7	2(50.0)	18(43.9)	20(44.4)	0.549	0.760

7-10	2(50.0)	18(43.9)	20(44.4)		
>10	0(0.0)	5(12.2)	5(11.1)		
Types of anemia					
MCHC	0 (0.0)	04(8.8)	04(8.8)	.513	.680
NCNC	04 (100)	37(90.2)	41 (91.2)		
Platelet					
<20000	1(25.0)	14(34.1)	15(33.3)	1.989	0.575
20000-50000	3(75.0)	17(41.5)	20(44.4)		
50000-100000	0(0.0)	8(19.5)	8(17.8)		
>150000	0(0.0)	2(4.9)	2(4.4)		
PS					
0 -25	01 (25.0)	13 (31.7)	14 (31.1)	1.637	.802
26-50	0(0.0)	3(7.3)	3(6.7)		
51-100	3(75.0)	25(61.0)	28(62.2)		
BMA					
0 – 25	0 (0.0)	0 (0.0)	0 (0.0)	14.588	**0.006**
26- 50	0(0.0)	1(2.4)	1(2.20)		
51-75	0(0.0)	8(19.5)	8(17.8)		
76 – 100	02 (50.0)	26(63.4)	28(62.2)		
Diluted	02 (50.0)	06 (14.6)	08(17.8)		
Immunopheno typing					
CALLA + B-ALL	O4 (100)	30(73.1)	34 (75.5)	1.596	.809
Pro B- ALL	0(0.0)	01(2.4)	01(2.2)		
T-ALL (early)	0(0.0)	05(12.1)	05(11.1)		
T-ALL (cortical)	0(0.0)	05(12.1)	05 (11.1)		
Total	4(100.0)	41(100.0)	45(100.0)		

Table 2: Relation Between MLL Aberration Signals with Clinicohema to logical Parameters at Day-0

Clinico - hematological Parameters	**MLL split signal**		**Total number**	**Test of significance**	
	Positive	**Negative**		**Chi square**	**Pvalue**
Sex					
Male	0(0.0)	30(68.2)	30(66.7)	0.153	0.33
Female	1(100)	14(31.8)	15(33.3)		
Age					
1-5	1 (100)	18(40.9)	19(42.2)	1.400	0.497
5-10	0 (0.0)	18(40.9)	18(40.0)		
10-16	0 (0.0)	8(18.2)	8(17.8)		
Clinical features					
Abdominal distension	0 (0.0)	06(13.6)		1.567	0.563
Fever	1(100)	23(52.2)			
Bone pain	0(0.0)	02(4.5)			
Pallor	0(0.0)	07(15.9)			
Petechiae	0(0.0)	03(6.8)			
Weight loss	0(0.0)	03(6.8)			
Hepatosplenomegaly					
Present	1(100.0)	38(86.4)	39(86.7)	0.692	1.00
Absent	0(0.0)	6(13.6)	6(13.3)		
Lymphadenopathy					
Present	1(100)	30(68.2)	31(68.9)	0.497	1.00
Absent	0(0.0)	14(31.8)	14(31.1)		
TLC					
<4,000	0(0.0)	3(6.8)	3(6.7)	0.978	0.913
4,000-11,000	0(0.0)	4(9.1)	4(8.9)		

11,000-50,000	1(100)	22(50.0)	23(51.1)		
50,000-1,00,000	0(0.00)	11(26.8)	11(24.4)		
>1,00,000	0(0.0)	4 (9.7)	4(8.9)		
Hemoglobin					
< 7	0 (0.0)	20(48.7)	20(44.4)	0.564	.754
7-10	01(100)	19(46.3)	20(44.4)		
>10	0(0.0)	05(12.1)	05(11.1)		
Types of anemia					
MCHC	0(0.0)	4(9.7)	4(8.8)	0.752	0.911
NCNC	1(100)	40(97.2)	41(91.2)		
Platelet count					
<20,000	0(0.0)	15(34.1)	15(33.3)	1.278	.734
20,000-50,000	1(100)	19(43.2)	20(44.4)		
50,000-1,00,000	0(0.0)	8(18.2)	8(17.8)		
>1,50,000	0(0.0)	2(4.5)	2(4.4)		
PS Blast count					
0-25	0(0.0)	14 (31.8)	14(31.1)	0.621	.961
26-50	0(0.0)	3(6.7)	3(6.7)		
51-100	1(100)	27(61.4)	28(62.2)		
BMA blast count					
0-25	0(0.0)	0 (0.0)	0 (0.0)		
26- 50	0(0.0)	1(2.2)	1(2.2)	.682	.954
51- 75	0(0.0)	8(17.8)	8(17.8)		
76 – 100	1(100)	27 (61.3)	28 (62.2)		
Diluted	0(0.0)	8(17.8)	8(17.8)		
Immunophe no typing					
Pro B- ALL	1(100)	0 (0.0)	0 (0.0)	45.0	**.048**
CALLA + B-ALL	0 (0.0)	34(77.2)	34 (75.5)		
T-ALL (early)	0(0.0)	05(11.3)	05(11.1)		
T-ALL (Cortical)	0(0.0)	05(11.3)	05(11.1)		
TOTAL	01 (100)	44(100)	45(100)		

Table 3: Correlation of clinico-hematological parameters in ETV6-RUNX1 positive cases and their outcome

Age (Yr)	Sex	HSM	LAP	TLC (X1000/ul)	Hb (g/dl)	Platelets (x1000/ul)	Blast (%)	IPT	MRD	Outcome
09	F	Present	Absent	84.06	4.9	24	Diluted	CALLA+	Negative	Alive
12	F	Present	Absent	43.80	7.4	30	95	CALLA+	Negative	Alive
14	F	Present	Present	14.16	6.4	5	Diluted	CALLA+	Negative	Alive
05	M	Absent	Present	23.20	7.2	21	90	CALLA+	Negative	Alive

Table 4: Correlation of clinico-hematological parameters in MLL positive case and their outcome

Age (Yr)	Sex	HSM	LAP	TLC (X1000/ul)	Hb (g/dl)	Platelets (x1000/ul)	Blast (%)	IPT	MRD	Outcome
04 month	F	Present	Present	11.9	7.9	31	82	Pro B-ALL	Negative	Died

DISCUSSION

Acute lymphoblastic leukemia (ALL) is a malignant neoplasm characterized by the clonal accumulation of immature blood cells in the bone marrow. ALL comprises of heterogeneous group of diseases with different morphologic, cytogenetic, and molecular subgroups, which carry significant therapeutic implications.[9] Studies in the pediatric population have identified genetic syndromes that predispose to a minority of cases of ALL, such as Down syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia and Nijmegen breakdown syndrome.Other predisposing factors include exposure to ionizing radiation, pesticides, certain viruses such as Epstein-Barr Virus and Human Immunodeficiency Virus. However, in the majority of cases, it appears as

a de novo malignancy in previously healthy individuals.[10] Chromosomal aberrations are the hallmark of ALL. Characteristic translocations include t(12;21) [ETV6-RUNX1], t(1;19) [TCF3-PBX1], t(9;22) [BCR-ABL1] and rearrangement of MLL.[11] The cryptic t(12;21) (p13;22) is the most frequent translocation in B-lineage childhood acute lymphoblastic leukemia, results in a fusion transcript of the TEL gene on 12p13 and the AML1 gene on 21q22.[12] The t(1;19) (q23;p13) fusion protein is comprised of the transactivation domains of TCF3 and a DNA binding domain of the homebox protein PBX1 , converting PBX1 into a transactivating factor and reducing expression of the TCF3 encoded transcription factors E12 and E47, required for the early lymphoid development.[13]

MLL gene rearrangements MLL (mixed-lineage-leukemia) gene rearrangements at 11q23 are present in 80% of all infant B-ALL cases. The MLL gene encodes for a protein with histone methyltransferase activity, which is essential for hematopoietic regulation of HOXA and MEIS1gene expression.The most common gene rearrangements include t(4;11)(q21;q23) encoding MLL-AFF1(AF4), t(9;11)(p22;q23) encoding ML L-MLLT3(AF9), t(11;19)(q23;p13.3) encoding MLL-ENL, and t(10;11)(p13-14;q14-21) encoding MLL-MLLT10(AF10).[14] In the present study, the presence of cytogenetic abnormality, ETV6-RUNX1, TCF3-PBX1, MLL rearrangements were studied in 45 newly diagnosed paediatric ALL cases prior to any treatment by Fluorescence in situ hybridisation. The study also evaluated the correlation of this translocation with clinico-haematological parameters. The study population included both males and females with the age-group ranging from 1 year to 18 years with a mean age of presentation being 6.8 years. The majority (82%) of the children fell in the age group ranging from 1-10 years with 66.6% male predominance. Findings in our study are similar to study by PBiswas et al[15]and Mazlomi SH et al[16] in India.The common clinical presentations of the 45 cases in the study were fever, abdominal distention, lymphadenopathy, pallor and weight loss, bone pain and petechiae. In a study by Ajuba et al[17]conducted in Africa in 2016, showed that the cases of interest had clinical presentations like fever, weakness, weight loss, abdominal distention, bleeding etc in which fever was the most common presenting symptom among their cases (100%) and appears similar to our study.All the cases had either hepatosplenomegaly, lymphadenopathy or both, which is similar to study by Ajuba et al[17]and Pandita et al.[18]Hepatosplenomegaly and lymphadenopathy is a reflection of tumour burden and is developed due to infiltration of leukemic cells.[19]No single child presented with CNS & testicularmani festation. The haematological parameters included were total leucocyte count, haemoglobin, platelet count, type of anemiaand blasts percentage. The total leucocyte count ranged from 2,000 cells/uL to 3,00,000 cells/ul with mean value of 46,784 cells/ul. Majority of the cases (51.1%) of cases showed moderate elevation of leucocytes (10,000-50,000/ul). A few cases (8.9%) showed hyperleu kocytosis. The haemoglobin values ranged from 2.8g/dl to 12.2g/dl with a mean value of 7.2 g/dl.The platelet count ranged from 5,000 to 3,70,000/ul with a mean platelet count of 40,980/ ul. 77.7 % cases had low platelet count (<50000/ul). The typeo fanae miap resent included bothnor mocyticnormochromic and micr ocytic hypochro micanemia. 91.2% of children had normocyticnormochromicanemia.There is a paucity of literatures available correlating totype of anaemia developed in ALL children.The blast percentage in peripheral smear showed a mean of 61.6%. All the cases showed blast in peripheral smear and majority of the cases (62.2%) showed blast percentage of more than 50%. This shows that most of the children had increased tumour burden. In our study,48.8%showed moderately elevated leucocyte count, low haemoglobin and low platelet counts. The haematological parameters were similar to the findings reported in thestudy by Hutspardol et al.[20] All cases analysed, showed 78% were precursor- B cell ALL and 22%were T cell ALL. Among the precursor B cell ALL, 99% were CALLA positive B cell ALL. Study conducted by Khalid et al[21],Siddhaiahgari et al[22]and Aydin et al[23]shows CALLA positive B cell as the predominant immunotype, which is in line with our study. Presence of CD10 marker decides the prognosis and treatment response.

Cytogenetic Analysis by FISH To Detect ETV6-RUNX1 Fusion: In our study,8.8% had shown ETV6-RUNX1 fusion pattern. This percentage is similar to the study by Goud et al[24] in Oman. However, it is lower compared to other studies i.e. Amare et al[25]and Iqbal Z et al. [26] This shows that variations exist in the frequencies of ETV6-RUNX1 fusion oncogenes among different geographic regions/ racial groups.

Cytogenetic Analysis by FISH To Detect MLL Split Signal: In our study 2.2% case was positive for MLL split signal. Our result was similar tothe findings of Jarosova et al [27]in 2016 in Czech Republic which shows 2.3% cases of MLL aberrations and in Amare et al[25]in 2016 in India shows 1.3% positive cases for MLL aberration.

Cytogenetic Analysis by FISH To Detect TCF3-PBX1 Fusion: No case was found positive for TCF3-PBX1 fusion in our study. This may be due to the

small sample size and this limits conclusions about the frequency of this translocation. A study conducted by Anderson et al [28]in 2011 in Denmark showed 1.8% cases of TCF3-PBX1 fusion, representing a very low frequency of this translocation.

Correlation Of ETV6-RUNX1 Status with Clinico-Hematological Parameters: The mean age group of children with ETV6-RUNX1 positive was 6.8 years. Among the four positive cases, three were female children (75%) which showed a female preponderance. It shows its association with good prognostic factor. Fever and pallor were the commonest symptom in children who were positive for ETV6-RUNX1 fusion and it may be attributed to the release of inflammatory cytokines to the tumour antigen and infiltration of bone marrow or extramedullary sites by blasts. As a result, initial symptoms may be due to the presence of anemia, neutropenia, or thrombocytopenia. Among the four cases which were positive for ETV6-RUNX1 translocation, 3/4 (75%) had hepatosplenomegaly and 2/4 (50%) had lymphadenopathy. Hepatosplenomegaly and lymphadenopathy are the poor prognostic factors whereas this translocation is associated with good prognosis. The variation may be because of less number of cases in our study which would then not be truly representative of its frequency. All the cases had low hemoglobin level (< 7g/dl) associated with good prognosis. This shows ETV6-RUNX1 positivity is associated with good prognostic factor. Thrombocytopenia was seen in all the four cases i.e. platelet count < 50,000/uL is a poor prognostic marker. This may be due to presentation as splenic sequestration in cases with splenomegaly. All the four cases (100%) had blasts percentage greater than 50%. No case had a subleukemic picture in relation to ETV6-RUNX1 positivity. We were unable to find a study to correlate with blast percentage in case of ALL despite extensive review. All the four cases (100%) were CALLA positive B cell ALL immunophenotypically. The study by Hustpadrol et al[20]in Thailand also showed similar findings in which predominant immunophenotype was B cell ALL in all the cases (100%) of ETV6-RUNX1 fusion. In the study by Ramirez et al[29] in 2001 in Spain also showed all the cases (100%) positive for ETV6-RUNX1 fusion were predominantly B cell ALL. On follow up, all the four cases were MRD negative. Study by Aydin et al [23]and Guru et al [30] also reported similar type of pattern where all the positive cases (8/42) for ETV6-RUNX1 fusion, were MRD negative at day 35 andin remission.

Correlation of MLL Split Signal Status with Clinico-Hematological Parameters: One case which was positive for MLL split signal was4 month year old. Study by Caranza et al [31] also reported approximately 75% patients associated with MLL split signal were younger than 1 year age.The common clinical manifestations at admission were fever, pallor, bleeding manifestations. There is paucity of literatures available in correlating the clinical features with MLL split signal cases though pallor and easy fatiguability is mentioned as the common symptom in general in ALL cases.[32] The positive case of MLL split signal had hepatosplenomegaly and lymphadenopathy (100%) similar to the study conducted by Marchesi et al.[33] The positive case of MLL split signal had total leucocyte count of 11,900 cells/ul, platelet count 31,000/ul and low haemoglobin level (7.9g/dl) which was similar to the findings present in a study conducted by Pandita et al in 2015 in Jammu and Kashmir[18] reported 10% cases of MLL split apart signal had platelet count < 20,000/cu.mm, hemoglobin level < 6g/dl and TLC of 60,000/cu.mm. This case was Pro B cell ALL immunophenotypically and was MRD negative. The study conducted by Poppe et al[34]in Belgium in 2005 also showed similar findings in which the single case of MLL split signal had immunophenotype of Pro B cell ALL. In the study by Woo Y H et al in Korea reported 71.4% cases of MLL split apart signal were MRD negative.[35] Gao et al in China also reported 66.6 % cases of MLL split apart signal were MRD negative.[36] No case was found positive for TCF3-PBX1 translocation and hence statistically significant data could not be studied. It may be attributed to limited sample size and study time. Bigger sample size may be more representative to assess frequency of TCF3-PBX1 fusion in pediatric age group.

CONCLUSION

The observations and results showed that the lower frequency of ETV6-RUNX1 fusion gene was seen in our study as compared to other studies could be because of female predominance. The presence of ETV6-RUNX1 fusion gene associated with good prognostic factors like female predominance, age > 1year, total leukocyte count <50,000/ul, low hemoglobin level < 7g/dl. Although we could not find the statistical significance between the clinico - haematological parameters and presence of fusion gene. Thus, bigger sample size and longer follow up period actually helpful in assessing its prognostic impact. The MLL split signal gene was present in a single case (2.2%), a 4 month old female child. This case was associated with lymphadenopathy, hepatosplenomegaly, mild elevation of TLC (11.9 x103/ul), low haemoglobin (7.9%) and low platelet count (31,000/ul). The child did not survive after the three months of initial diagnosis. The MLL signal was

thus associated with poor prognostic parameters like age < 1year, systemic involvement, low hemoglobin and low platelet count. The frequency of detection of MLL split apart signal was similar to reported in other studies. No statistical significance is established regarding MLL because a single case was positive for MLL split apart gene. A bigger sample size should be more representative for a definitive conclusion. No comment can be made regarding TCF3-PBX1 fusion as no case was found to be positive. The technique FISH (Fluorescence in situ hybridisation) used in the study was relatively simple to perform, signals were easily visible and readily readable. It allowed direct visualisation of genetic mutation in tumour cells/blasts as well as easy identification of signal pattern associated with underlying aberration. We recommend that FISH technique should be used for detection of clinically relevant cytogenetic abnormalities such as ETV6-RUNX1 translocations, TCF3-PBX1 translocations and MLL split apart signals as a mandatory investigation in the diagnostic workup of newly detected paediatric ALL cases. This will help in Risk stratification, prognostication and modifying treatment protocols in these patients.

REFERENCES

1. Aplan P, Mrozek K, Harper DP. Cytogenetics and molecular genetics of Acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2009;23(5):991-1010.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCON estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer Journal for Clinicians. 2018;68(6):394-424. Available from: https://doi.org/10.3322/caac.21492.
3. Mullighan CG. The molecular genetic makeup of Acute lymphoblastic leukemia. Hematol Am Soc Hematol Educ Program. 2012;1:389-386.
4. Cooper SL, Brown PA. Treatment of Pediatric Acute Lymphoblastic Leukemia. Pediatr Clin North Am. 2015;62(1):61-73.
5. Woo JS, Alberti MO, Tirado CA. Childhood B –acute lymphoblastic leukemia: a genetic update. Exp Hematol Oncol. 2014;3:16.
6. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943-1955.
7. Kannan TP, Zilfalil BA. Cytogenetics: Past, Present And Future. Malays J Med Sci. 2009;16(2):4-9.
8. Schultz KR, Pullen DJ, Sather HN, Shuster JJ, Devidas M, Borowitz MJ, et al. Risk and response based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (COG). Blood.2007;109(3):926-935.
9. Kebriaei P, Anastasi J, Larson RA. Acute lymphoblastic leukemia: diagnosis and classification. Best Pract Res Cl Ha. 2003;15(4):597-621.
10. Meyer C, Kowarz E, Hofmann J, Rennevilla A, Zuna J, Trka J, et al. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009;23(8):1490-1499.
11. Terwilliger T, Hay MA. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood cancer J. 2017;7:e577.
12. Yehuda-Gafni O, Cividalli G, Abrahmov A, Weintrob M, Neriah SB, Cohen R, et al . Fluorescence in situ hybridization analysis of the cryptic t(12;21) (p13.q22) in childhood B-lineage acute lymphoblastic leukemia. Cancer Genet Cytogenet. 2002;132:61-64.
13. Hunger SP. Chromosomal translocations involving the E2A gene in acute lymphoblastic leukemia: clinical features and molecular pathogenesis. Blood. 1996;87:1211-1224.
14. Armstrong SA, Staunton JE, Silverman LB, Pieters R, Boer ML, Minden MD, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30:41-47.
15. Biswas S, Chakrabarti S, Chakraborty J, Paul PC, Konar A, Das S. Childhood acute leukemia in West Bengal, India with an emphasis on uncommon clinical features. Asian Pac J Cancer Prev. 2009;10:903-906.
16. Mazloumi SHM, Kumari P, Madhumathi DS, Appaji L. Rare and recurrent chromosomal abnormalities and their clinical relevance in pediatric acute leukemia of south Indian population. Indian J Med Paediatr Oncol. 2012;33(3):166-169.
17. Ajuba I, Madu A, Okocha C, Ibegbulam O, Okpala I, Nna O. Frequency and clinical impact of ETV6/RUNX1, AF4-MLL, and BCR/ABL fusion genes on features of acute lymphoblastic leukemia at presentation. Niger J Clin Pract. 2016;19(2):237.
18. Pandita A, Harish R, Digra SK, Raina A, Sharma AA, Koul A. Molecular cytogenetics in childhood acute lymphoblastic leukemia: A hospital based observational study. Clin Med Insights Oncol. 2015;9:39-42.
19. Carlson HC, Breen JF. Amyloidosis and plasma cell dyscrasias: Gastrointestinal involvement. Semin Roentgenol. 1986;21(2):128-138.
20. Hutspardol S, Pakakasama S, Kanta K, Nunta karan L, Anurathapani U, Sirachainan N, et al. Interphase-FISH screening for eight common rearrangements in pediatric B-cell precursor ALL. Int J Lab Hematol. 2013;35(4):406-415.
21. Khalid S, Moiz B, Adil SN, Khurshid M. Retrospective review of pediatric patients with acute lymphoblastic leukemia: a single center experience. Indian J Pathol Microbiol. 2010;53(4):704-710.
22. Siddaiahgari SR, Awaghad MA, Latha MS. Clinical immunophenotype and cytogenetic profile of ALL in children at tertiary health care centre in India. Muller J Med Sci Res. 2015;6:112-118.
23. Aydin C, Cetin Z, Manguoglu AE, Tayfun F, Clark OA, Kupesiz A, et al. Evaluation of ETV6/RUNX1 fusion and additional abnormalities involving ETV6 and/or RUNX1 genes using FISH technique in patients with childhood acute lymphoblastic leukemia. Indian J Hematol Blood Transfus. 2016;32(2):154-161.

24. Goud TM, Salmani KK, Harasi SM, Musalhi MA, Wasifuddin SM, Rajab A. Importance of FISH combined with morphology, immunophenotype and cytogenetic analysis of childhood/ adult acute lymphoblastic leukemia in Omani patients. Asian Pac J Cancer Prev. 2015;16:7343-7350.
25. Amare PS, Jain H, Kabre S. Cytogenetic Profile in 7209 Indian Patients with de novo Acute Leukemia. J Cancer Ther. 2016;7:530-544.
26. Iqbal Z, Akhtar T, Awan T, Aleem A, Sabir N, Rasool M, et al. High frequency and poor prognosis of late childhood BCR-ABL positive and MLL-AF4 positive ALL define the need for advanced molecular diagnostics and improved therapeutic strategies in pediatric B-ALL in Pakistan. Mol Diagn Ther. 2015;19(5):277-287.
27. Jarosova M, Volejnikova J, Porizkova I, Holzerova M, Pospisilova D, Novak Z, et al. Chromosomal aberrations in childhood acute lymphoblastic leukemia: 15-year single center experience. Cancer Genet, 2016;209(7-8):340-347.
28. Andersen KM, Autio K, Barbany G, Borgstrom G, Cavelier L, Golovleva I, et al. Paediatric B-cell precursor acute lymphoblastic leukemia with t(1;19)(q23;p13). Br J Haematol. 2011;155:235-243
29. Ramirez AM, Urioste M, Contra T, Cantalejo A, Tavares A, Portero JA, et al. FISH study of TEL/AML1 fusion and other abnormalities involving TEL and AML1 genes. Correlation with cytogenetic findings and prognostic value in children with ALL. Haematologica. 2001;86(12):1245-1253.
30. Guru FR, Muzamil J, Bashir S, Mahajan A. Acute lymphoblastic leukemia, the Indian scenario. MOJ Cell Sci Rep. 2018;5(1):33-37.
31. Carranza C, Granados L, Morales O, Jo W, Villagran S, Tinti D, et al. Frequency of the ETV6-RUNX1, BCR-ABL1, TCF3-PBX1, and MLL-AFF1 fusion genes in Guatemalan pediatric acute lymphoblastic leukemia patients and their ethnic associations. Cancer Genet. 2013;206:227-232.
32. O'Brien MM, Seif AE, Hunger SP. Acute lymphoblastic leukemia in children. In: Greer JP, Rodgers GM, editors. Wintrobe's Clinical Hematology. 14th ed. Philadelphia,USA: Wolters Kluwer;2019. p. 5042-5114.
33. Marchesi F, Girardi K, Avvisati G. Pathogenetic, clinical, and prognostic features of adult t(4;11)(q21;q23)/ MLL-AF4 Positive B-cell acute lymphoblastic leukemia. Adv Hematol. 2011;2011:621-627.
34. Poppe B, Cauwelier B, Limbergen VH, Yigit N, Philippe J, Verhasselt B, et al. Novel cryptic chromosomal rearrangement in childhood acute lymphoblastic leukemia detected by multiple color fluorescent in situ hybridization. Haematologica. 2005;90:1179-1185.
35. Woo YH, Kim DW, Park H, Seong W, Koo HH, Kim SH. Molecular cytogenetic analysis of gene rearrangements in childhood acute lymphoblastic leukemia. J Korean Med Sci. 2005;20:36-41.
36. Gao C, Zhao XX, Li WJ, Cui L, Zhao W, Liu SG, et al. Clinical features, early treatment responses, and outcomes of pediatric acute lymphoblastic leukemia in China with or without specific fusion transcripts: a single institutional study of 1,004 patients. Am J Hematol. 2012;87(11):1022-1027.