Induction Chemotherapy Response in Childhood Acute Lymphoblastic Leukaemia and its Correlation with Cytogenetic and Molecular Features

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ABSTRACT

Objective: To study the correlation of cytogenetic and molecular abnormalities on induction chemotherapy in childhood acute lymphoblastic leukaemia (ALL).

Study Design: Analytical study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology (AFIP), from March 2021 to August 2021.

Methodology: Patients aged 1-18 years with newly diagnosed acute lymphoblastic leukaemia were inducted. Patients aged less than 1 year and more than 18 years were excluded from the study. The diagnosis was based on morphology, cytochemistry, flow cytometry, and cytogenetic/molecular analysis. Risk stratification was done on the basis of age, TLC, and cytogenetic/molecular defects. The UKALL 2011 protocol was used for treatment with regimen-A for standard risk and regimen-B for high-risk patients. Bone marrow was repeated on day 29 of induction therapy and blast percentage was assessed to establish post-induction remission. Association between cytogenetic / molecular abnormalities and post-induction remission status was analysed using chi-square test.

Results: There were total 142 patients with mean age of 6.4 $\pm$ 3.6 years and a male- to-female ratio of 2.7:1. Immunophenotyping revealed 85.9% cases as B-cell ALL and 14.1% as T-cell ALL. The most frequent cytogenetic and molecular abnormalities were hyperdiploidy (19%), t(9;22)/BCR-ABL1(p190) (10.6%), complex karyotype (5.6%), E2A-PBX1 (8.5%), and TEL-AML1 (4.9%). A total of 127/142 (89.4%) achieved haematological remission after induction therapy with two deaths during induction therapy (1.4%). Post-induction remission rate in patients with favorable cytogenetic/molecular defects was 100% and in children with bad prognostic changes, the rate of remission was 69.2%. Chi-square test showed a significant association between cytogenetic/molecular abnormalities and post-induction remission (p-value <0.001).

Conclusion: Cytogenetic and molecular abnormalities have a significant association with post-induction remission in children with acute lymphoblastic leukaemia.

Key Words: *Acute lymphoblastic leukaemia, Cytogenetics, Chemotherapy, Induction, Remission.*

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INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is one of the most frequent childhood malignancies accounting for about one-third of childhood cancers.[1] Disease prognosis depends upon many factors including age, total leukocyte count (TLC), immunophenotype and cytogenetic abnormalities. Cytogenetic abnormalities at diagnosis have a significant association with distinct immunophenotype and with disease outcomes.[2]

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Detection of blasts morphologically as well as by minimal residual disease (MRD) is important in both the risk group allocation and assessing disease prognosis.[3] Blast percentage in post-induction therapy bone marrow is an important factor that can predict relapse-free and overall survival in patients with ALL.[4] Survival rate in paediatric ALL has improved over time, especially due to risk-based therapeutic protocols. Risk stratification is based on age, gender, TLC at diagnosis, cytogenetic, and molecular changes. Post-induction blast percentage is one of most important prognostic factors in patients with acute lymphoblastic leukaemia.[5]

The aim of this study was to find out the more common cytogenetic and molecular defects in the Pakistani population and their association with post-induction remission in patients of childhood ALL.

METHODOLOGY

An analytical study was conducted at the Armed Forces Institute of Pathology Rawalpindi, for six months duration from March 2021 to August 2021 after approval from the ethical review committee. Patients with suspicion of acute leukaemia on CBC findings were selected for bone marrow aspiration. A total of 142 patients from both genders, aged 1-18 years and newly diagnosed with ALL based on cytology, immunophenotyping, and cytogenetics/molecular defects were selected for the study. Patients aged less than 1 year, aged more than 18 years, and patients with other malignancies were excluded from the study.

Patients' particulars and laboratory parameters noted for the study included age and gender, findings of complete blood count (CBC), bone marrow aspirate and trephine biopsy, immunophenotyping, cytogenetic analysis, molecular analysis, and cerebrospinal fluid (CSF) findings. CBC was done on Sysmex XN-3000 automated haematology analyser. Cytological parameters of bone marrow aspirate and trephine biopsy were noted after assessment on light microscopy. Flow cytometry was done to confirm diagnosis as well as to determine the immunophenotype (B / T cell ALL) of the disease. Conventional karyotyping was done on MetaSystems automated chromosome analyzer to assess cytogenetic abnormalities. Molecular abnormalities were detected by real-time PCR with the following gene panel for screening: *E2A-PBX1, TEL-AML1, MLL-AF4/KMT2A-AFF1, MLL-AF9, MLL-ENL, BCR-ABL1* (*p210 and p190*) e13 a2.e14 a2 and e1 a2 fusion transcripts. CSF was obtained in the patients and analysed for routine examination (RE) and cytological analysis (for blasts in CSF).

The diagnosis was followed by risk stratification into standard risk and high risk based on NCI criteria. Patients aged 1-10 years and TLC <50 x 10^9/L were at the standard risk. High-risk group included patients aged >10 years or TLC >50 X 10^9/L as well as patients with poor prognostic cytogenetic/molecular alterations.

Patients with the confirmed diagnoses were treated with UKALL-2011 chemotherapy regimen. Regimen A for standard-risk patients, regimen B for high-risk patients and patients with *t(9;22)/BCR-ABL1* with TKIs. After induction chemotherapy, remission status was assessed on day 29 of treatment with remission defined as bone marrow blast percentage <5%, absolute neutrophil count (ANC) > 1.0 x 10^9/L, platelet count > 100 x 10^9/L, transfusion independence and no extramedullary disease (confirmed on imaging).

Statistical analysis was done on SPSS version 26 and graphs were made using the latest version of Microsoft Excel. Kolmogorov-Smirnov normality test was used to assess the normal distribution of the quantitative variables. Mean and standard deviation were used to express the variables with normal distribution and the variables without normal distribution of data were expressed in median values and interquartile ranges (IQR). Qualitative variables were expressed as frequencies and percentages after grouping patients on basis of age at diagnosis, TLC at diagnosis, and cytogenetic/molecular abnormalities. Chi-square test was applied to analyse the association of post-induction remission with cytogenetic/molecular abnormalities detected. A p-value <0.05 was considered significant.

RESULTS

A total of 142 patients were newly diagnosed with ALL. Females included 26.8% (n=38) patients and males were 73.2% (n=104) with male-to-female ratio of 2.7 to 1. The mean age was 6.4 $\pm$ 3.6 years. CBC revealed mean haemoglobin (Hb) of 7.3 $\pm$ 2.13 g/dL, median TLC of 26.1 x 10^9/L (IQR = 8.2 – 113), and median platelet count (Plt) of 36.5 x 10^9/L (IQR = 21 – 69). Out of 142 patients included in the study, post-induction remission was achieved in 89.4% (n=127), remission was not achieved in 9.1% (n=13), and death during induction therapy occurred in 1.4% (n=2).

Patients aged 1-10 years were 50.7% (n=72) and patients aged >10 years were 49.3% (n=70). Immunophenotypically 85.9% (n=122) were diagnosed B-ALL and 14.1% (n=20) were diagnosed T-ALL. TLC at diagnosis <50 x 10^9/L was observed in 59.2% (n=84) and count $\geq$50 x 10^9/L was seen in 40.8% (n=58). CSF RE, cytology and flow cytometry for blast cells showed CNS disease was in 2.8% (n=4). Patients with good prognostic cytogenetic abnormalities *i.e.*, hyperdiploidy and hypertetraploidy, made up 20.4% (n=29) as compared to patients with bad prognostic cytogenetics *i.e.*, t (1;9), t(9;22), and complex karyotype, in 16.9% (n=24).

Cytogenetic analysis showed most frequent karyotypes to be normal in 62.7% (n=89), hyperdiploidy in 19% (n=27), t (9;22) in 10.6% (n=15), and complex karyotype in 5.6% (n=8). Real-time PCR for genetic abnormalities showed no abnormality in 73.9% (n=105), mutations of BCR-ABL1 (p190), E2A-PBX1, TEL-AML1, and MLL-MLLT10 were seen in 10.6% (n=15), 8.5% (n=12), 4.9% (n=7), and 2.1% (n=3) patients respectively (Table I).

Table I: Frequency of cytogenetic and molecular abnormalities in the study.

Karyotype	Frequency	Percentage
Cytogenetic Abnormalities	89	62.7%
Normal		
Hyperdiploidy	27	19%
t(9;22)	15	10.6%
Complex	8	5.6%
Hypertetraploidy	2	1.4%
t(1;9)(q21;34)	1	0.7 %
Molecular Abnormalities		
Genetic defect	Frequency	Percentage
BCR-ABL1(p190)	15	10.6%
E2A-PBX1	12	8.5%
TEL-AML1	07	4.9%
MLL-MLLT10	03	2.1%
No defect detected	105	73.9%

Based on patients grouping by age, TLC, and cytogenetics, post-induction remission was better in patients with age 1-10 year (98.3%), TLC <50 x 10^9/L (98.7%), good cytogenetics (100%),

and favourable molecular defects (100%). Independent samples t-test gave a p-value <0.05 for above-mentioned parameters.

Based on cytogenetic and molecular abnormalities, patients with hyperdiploidy, hypertetraploidy, and TEL-AML1 had post-induction remission rates of 100%. Patients with poor prognostic cytogenetic/molecular abnormalities had remission rates of 75% with complex karyotype, 33% with t (9; 22), 100% with t(1;9), E2A-PBX1 and MLL-MLLT10 each.

Patients grouped in good prognostic cytogenetic/molecular abnormalities had 100% post-induction remission and patients grouped in bad prognostic abnormalities had a post-induction remission rate of 69.2%.

Pearson Chi-square test analysis showed a p-value of <0.001, thus showing a significant association between favourable cytogenetic / molecular features and post-induction remission in children with ALL.

DISCUSSION

ALL alone accounts for almost ¾ of the leukaemias. Pathophysiology of ALL includes clonal proliferation of lymphoid precursors resulting in bone marrow replacement.[1] The results of this study showed mean age of 6.4 $\pm$ 3.6 years and a male-to-female ratio of 2.7. These findings were comparable to the outcomes reported in the literature. Korejo *et al.* reported mean age of 5.7 $\pm$ 0.23 years and male-to-female ratio of 2.4 in a study conducted in Pakistan in 2019.[6]

Immunophenutypically B-cell ALL was seen in 85.9% and T-cell ALL in 14.1%, similar to the results reported in the literature.[17] Study showed normal karyotype in 62.7%, Chennamaneni *et al*. reported normal karyotype in 61.6%.[2] In this study, frequencies of hyperdiploidy, BCR-ABL1 gene-defect, and TEL-AML1 gene-defect were 19%, 10.6%, and 4.9% as compared to the literature citing frequencies of 25%, 5%, and 25% respectively in childhood ALL.[7]

In this study, post-induction remission was seen in 89.4% of patients. These results were similar to the facts reported in the literature. In 2012 Schrappe *et al*. and in 2020 Maloney *et al*. have reported remission rates of 75% and 98% respectively in patients of childhood ALL.[8,9]

ALL had history of poor outcomes in 1950s that has improved enormously because of better management protocols devised after risk stratification based on prognostic factors.[10] In this study, higher rate of post-induction remission was seen in patients with standard risk. Results showed statistically significant differences among patients divided on the basis of age, TLC, and cytogenetic/molecular defects. Literature has also reported some new prognostic factors known for unfavourable outcomes including male gender, age more than nine years, low CD10 expression, mediastinal enlargement, and CNS involvement while DNA index (DI) >1.16 is associated with favourable outcomes.[5]

This study also demonstrated that following risk stratification, patients grouped into favourable cytogenetic and molecular changes had higher rates of post-induction remission (100% in this study) than patients in other prognostic groups. These findings are in line with the literature. In 2019, a study conducted in Pakistan showed patients with hyperdiploidy (favourable cytogenetic defect) had remission rate of 94.7%.[6] in 2013, Dastugue *et al*. reported a post-induction remission rate of 99.6% in patients of childhood ALL with hyperdiploidy.[11]

Statistical analysis also showed that a positive association exists between favourable cytogenetic/molecular defects and post-induction remission in childhood ALL. Lazaryan *et al*. conducted a study and proved complex karyotype, monosomy 7, and t(11;19) to be associated with unfavourable outcomes in patients undergone with stem cell transplant (SCT).[12] Pullarkat *et al*. conducted a study and concluded the cytogenetics to be the single most important prognostic component that can predict response to treatment. The study also urged the development of new targeted therapies that can act on specific cytogenetic/molecular defects for better outcomes in adult ALL.[13] Shi *et al*. 2020 reported cytogenetic and molecular abnormalities as strong independent prognostic factors for ALL and can be used in both risk stratification as well as in making choice of therapy.[14] Genescà *et al*. has also published research that stresses upon different cytogenetic abnormalities' role in risk stratification at diagnosis of ALL.[15]

In light of studies on treatment outcomes, the most frequent cytogenetic changes have been divided into favourable and unfavourable groups.[2,3] Cytogenetic abnormalities involving an altered number of chromosomes as well as modified structural abnormalities are detected in upto 85% of patients diagnosed. Chromosome defects grouped on basis of number defects include hypodiploidy (<46 chromosomes), hyperdiploid (47-50 chromosomes), hyper-hyperdiploid (>50 chromosomes). Structural abnormalities include Philadelphia chromosome formed as a result of t(9;22), t(1;19), t(4;11), t(8;14), t(12;21), complex karyotype (defined as five or more chromosome abnormalities), and t(5;14). Cytogenetic abnormalities classified on the basis of prognosis are shown below (Table II).[16,17]

Table II: Cytogenetic and molecular abnormalities grouping by prognosis.

Prognosis	Karyotype	Molecular/chromosome defect	Remarks
Good	Hyperdiploidy	>50 chromosomes	25% - childhood ALL 5% - adult ALL
	t(12;21)	TEL-AML1/ETV6-RUNX1	25% - childhood ALL 2% - adult ALL
	t(11;19)	MLL-ENL	Good prognosis in T-ALL
Bad	Hypodiploidy	<46 chromosomes	-
	Complex	>5 chromosome defects	-
	t(9;22)	BCR-ABL1/Philadelphia chromosome	5% - childhood ALL 25% - adult ALL
	t(4;11)	MLL-AF4	80% - infant ALL 6% - adult ALL
	t(8;14)	MYC/IGH	5% of patients
	t(1;19)	E2A-PBX1/TCF3-PBX1	Associated precursor B-ALL
	t(5;14)	IL3-IGH	Associated B-ALL Peripheral blood eosinophilia

Detection of different cytogenetic and molecular defects can be done by conventional chromosomal analysis, *Fluorescence In Situ Hybridization* (FISH), polymerase chain reaction (PCR), and next-generation sequencing (NGS). Conventional analyses and FISH are cost-effective with FISH having the advantages of shorter period required and being able to detect more subtle abnormalities. Samples that can be used for cytogenetic analysis are cells from blood, bone marrow, skin (fibroblasts), lymph nodes, and serous effusions. Modern method like single nucleotide polymorphism array (SNP-A) analysis has proven to be of more utility in the detection of additional defects that were not detected by conventional method.[18,19]

The limitations of this study include the use of conventional cytogenetic analysis and a relatively smaller gene-panel for molecular characterisation of ALL that have lesser yield as compared to newer techniques. Another shortcoming is to limit the study till the assessment of post-induction remission instead of long-term follow-up to study disease-free survival and overall survival in patients.

Newer studies with longer follow-up may be planned by grouping based on favourable cytogenetic/molecular changes and post-induction remission status to study overall survival among patients of childhood ALL.

CONCLUSION

The study of childhood ALL in the Pakistani population showed the most common cytogenetic defect to be hyperdiolpoidy. Molecular defect of BCR-ABL1 had frequency of 10.6% *i.e.*, more than that reported in literature and TEL-AML1 4.9% *i.e.*, less than that reported in the literature. Patients with favourable cytogenetic/-molecular defects had highest rate of post-induction remission and a positive association was observed between cytogenetic/-molecular changes and post-induction therapy results.

ETHICAL APPROVAL:
Ethical approval was given by the Institutional Review Board (IRB), Armed Forces Institute of Pathology (AFIP) at the IRB meeting held on 03-Feb-2021
(IRB/22/876; dated: February 03, 2021).

PATIENTS' CONSENT:
Written informed consent were taken from all the patients.

COMPETING INTEREST:
There is no potential competing interest.

AUTHORS' CONTRIBUTION:
NAR: Conceived and designed study along with data collection, statistical analysis and result compilation.
AM, HMR: Data collection, results, discussion, and literature review.
SZ, IA, SR: Discussion and literature review.
All the authors have approved the final version of the manuscript to be published.

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