A Single Institution's Experience with Cytogenetic and MRD Outcomes in Pediatric Acute Lymphoblastic Leukemia

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ABSTRACT

Objective: To determine the frequency of cytogenetic type and its significance in the prognostic outcome of the pediatric patients in acute lymphoblastic leukemia (ALL), aged 1 to 15 years, and also determine the importance of minimal residual disease (MRD) in the management of the condition.

Study Design: An observational study.

Place and Duration of Study: Pediatric Oncology Ward, Shaukat Khanum Cancer Hospital, Lahore, from January 2015 to July 2017.

Methodology: Patients aged 1-15 years, diagnosed with ALL, were included. Studied variables were cytogenetic type and MRD outcome in patients with ALL. Patients under one year of age and more than 15 years, or those having co-morbidities, were excluded.

Results: Total 150 patients' data were retrieved from the Hospital database. One hundred and thirty-three belonged to age 1 to 5 years group (89%) and 17 (11%) were in 5 to 10 years group. The mean age of the patient was 4.3 $\pm$3.1 years. One hundred and two (68%) were males; whereas, 48 (32%) were females. Pre B acute lymphoblastic leukemia was diagnosed in 139 (93%) patients and 11(7%) were diagnosed with Pre T acute lymphoblastic leukemia. Standard risk was observed in 120 (80%) patients and 30 (20%) patients were on high risk as per National Cancer Institute (NCI) Guidelines. Regimen A was used in 125 (83.3%), Regimen B in 16 (10.7%), and Regimen C in 9 (6%) patients. BCR-ABL was positive in 2 (1.30%), TEL-AML in 68 (45%), MLL in 5 (3.30%), and normal in 54 (36%). MRD at day 29 was negative in 40 (93%) and positive in 3 (7%). The karyotyping was done in 128 (85%) patients, out of which 68 (53%) were hyperploids, 41 (32%) euploid, and 19 (15%) were hypoploid. Death was observed in 22 (15%) patients. Nineteen (86%) deaths were due to fungal and bacterial sepsis; and disease-related deaths were noted in 3 (14%) patients.

Conclusion: The role of MRD and cytogenetics in risk assessment has improved in the early prognosis determination.

Key Words: *Cytogenetics, Acute lymphoblastic leukemia (ALL), Minimal residual disease (MRD).*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is among one of the most common hematological malignancies seen in children. Children with acute leukemia mostly present with pallor, bone pain, bleeding manifestations, and hepatosplenomegaly.[1,2] In the last three decades, many changes were made in treatment and outcomes of acute lymphoblastic leukemia patients, and at the start of the 21st century almost 80% of patients were expected to survive without relapse after first-line treatment.[3-6] Factually, the treatment intensity received by a patient with acute lymphoblastic leukemia was relapse risk-based, which was predicted by a combination of clinical, cytogenetic, and morphological early response criteria.[3,5,6] Cytogenetics and minimal residual disease (MRD) is of great importance in the prediction of outcome in acute lymphoblastic leukemia patients.[7,8] The t(9;22) (q34;q11.2) chromosomal translocation, *i.e.* the Philadelphia (Ph.) chromosome, and the resulting breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1 (BCR-ABL1) fusion protein1 occur in 20 to 30% of adult patients with acute lymphoblastic leukemia (ALL) and in 3 to 5% of patients with childhood ALL, because of its high rate of relapse with chemo-therapy.[9,10]

Over the past decade, tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1 have been added to chemotherapy regimens; a Children Oncology Group (COG) showed that combined Imatinib with intensive post-remission induction chemotherapy yielded a significant improvement in event-free survival (EFS) rates for childhood Ph.-positive ALL.

The primary objective of this study was to see the different cytogenetic abnormalities in Pakistani population-based on karyotyping and fluorescent *in situ* hybridisation (FISH) analysis and its significance in the prognostic outcome of the pediatric patients in ALL aged 1 to 15 years. The secondary objective was to see the results of MRD and its significance in the patients at the end of induction.

METHODOLOGY

The study design was observational retrospective. The site of the study was Department of Pediatric Oncology,

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Shaukat Khanum Memorial Cancer Hospital, Lahore. Information was retrieved from all patients registered in Pediatric Oncology Department from January 2015 to July 2017, after taking International Research Board (IRB) approval. The study enrolled all the patients aged 1-15 years who were diagnosed with acute lymphoblastic leukemia. Exclusion criteria were age less than 1 year and greater than 15 years or the patient with comorbidities. Studied variables were cytogenetic type and MRD outcome in patients with ALL. Since the study centre started doing MRD in January 2017, so the sample for MRD included patients from January 2017 till July 2017. Patients were categorised according to National Cancer Institute (NCI) risk stratification on the basis of age, WBC count, phenotype, and karyotyping. All patients were diagnosed on flow cytometry either on bone marrow or peripheral blood at baseline. Cytogenetic and karyotyping was also done at baseline. High risk karyotyping and cytogenetic were defined as hypoploidy, MLL and BCR-ABL translocations. Bone marrow biopsy was done at day-8, day-15 and day-29 at the end of induction. Bone marrow in remission was defined as bone marrow having less than 5% blast cells on examination. Additional testing at day 29 was done on bone marrow in the form of minimal residual disease (MRD), which can detect even few microscopic blast cells and its normal value was less than 0.001%.

Protocol used for treatment was UKALL interim guidelines 2013. All cases received either regimen A with 3 drugs induction (vincristine, dexamethasone, pegylated asparagine) or regimen B and C in induction with 4 drugs (vincristine, dexamethasone, pegylated asparagine, donorubicin) on the basis of their risk stratification. The MLL gene abnormalities and hypoploidy was treated with regimen C because of its high risk stratification. The patients having BCR-ABL translocations were treated with the combination of tyrosine kinase inhibitors due to the better survival rates with combination therapy. Patients who had either bone marrow not in remission or positive MRD post induction were escalated according to UKALL Interim Guidelines 2013 for ALL.

All the collected data were formalised electronically and analysed later by using Microsoft Excel version 10 and SPSS version 19. Descriptive statistics were applied to calculate mean and standard deviation for quantitative variables like age. Frequency distribution and percentages were calculated for qualitative variables like gender, cytogenetic type, MRD findings and remission.

RESULTS

One hundred and fifty patients' data were retrieved from the Hospital database, out of whom 133 (89%) children belonged to age 1 to 5 group; whereas, 17 (11%) were in 5 to 10 years age group. The mean age of the patients was 4.3 years. Most of the patients were males (n=102, 67%); whereas, 48 (33%) were female. Pre B acute lymphoblastic leukemia was seen in 139 (93%) and 11 (7%) had, Pre T ALL. Standard risk was in 120 (80%) patients and 30 (20%) patients were categorised high, risk as per National Cancer Institute (NCI) guidelines. Regimen A treatment was given in 125 (83%) patients, Regimen B in 16 (11%), and Regimen C in 9 (6%). FISH showed BCR-ABL in 2 (1.30%), TEL-AML in 68 (45%), MLL in 5 (3%), normal in 54 (36%) and not done in 21 (14%) due to its unavailability at that time. MRD at day-29 was done in 43 patients, which was negative in 40 (93%) and positive in 3 (7%). Karyotyping was done in 128 (85%) out of which 68 (53%) were hyperpoild, 41 (32%) euploid and 19 (15%) were hypoploid.

Table I: Clinical and diagnostic findings in children with ALL.

Findings	n	%
MRD at day 29		
Performed in patients	43	29%
Negative	40	93%
Positive	3	7%
FISH		
BCR-ABL	2	1.30%
TEL-AML	68	45.30%
MLL	5	3.30%
NORMAL	54	36%
Not done	21	14.10%
Bone marrow remission at day 15		
Yes	125	83%
No	20	13%
Not done	5	3%
Bone marrow remission at day 29		
Yes	142	95%
No	5	3%
Not done	3	2%

Death was seen in 22 (15%) patients (Table I). Total 19 (86%) deaths were due to fungal and bacterial infections leading to sepsis. Disease related deaths were noted in 3 (14%) patients only. All patients were of Pre B acute lymphoblastic leukemia phenotype standard risk as per National cancer institute (NCI) criteria, and there was no patient with hypoploidy, and high risk MLL, BCR-ABL cytogenetic. There were total 3 disease related deaths out of which minimal residual disease was positive in 2 (67%) at the end of induction, and 1 (33%) patient had day-29 bone marrow positive with blast cells had not achieved remission. Out of these, three disease related deaths, two patients (67%) patients were escalated to Regimen C due to induction failure, however, 1 (33%) patient died of central nervous system relapse at maintenance therapy.

DISCUSSION

This study was done to highlight the Institutional experience with Cytogenetic and MRD outcomes in acute lymphoblastic leukemia pediatric patients. These results not only showed the profiling of patients with acute

lymphoblastic leukemia in children like risk stratification, cytogenetic type, and assigned treatment accordingly, but also identified that the low risk of relapse can be achieved by rapid clearance of MRD at the end of induction therapy. This finding is supported by other published studies.[3,10]

The incidence of relapse was low in comparison with the previous studies.[3,11] The significance of the rapid morphological clearance (RMC) of blasts in peripheral blood (PB) and bone marrow (BM) during the initial levels of chemotherapy was demonstrated by various published studies.[12,13] Early in therapy the assessment of RMC at different times was used as a tool for risk group provision in acute lymphoblastic leukemia therapy.[14,15]

This method has recently been replaced by minimal residual disease (MRD) assessment for the identification of the risk group. A poor prognosis was observed among patients who failed after therapy induction in achieving the morphological remission. Somewhat dismal outcomes were not observed in all these patients, and due to the induction remission therapy duration variance among different studies, it is difficult to compare the outcomes among them.[14]

Often high-risk features were observed among induction failure patients; these features include T cell immune phenotype, MLL-rearrangement (MLL-r), BCR-ABL translocation, age, and higher WBC count. The finding of our study showed high percentages of TEL-AML. This finding is supported by other available published studies.[15,16] The other significant result of our study was the large number of children were under 5 years of age. Hence the TEL-AML was the commonest cytogenetic under 5 years of age children. The worse outcome was highly correlated to the presence of residual leukemia in BM on 8th day of induction or some time 15th day.[17]

Without any shadow of doubt, nowadays MRD is considered to be the most powerful prognostic factor in childhood acute lymphoblastic leukemia.[18] The sensitivity and specificity of MRD has been assessed by many studies at various time points with variety of therapy protocols by using PCR-based technology.[19-20] Hence, the utility of MRD in prognosis determination of de novo acute lymphoblastic leukemia patients for first relapse and in re-induction of chemotherapy is very crucial and vital.[13] Other findings of our study include, protocols with regimen A as most commonly observed in the present study population.

This study is only one of its own kind in the region, so more studies in the domain will definitely help in improving the therapy success rate and protocol development in acute lymphoblastic leukemia patients. There was a limitation of this study because of the unavailability of MRD before January 2017 at the study centre, so it was not done in all the patients included in this study. However, sample size was adequate to have a concrete evidence about the prognostic significance of MRD in pediatric leukemic patients.

CONCLUSION

Cytogenetics and MRD are imperative in risk stratification of a patient with acute lymphoblastic leukemia. An accurate risk stratification of a patient, with acute lymphoblastic leukemia, guides about the type of chemotherapy regimen to be given; and this in turn, reduces the risk of disease relapse due to the perfect choice of treatment. This is also helpful in preventing the undue administration of intensive chemotherapy drugs to the patients, and thus preventing them from chemotherapy-related toxicities and deaths.

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