

Immunophenotyping and cytogenetic profile of children with acute lymphoblastic leukemia and its prognostic significance: A study from a tertiary care center from Kashmir.

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ABSTRACT

Background: The complete cytogenetic and immunophenotyping data in children suffering from acute lymphoblastic leukemia (ALL) in Jammu and Kashmir is scarce. To bridge the knowledge gap, the present study was aimed to evaluate the immunophenotype and cytogenetic profile of pediatric ALL patients treated in our hospital.

Participants and methods: This hospital-based observational study was conducted on 180 pediatric patients aged between 1 to 18 years who had visited the Pediatric Unit of the Department of Medical Oncology at Sher-I-Kashmir Institute of Medical Science, Srinagar, Jammu, and Kashmir between January 2015 to December 2019.

RESULTS: Among the study participants, 57.8% were male, and 42.2% were female with a mean age of 9.2 (± 3.5) years and a median of 8 years. The majority of patients (68%) were below ten years. The male: female ratio was 1.3:1. Central nervous system disease at diagnosis was seen in 7.8% of patients. The mean and the median TLC were 56000/ μ l and 12000/ μ l, respectively. The TLC was more than 20000/ μ l in 63.3% of patients. The immunophenotyping analysis revealed Pre B ALL in 77.8%, B cell in 9.4% and T cell ALL in 12.8% patients. The difference in the median survival time for patients with Pre B ALL, B cell ALL, and T cell ALL was not statistically significant ($p=0.317$). Conventional cytogenetic revealed normal cytogenetics in 87.6%, hyperdiploidy in 8.5%, and hypodiploidy in 3.9%. The median survival time in patients who had a normal karyotype, hyperdiploidy, and hypodiploidy were not statistically ($p=.381$). FISH for TEL AML, MLL, BCR-ABL gene rearrangement analysis was positive in 23.3%, 4.4%, 11.1% patients respectively. The median survival was statistically significant with respect to MLL gene rearrangement ($p=0.001$) clearly indicating that all patients with MLL positivity eventually relapse and die. However, the median survival with respect to BCR-ABL and TELAML was not statistically significant.

Conclusion: In summary, our study has reported the complete cytogenetic and immunophenotyping profile of the children suffering from ALL in Jammu and Kashmir. The study clearly shows that the MLL gene rearrangement positivity had a significant impact on the survival outcome. All patients positive for MLL gene rearrangement died. The immunophenotyping and conventional cytogenetic analysis had no statistically significant impact on survival with appropriate risk stratification.

Keywords: ALL, cytogenetics, immunophenotyping, overall survival, SKIMS

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INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is a neoplastic disease that results from a somatic mutation in a single lymphoid progenitor cell at one of the several discrete stages of development. ALL is the most common malignancy in children¹. It accounts for 25% of all childhood cancers and approximately 75% of all cases of childhood leukemia.

Remarkable advances have been made over the last fifteen years in the treatment of ALL and the understanding of its biology. Pediatric ALL is often cited as one of the true success stories of modern medicine. Despite advances in the

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treatment, 20 to 30% of children with ALL in whom remission is achieved after initial induction chemotherapy, subsequently relapse. An array of clinical and lymphoblastic biological features have been identified as prognostically significant in childhood ALL, including age, presenting leukocyte count, immunophenotype, chromosomal abnormalities, the presence of overt central nervous system leukemia and the rapidity with which the patients demonstrate a response to initial induction chemotherapy⁶⁻¹³.

Risk factors are utilized for stratification of therapy^{7,9,10,11,14,15,16}. More intensive therapies are administered to patients considered to have the highest risk of relapse. In contrast, some of the more morbid components of the therapy are modified or eliminated for children considered to have the best prognosis. The goal of risk-adapted therapy is to "treat away" adverse presenting features, so that cure rates are similar for patients with high risk and low-risk disease^{17,18}. Several chromosomal aberrations, including translocations, have been observed in acute lymphoblastic leukemia which results in the formation of fusion genes such as TEL-AML1, MLL-AF4, and BCR-ABL, which result in a protein with altered properties. All these chromosomal rearrangements have great therapeutic and prognostic significance. Fusion gene TEL-AML1 represents a subgroup of acute lymphoblastic leukemia cases associated with good prognosis.¹⁵

T-ALL constituted approximately 15 % of pediatric ALL and was associated with adverse outcomes.¹⁶ In a study by Noronha, E. P. *et al* a total of 8 subtypes of immunophenotypic T-ALL were defined by using multiparametric flow cytometry: early T-cell precursor, early cortical, cortical, late cortical, immature, simple positive mature, double-positive mature, and CD4/CD8 double negative mature. *CDKN2A/B*^{del} (71.4%), *NOTCH1*^{mut} (47.6%), and *FBXW7*^{mut} (17%) were the frequent gene alterations observed in pediatric ALL. *FLT3*^{mut} (22.2%) and *SUZ12*^{del} (16.7%) were frequently found gene alterations in the case of ETP-ALL ($p < 0.001$) whereas *CDKN2A/B*^{del} was rarely observed.¹⁷

To diagnose various types of leukemias, Immunophenotyping, and cytogenetic investigations have a crucial role. As a well-established fact cytochemistry has pivotal importance in neoplasm profiling. The most

significant function of immunophenotyping is in the diagnosis of the chronic lymphoproliferative malignancies and of acute leukemia which is not myeloid.¹⁸ In spite of a national registry that notes the instances of childhood lymphoblastic leukemia, still several cases remain unregistered due to lack of awareness and delay in recognition of the disease in children. The lack of infrastructure and late referral adversely affect survival outcome.¹⁹

In Kashmir, no study has been reported that has methodically recorded the cytogenetics and immunophenotyping data of the children suffering from ALL. With this background, we conducted a study to assess the immunophenotyping and cytogenetic factors and the impact they have on the outcome.

MATERIAL AND METHODS

The study was a hospital-based, observational study conducted between January 2015 to December 2019 in the pediatric unit of the Department of Medical Oncology at Sher-I -Kashmir institute of Medical science, Srinagar Jammu and Kashmir. The study included analysis of data of children in the age group of 1 to 18 years, diagnosed as acute lymphoblastic leukemia (ALL), who visited the department during the study period for their treatment. All the cases of ALL below age of 1 year (infant ALL) and those who had already relapsed at the time they presented to our Centre were excluded from the study. The data was collected from 180 patients regarding their age, gender, laboratory indices like hemoglobin, leukocyte count, platelet count, CSF cytology (positive or negative for blasts), Immunophenotypic data (Pre B ALL, B cell ALL, T cell ALL) by flow cytometry, Cytogenetics data (hyperdiploidy, normal, hypodiploidy) by the conventional karyotyping method and Fluorescent In Situ Hybridization (FISH) technique was used to determine presence or absence of BCR-ABL, TEL AML, MLL gene rearrangements. In the 180 patients, the status of blasts in peripheral blood at day-8 and bone marrow status at day 33 was assessed to determine the response to therapy. The outcome in terms of survivor and non-survivor was determined. The last point of contact in survivors and non-survivors was recorded. All the patients were risk-stratified, and the treatment was modified accordingly. The treatment protocol used was BFM 95 protocol, and treatment was risk adopted, either standard,

moderate, or high risk. Analysis of disease outcome was examined as overall survival (OS). OS was measured from the date of initial diagnosis of ALL to date of death from any cause or date of the last contact using the Kaplan-Meier method which is a nonparametric (actuarial) technique for estimating time-related events (the survivorship function).

RESULTS

This study was observational in nature. The first patient was enrolled in January 2015, and the last patient was enrolled in December 2019. The data of 180 patients with ALL who visited our Centre during the study period revealed the following results. The age range of the study population varied between 1.5 to 18 years. The mean and the median age were 9.2 and 8.0 years, respectively. The majority of patients (68%) were below 10 years. There were 57.8% boys and 42.2% of girls. The male: female ratio was 1.3:1. The common clinical manifestations at admission were fever (80%), pallor (70%), bleeding manifestations (40%) and musculoskeletal aches and pain (20%). The clinical examination revealed hepatomegaly (70%), splenomegaly (50%), lymphadenopathy (45%), and testicular enlargement (1%) of patients. Central nervous system disease at diagnosis was seen in 7.8% of patients. The Total leukocyte count (TLC/ μ l) ranged from 100/ μ l to 11 lakhs/ μ l. The mean and the median TLC were 56000 / μ l and 12000 / μ l respectively. The TLC was more than 20000 / μ l in 63.3% of patients. The platelet count at presentation was less than 30.0×10^9 /L in 38% patients, more than 30.0×10^9 /L to 50.0×10^9 /L in 40% patients and greater than 50×10^9 /L in 32% patients. Risk stratification of the patients has been done in this study into high risk, moderate risk, a standard risk, and treatment was modified accordingly, giving more intense therapy to the high-risk patients. Patients having any one of the following criteria leukemic cells < 1000 micromol/L in the peripheral blood on day 8 after 7-day prednisone pre-phase, WBC $< 20\ 000$ micromole/L, age $> 1 < 6$ years, a complete remission on day 33 (M1-marrow), no translocation t(9;22) or BCR/ABL recombination, no translocation t(4;11) or MLL/AF4 recombination, no T cell phenotype were considered as standard risk. The Medium risk group constituted of leukemic cells < 1000 micromol/L in the peripheral blood on day 8 after 7-day prednisone pre-phase, complete remission

on day 33 (M1-marrow), no translocation t(9;22) or BCR/ABL recombination, no translocation t(4;11) or MLL/AF4 recombination. All 4 criteria must be met as well as at least one of the following leukocytes > 20000 micromols/, age < 1 year, age > 6 years. The high-risk group constituted 1000micromol/L leukemic cells in the peripheral blood on day 8, no complete remission on day 33, translocation t(9;22) or BCR/ABL recombination, translocation t(4;11) or MLL/AF4 recombination(Each criterion alone qualifies as high risk regardless of age and WBC).

The Immunophenotyping analysis revealed Pre B ALL in 77.8%, B cell in 9.4%, and T cell ALL in 12.8% patients (Figure 1). Conventional cytogenetic revealed normal cytogenetics in 87.6%, hyperdiploidy in 8.5%, hypodiploidy in 3.9% (Figure 2). FISH for TEL AML, MLL, BCR-ABL gene rearrangement analysis was positive in 23.3%, 4.4%, 11.1% patients respectively.

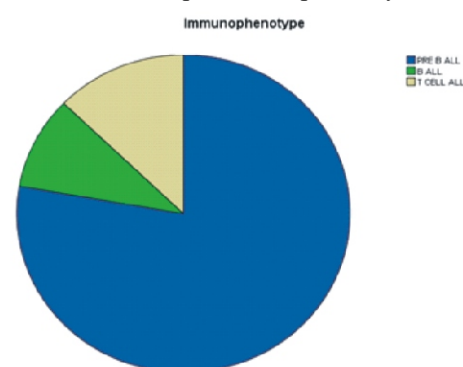


Figure 1: Immunophenotyping profile of the study participants

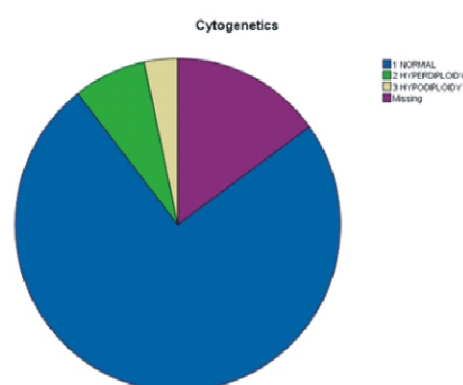


Figure 2: Conventional cytogenetic profile of the study participants

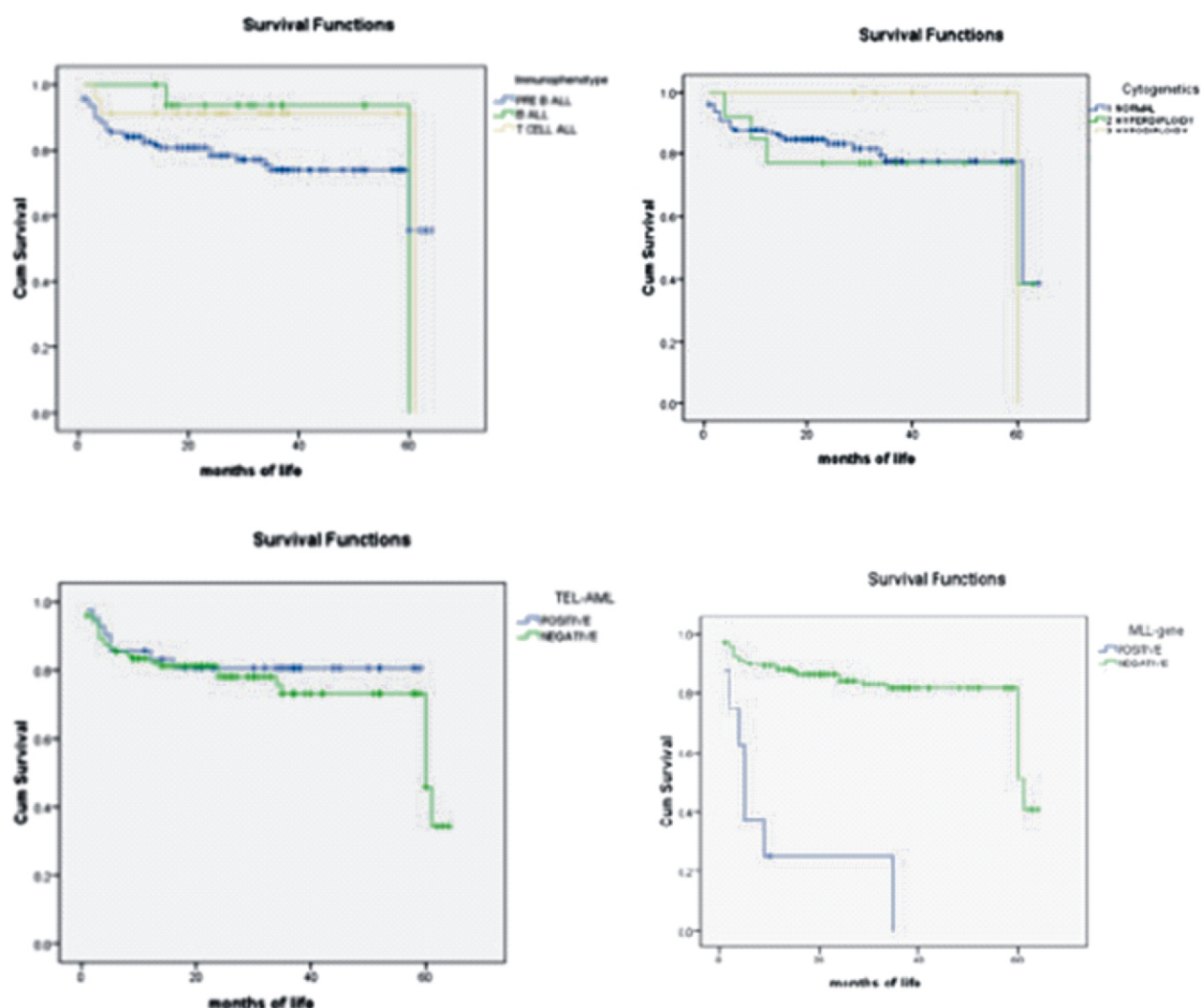


Figure 3: Kaplan-Meier analysis of immunophenotyping analysis (upper left panel), cytogenetics data (upper right panel), TEL AML data (lower left panel), and MLL-gene analysis (lower right panel)

The peripheral blood status on day 8 showed complete disappearance of blasts in 96% of patients. The bone marrow status at day 33 revealed complete remission in 87% of patients. The outcome of 180 patients revealed 142 survivors and 38 non-survivors. Among the 38 non-survivors, 27 patients had either relapsed or died, 9 patients died due to treatment-related toxicity. Bone marrow was the commonest site of relapse. So in our study, the overall survival at a median of 2.5 years was 78.9%.

The overall survival range (in months) was 1 month to 60 months with a mean of 27.25 ± 20.5 (median, 24.5). Kaplan Meier survival analysis is shown in Figure 3. As shown in the figure, the difference in the median survival time for

patients with Pre B ALL, B cell ALL, and T cell ALL was not statistically significant ($p > 0.05$). There was no significant difference in survival time in relation to karyotype, hyperdiploidy or hypodiploidy, or with respect to TEL AML and BCRABL gene. However, MLL gene rearrangement was statistically significantly related to survival time ($p = 0.001$), clearly indicating that all patients with MLL positivity eventually relapse and die.

DISCUSSION

ALL is the most common malignancy diagnosed in children, representing nearly one-fourth of all pediatric cancers. The annual incidence of ALL is about 30 cases per million people, with a peak incidence in children aged 2-5

years. Several studies from India have reported a higher prevalence of high-risk factors among the Indian population. In our study, the mean and the median ages were 9.2 (± 3.5) years and 8 years respectively. The majority of patients (68%) were below 10 years. There were 57.8% boys and 42.2% of girls. The male: female ratio was 1.3:1 which is lower than reported from Indian studies. An Indian study by Padhi et al (2011) has shown that males are a higher prevalence of ALL.²⁰ Similarly, another study reported from a tertiary care hospital in India has shown a male preponderance of 70.87%.²¹ In a study by **Padmanjali KS²² et al** from AIIMS showed a male preponderance (male: female-3.8: 1) and immunophenotyping analysis revealed 30.6% of patients have T-ALL which was much higher than western data. The immunophenotyping analysis revealed Pre B ALL in 77.8%, B cell in 9.4%, and T cell ALL in 12.8% patients. The median survival time in patients with Pre B ALL, B cell ALL, and T cell ALL was not statistically significant.

Waghay M et al²⁴ in a study, during Jan 1984-1985, on 25 children less than 15 years old (M:F ratio 3.3), with a median age of 6 years, admitted in Children's Hospital (Hyderabad) with acute lymphoblastic leukemia 31% of the 13 patients had a normal karyotype. The most common abnormal pattern was a hyperdiploid karyotype with greater than 50 chromosomes observed in 38% of the patients. Two patients had a Ph chromosome. Gladstone B et al²⁵ in a study on cytogenetic analysis, performed in 30 patients in the department of Medical Oncology, Tata Memorial Hospital Bombay (India), with T cell acute lymphoblastic leukemia chromosomal abnormalities were detected in 27 cases (90%) and hypodiploidy was detected in 19 patients (63.3%), followed by pseudodiploidy in six patients (20%) and hyperdiploidy in two patients (6%). In a study by Kamat et al²⁶ from India the immunophenotype data analysis of 124 patients of acute lymphoblastic leukemia revealed that 33% of patients had T-cell ALL which was associated with a higher age group, mediastinal mass, and high WBC count. The Pre B ALL was predominant between 2 and 9-yr age groups. In a study by Bhargava M et al²⁷ revealed T-ALL (37.7%), N-ALL (33.1%) and C-ALL (21.5%). The early age peak was absent, males predominated in all the subgroups and T-ALL had increased incidence of thymic mass. Leucocyte counts of 50,000 X 10⁶/l were equally

frequent in the three groups. T-ALL showed marked heterogeneity by showing a variety of markers such as T-helper/inducer, T-suppressor/cytotoxic, p-24, and CALLA. These results show a high prevalence of unfavorable prognostic factors. In a study by Advani S et al²⁸ at the Tata Memorial Hospital, Mumbai on 430 previously untreated patients < 25 years of age with ALL revealed that most patients had hepatosplenomegaly (80%) and or lymphadenopathy (79%) and 21% were of T-cell immunophenotype, but very few (1.3%) had CNS disease.

In our study, conventional cytogenetic revealed normal cytogenetics in 87.6%, hyperdiploidy in 8.5%, hypodiploidy in 3.9%. The median survival time in patients, who had normal karyotype, hyperdiploidy, and hypodiploidy were not statistically ($p=.381$). FISH for TEL AML, MLL, BCR-ABL gene rearrangement analysis was positive in 23.3%, 4.4%, 11.1% patients respectively. The median survival was statistically significant with respect to MLL gene rearrangement ($p=0.001$) clearly indicating that all patients with MLL positivity eventually relapse and die. However, the median survival with respect to BCR-ABL and TEL AML was not statistically significant. Amare P et al²³ reported in a study at the Department of Medical Oncology, Tata Memorial Hospital, Mumbai, India, from 1988 -1993 cytogenetic findings of 78 children (≤ 15 years) of ALL with chromosomal aberrations in (95%) cases. A lower frequency of hyperdiploidy (15.4%) in children and a higher frequency of hypodiploidy in children (38.4%) were found, in contrast to literature. Translocations were detected in one-third of pediatric cases. Common abnormalities seen were t(9;22), t(1;19), del(6q), abn(11q23), i(9p), abn(12q13), del(7q), and i(17q). In a similar study, Alkhayat et al have shown hypoploidy and MLL gene rearrangements associated with bad prognosis with short survival and high rate relapse as compared with the standard risk group ($P=0.031$) and the event-free survival was found to be statistically significant ($P=0.04$).²⁹ Gupta et al. in their study of 125 cases showed 44.8% were diploidy, 13.6% were low hypoploidy, 32.8% were high hyperploidy, and 2.4% were near-tetraploidy.³⁰ In a study by Amare et al., cytogenetic profiling of B-cell-precursor acute lymphoblastic leukemia (BCP-ALL) showed a low prevalence of ETV6/RUNX1. The study also showed the heterogeneity of ALL involving several factors

such as age, gender, the prevalence of distinct cytogenetic subgroups, etc.³¹

Kobayashi H et al³² reported 75 (31%) had normal diploidy, 47 (20%) hyperdiploidy with more than 50 chromosomes, 18 (8%) hyperdiploidy with 47-50 chromosomes, 77 (32%) pseudodiploidy, 22 (9%) hypodiploidy and one hypotetraploidy in the leukemic cells. Event-free survival (EFS) +/- standard error (SE) at 4 years was 74 +/- 7% in patients with hyperdiploidy > 50, 68 +/- 6% in those with normal diploidy, 55 +/- 13% in those with hyperdiploidy 47-50, 54 +/- 11% in those with hypodiploidy, and 22 +/- 5% in those with pseudodiploidy (log-rank, $p < 0.0001$). Pui CH et al³³ in a study showed hyperdiploidy greater than 50 has been associated with the most favorable prognosis. At the opposite end of the spectrum, the treatment outcome for patients with classical Ph+ or hypodiploid ALL is very poor even in programs of intensive chemotherapy. Cases with the t(4;11)(q21;q23) also had a very poor clinical outcome.

In a study by Kebriae P et al³⁴ the percentage of numerical chromosomal abnormalities in pediatric ALL was found to be hyperdiploidy in 45%, normal in 9%, hypodiploidy in 6% and pseudodiploidy in 40% patients. In another study by Chang HH et al³⁵ on 78 Taiwanese children under 18 years of age during 1993 to revealed 16 (20.5%) patients with normal diploidy, 28 (35.9%) with pseudodiploidy, 6 (7.7%) with hyperdiploidy (47-50), 19 (24.4%) with hyperdiploidy (> 50), and 9 (9.4%) hypodiploidy. Near-haploidy was not observed. Of the patients with abnormal karyotypes, recurrent structural abnormalities were determined in 31 (50%) of cases.

CONCLUSION

The present study has given an insight into the complete cytogenetics and immunophenotyping profile of the children suffering from acute lymphoblastic leukemia from Jammu and Kashmir. The study reveals that MLL gene rearrangement has significant impact on survival and all patients with positive MLL gene rearrangement died. With appropriate risk stratified treatments the effects of adverse prognostic factors can be minimized.

Conflict of interest: Nil

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