Molecular Cytogenetics in Childhood Acute Lymphoblastic Leukemia: A Hospital-Based Observational Study

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ABSTRACT

OBJECTIVE: This study was conducted to determine the frequency of chromosomal aberrations in children aged <19 years with newly diagnosed acute lymphoblastic leukemia (ALL), attending/admitted in the Department of Pediatrics and Radiotherapy, Government Medical College, Jammu. Furthermore, we aimed to study the correlation between the cytogenetic molecular abnormalities and the immediate clinical outcome (induction of remission).

MATERIALS AND METHODS: This was a prospective study conducted over a period of 2 years (May 2011 to May 2013) in a tertiary care hospital in India. Forty pediatric (1–19 years) patients (18 males, 22 females; M: F = 0.8 : 1) with newly diagnosed ALL were studied for molecular cytogenetic analysis. Written consent was obtained from the parents of the patients. Bone marrow aspiration was done for making the diagnosis of ALL. Children lost to follow-up and who failed to give consent were excluded from the survey. Host factors and clinical parameters were obtained from patients.

RESULTS: Bone marrow aspirate samples of 40 diagnosed cases of ALL were subjected to routine cytogenetic analysis, and reverse transcription-polymerase chain reaction (RT-PCR) technique was used for molecular analysis. Well-spread metaphase plates were obtained in 18/40 (45%) cases for analysis. RT-PCR revealed abnormal genes in 20/40 (50%) patients. The results of molecular cytogenetic analysis were correlated with patients' clinical and hematological parameters for risk stratification and immediate outcome (induction of remission). Eighteen out of 40 (45%) cases revealed no abnormality. Among the remaining 22 cases, 8 had *TEL–AML1* (20%), 6 had *BCR–ABL* (15%), 4 had *MLL–AF4* (10%), 2 had *E2A–PBX1* (5%) fusion genes, and 2 had hyperdiploidy. To conclude, a higher proportion of cases in this study showed adverse translocations such as t (9;22), t (4;11), and t (1;19) compared to that reported in literature.

CONCLUSION: RT-PCR assay was useful in detecting the prognostically significant oncogene fusion transcripts. In our study of 40 patients, we found that the pattern and frequency differ from those reported in Western literature. Our study reveals a lower frequency of hyperdiploidy (5%) and a higher frequency of *BCR–ABL* gene fusion (20%) in childhood ALL. Above all, in contrast to previous studies on childhood ALL, our study showed female predominance, with the male-to-female ratio being 0.8 : 1. Apart from the *BCR–ABL* fusion gene, none other was associated with poor prognosis. It is already well established that the characterization of the genetic entities at diagnosis is crucial for the understanding and the optimal treatment of ALL. Because the aberrations in our population differ significantly from those reported in Western populations, we may be required to tailor our protocols.

KEYWORDS: acute lymphoblastic leukemia, molecular cytogenetics, pediatric leukemia, Jammu

Introduction

A great number of cytogenetic aberrations, mainly translocations, specific to a certain character – or even a subtype of leukemia – some of which have been proven to be prognostically significant, are employed in the intent of treatment.[1] Acquired chromosomal abnormalities, structural or numerical, are observed in malignant bone marrow cells in >75% of patients with hematologic malignancies, with an increasing incidence reported due to the application of complementary detection methods provided by molecular cytogenetics.[2] Appropriate risk assessment is the most important requirement in the choice of therapy, ensuring that patients are neither over- nor undertreated. There is universal understanding that certain clinical features, genetic abnormalities of leukemic cells, pharmacodynamics, pharmacogenetics, pharmacogenomics variables of the innkeeper, and early treatment response have important prognostic and therapeutic implications.[3–5] Further refinements in therapy, including the role of risk-adapted treatment protocols, attempt to improve cure rates for high-risk patients, while determining the toxicity of therapy for low-risk patients, are required.[6–8]

Materials and Methods

This was a prospective study conducted over a period of 2 years (May 2011 to May 2013) in a tertiary care hospital in India. The

study was approved by the ethics committee of the University of Jammu, and conducted in accordance with the principles of the Declaration of Helsinki. Forty patients (18 males, 22 females; M: F = 0.8 : 1) with newly diagnosed pediatric (1–19 years) acute lymphoblastic leukemia (ALL) were studied for molecular cytogenetic analysis. Written consent was obtained from the parents of the patients. Bone marrow aspiration was done for making the diagnosis of ALL. Children lost to follow-up and who failed to give consent were excluded from the survey. Host factors and clinical parameters were obtained from patients. Detailed clinical examination, investigations, aspiration and examination of bone marrow, chemotherapy (according to risk stratification), and monitoring were conducted as per standard protocol and were not altered. Apart from routine histopathological examination for type and classification, 4 mL of bone marrow aspirate was collected for this study, 2 mL each for cytogenetic and molecular studies. The bone marrow aspirate (2 mL) was collected for cytogenetic study in a 10-mL sterile tube containing RPMI 1640 medium, heparin, and fetal calf serum. Furthermore, 2 mL of sample was collected in a 2.5-mL ethylenediamine tetraacetic acid-containing vial for molecular analysis. The above-collected samples were then subjected to karyotyping and reverse transcription-polymerase chain reaction (RT-PCR).

Results

The following host factors and clinical parameters were obtained from patients.

Age and sex. The age of patients at the time of diagnosis ranged from 6 months to 16.5 years. The majority of patients were below 10 years of age, mean age of the patients being 7.4 years. The majority of patients were females (22/40) and the male-to-female ratio was 0.8 : 1.

Nutritional status. Protein–energy malnutrition was seen in 8/40 patients (20%), with two having chronic grade 3 protein–energy malnutrition, two having acute grade 2 protein–energy malnutrition, and four having acute grade 1 protein–energy malnutrition.

Duration of symptoms. The duration of symptoms at presentation ranged from 1 week to 6 months. The majority of patients (24/40, 60%) presented with history of symptoms in the 4–12 weeks preceding the time of presentation. The prolonged duration of symptoms can be explained by the lack of proper health care facilities in the peripheral regions. Most of the patients were from far-flung areas.

Presenting symptoms. The common clinical manifestations at admission were fever, pallor, bleeding manifestations, hepatosplenomegaly, lymphadenopathy, and musculoskeletal pain. On examination, hepatosplenomegaly was observed in 34 patients, pallor in 22 patients, fever in 20 patients, generalized lymphadenopathy in 18 patients, and petechiae or bleeding manifestation in 8 patients. Joint symptoms were seen in six patients, whereas CNS disease at the time of presentation was observed in four patients. High incidence of CNS and joint disease can be explained by the delay in diagnosis.

Molecular cytogenetics. Using the morphological classification, 24 patients had L1 type ALL and the remaining 16 had L2 phenotype. Cytogenetic analysis using karyotyping revealed the occurrence of normal karyotype in 12 patients (30%). Among the remaining patients, six (15%) had abnormal karyotype (hyperdiploidy in two patients and Philadelphia chromosome in four patients [Ph+ve]). Absence of mitotic division was determined in 22 (55%) patients. Molecular analysis using RT-PCR revealed *TEL–AML1* as the most common fusion gene abnormality in the study cohort (8/40), followed by *BCR–ABL* fusion gene in (6/40), *MLL–AF4* in (4/40), and *E2A–PBX1* (2/40). No abnormal fusion gene was seen in the remaining 20 patients. *BCR–ABL*-positive patients tested positive for p190 protein. This test was conducted to differentiate ALL from CML.

Correlation between molecular cytogenetics and clinical features. The lowest hemoglobin level was noted in ALL with *BCR–ABL* fusion gene, with a base value of 3.5 g/dL, while the highest level was seen in ALL with *TEL–AML1* fusion gene, with a base value of 5.75 g/dL (Table 1). The lowest white blood cell count was seen in ALL with *TEL–AML1* fusion gene, with a base value of 7,825/mm^3 and the highest count was found in ALL with *BCR–ABL* fusion gene, with a mean value of 109,333/mm^3. The lowest platelet count was found in ALL with *MLL–AF4* fusion gene, with a mean value of 25,000/mm^3, while the highest level was seen in ALL with *TEL–AML1* fusion gene, with a base value of 165,000/mm^3.

Treatment. Treatment could be started in only 30 (75%) patients as per UK-ALL 2003 version 6 protocol because the remaining 10 (25%) patients did not opt for treatment (Table 2).

Bone marrow status at day 28/outcome. Of the 30 patients who received therapy, 20 (66.6%) were in complete remission (M1 status of the bone marrow on day 28).

Failure of induction of remission was seen in six (15%) patients, with day 28 bone marrow showing M2 status. Among those who did not go into remission after day 28 of therapy, four were already on regimen C because they had *BCR–ABL* and *MLL–AF4* fusion gene abnormalities. They were shifted to a higher center for further treatment, and imatinib was considered for their treatment. Two of the patients with M2 status of bone marrow on day 28 were shifted to regimen C from B, but their parents refused further treatment thereafter. Two patients each, among the six patients with failure of induction, had the abnormal fusion genes *BCR–ABL*, *MLL–AF*4, and *E2A–PBX1*. In the present study, there were four (13.3%) induction deaths. These patients were Ph+ve, with high initial total leukocyte count (TLC) between 90,000/mm^3 and 140,000/mm^3. Morphology revealed L1 type of blasts, as well as aggressive bulky disease at the onset in the form of massive hepatosplenomegaly and generalized lymphadenopathy. All the deaths occurred in the male group, aged >7 years. The cause of death was determined as sepsis (*Pseudomonas*, in two cases), massive intracranial bleeding (one case), and massive upper gastrointestinal bleeding (one case).

Table 1. Relative frequencies and clinical characteristics of fusion gene transcripts in all patients based on RT-PCR.

CHARACTERISTICS	TEL-AML1 (n = 8)	BCR-ABL (n = 6)	MLL-AF4 (n = 4)	E2 A-PBX1 (n = 2)	HYERDIPLOIDY (n = 2)	NONE (n = 18)
Frequency (%)	20	15	10	5	5	45
Age Range (years)	3.5–12	7–12 years	10–16.5	9	9	2–6
WBC count/cubic mm	2,500–17,000	90,000–1,500,000	60,000–70,000	90,000	8,000	25,000–100,000
WBC count/cubic mm (mean)	7,825	109,333	63,250	–	–	64,500
Haemoglobin (g/dl)	5–7.5	3–4	4–5	4.5	4.2	2.5–9
Haemoglobin (g/dl) (mean)	5.75	3.5	4.9	–	–	5.1
Platelet count/cubic mm	80,000–280,000	20,000–60,000	20,000–30,000	100,000	100,000	20,000–100,000
Platelet count/cubic mm (mean)	165,000	36,000	25,000	–	–	53,000
FAB group						
LI	3	3	0	0	1	5
L2	1	0	2	1	0	4
Gender Male/female						
Male	4	4	2	2	2	4
Female	4	2	2	0	0	14

Table 2. Outcome of patients according to molecular/cytogenetic abnormality (RT-PCR/karyotyping).

	TEL/AML1 (n = 8)	BCR/ABL (n = 6)	MLL/AF4 (n = 4)	E2A/PBX1 (n = 2)	HYPERDIPLOIDY (n = 2)	NONE (n = 18)
Treatment started	6	6	2	2	Nil	14
Successful induction of remission	6	Nil	Nil	Nil	–	14
Failure of induction of remission	Nil	2	2	2	–	Nil
Death	Nil	4	Nil	Nil	–	Nil
Relapse if any	Nil	Nil	Nil	Nil	–	2

Discussion

Acute lymphoblastic leukemia (ALL) is the most common neoplastic disease in children, which results from somatic mutation in a single lymphoid progenitor cell at one of the several discrete stages of development. It accounts for 25% of all childhood cancers and approximately 75% of all cases of childhood leukemia.[9] To improve the survival of patients with ALL in developing countries, it is important to conduct research into the biology, treatment response, and prognostic factors. Effective protocols from the Western world may not be optimal in developing countries because factors considered for assigning risk groups may differ in developing countries. There is a need to assess survival data and identify risk factors for relapse in our set of patients. Although multiple studies have been performed across the world, there is a paucity of literature available on the risk factors, pattern of relapsed disease, and outcome of children with ALL in India.[10–12]

The present study was an attempt to study the clinical features, laboratory parameters, and prognostic factors for immediate outcome (induction of remission) in 40 newly diagnosed ALL children hospitalized during a period of 2 years in the Jammu region. They were analyzed to enable identification of problems, risk factors, and prognostic factors that might be specific to this patient population. These patients were treated as per the UK-ALL 2003 version 6 protocol after clinical stratification and were given chemotherapy accordingly. Supportive therapy in the form of blood products, intravenous fluids, antibiotics, nursing care, and other necessary medication was given and patients were monitored subsequently for induction of remission or any adverse event and the final outcome.

Hyperleukocytosis (defined as TLC $>100 \times 10^9$/L) was observed in six (15%) patients; this result is similar to studies by researchers from other Indian centers, who have reported hyperleukocytosis in 15.3%–23.2% of cases. Normal cytogenetics was reported in 12 (30%) patients, consistent with results of other studies.[13,14] The present study revealed less frequency of hyperdiploidy (5%), contrary to the frequency reported in literature. In the present study, the most common chromosomal aberration detected was *TEL–AML1* (8/40),

followed by *BCR–ABL* (6/40), *MLL–AF4* (4/40), and *E2A–PBX1* (2/40).

Complete remission was thus attained in 66.6% patients. The low remission rate in the present study may be attributed to the higher frequency of unfavorable chromosomal aberrations, especially *BCR–ABL* and *MLL–AF4*, and relatively lower frequency of good prognostic chromosomal aberrations, such as hyperdiploidy. Mortality rates (10%) were similar to those reported in several Indian studies but significantly higher than those in Western studies. This may be explained on the basis of the higher percentage of unfavorable chromosomal aberrations and T-cell ALL in the Indian population.[15]

What this study adds. The following results have been obtained in this study:

1. Higher percentage (15%) of *BCR–ABL* fusion gene in childhood ALL
2. Lower percentage (5%) of hyperdiploidy in childhood ALL
3. Lower age group for *BCR–ABL* type
4. Increased duration between onset of symptoms and diagnosis
5. Hepatosplenomegaly was the most common finding at presentation in 85% of patients.
6. Patients carrying fusion genes with poor prognosis, namely, *BCR–ABL/E2A–PBX1*, were from the border areas of Sunderbhani and Samba, where shelling, blasts, and firing are common from across the border. This indicates a possible common environmental factor that may be prevalent in these areas.

Author Contributions

AP wrote the first draft of the manuscript. AR, AK, and AAS helped in writing the manuscript and did primary corrections in the manuscript. RH and SKD made final corrections to the manuscript before submission. All authors reviewed and approved of the final manuscript.

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