***BCR-ABL* Translocation in Pediatric Acute Lymphoblastic Leukemia in Southern India**

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Abstract Cytogenetics and polymerase chain reaction (PCR) based assays provide important information regarding biologically defined and prognostically relevant subgroups in acute leukemias. We utilized karyotyping and molecular analysis by reverse transcriptase PCR for the *BCR-ABL* translocation, in addition to morphological study, cytochemistry and immunophenotyping, to study 24 cases of pediatric acute lymphoblastic leukemia (ALL). Our objective was to determine the frequency of the *BCRABL* translocation in childhood ALL from southern India. Karyotyping showed one case of hyperdiploidy, one case of *t* (12; 21) translocation and one case of 46, XY-21+mar. The *BCR-ABL* translocation was found in 8.3% of these cases. One of these was a cryptic translocation, the karyotype being normal. *BCR-ABL* positivity in ALL is associated with aggressive disease and has been shown to be a poor prognostic factor, especially in children.

Keywords Acute lymphoblastic leukemia · *BCR-ABL* translocation · Karyotyping · Immunophenotyping

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy; dramatic advances in its treatment over the past three decades have changed it from a universally fatal to an almost curable disease in 85% of cases. Survival rates after treatment for acute lymphoblastic leukaemia in children in India are much lower, even in specialized cancer centers [1]. The worse outcome of treatment in Indian patients compared with recent western series was probably due to the higher rate of toxic deaths in the Indian patients, and possibly also due to their more extensive disease. Current figures for 5 year survival in childhood ALL in India are 45–50% [2]. As pediatric oncologists have become more successful at treating ALL, much of the clinical research efforts have focused on stratifying patients into various risk groups based on known prognostic features, so that patients with lower risk disease could be treated less intensively with much less side effects and toxicities, while patients with a higher risk of treatment failure could be targeted for more aggressive therapies [3]. ALL is a heterogeneous group of disorders that can be subtyped by morphology, immunophenotyping, cytogenetic study, and molecular techniques. However substantial information on these aspects of childhood ALL in India is lacking. The Philadelphia chromosome (Ph) is the hallmark of chronic myeloid leukemia and also occurs in approximately 5% of children and 20% of adults with ALL [4]. This reciprocal translocation juxtaposes the *BCR* gene on chromosome 22 with the *ABL* gene on chromosome 9, producing a fusion gene that encodes the *BCR-ABL* fusion

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Table 1 PCR primers used in this study

BCR-ABL (CML—b2a2- and b3a2-385 bp) (ALL—e1a2-485 bp)

Sense primer B2B (CML A): 5'-ACA GAA TTC GCT CAT CAA TAA G-3' (5'Ph+CML primer)

Sense primer BCR-C (CML B): 5'-ACC GCA TGT TCC GGG ACA AAA-3' (5'Ph+ALL primer)

Anti-sense primer CA3 (CML C): 5'-TGT TGA CTG GCG TGA TGT AGT TGC TTG G-3' (3' ABL primer)

RAR-αInternal control- (IC) (163 bp)

Sense primer RAR-α (CML D): 5'-TCC CCA GCC ACC ATT GAG ACC-3' (5' IC)

Anti-sense primer R4a (CML E): 5'-AGC CCT TGC AGC CCT CAC AG-3' (3' IC)

protein. Ph-positivity in ALL is associated with aggressive disease and has been shown to be a poor prognostic factor, especially in children. Thus, Ph positive patients are candidates for more aggressive treatment regimens such as bone marrow transplantation. Even with transplant the relapse rate for Ph+ALL is quite high, ranging from 40 to 80% [5]. Our objective was to determine the frequency of the *BCR-ABL* translocation in childhood ALL from Southern India.

Materials and Methods

Patients

Twenty-four cases of pediatric acute lymphoblastic leukemia were studied; material was provided by The Institute of Child Health and Hospital for Children, Egmore. ALL was classified by morphology, cytochemistry and immunophenotypic findings. The cytogenetic study was done to detect the Ph and reverse transcription (RT)-PCR was carried out to detect the presence of the *BCR-ABL* translocation. Peripheral blood and/or bone marrow smears were used for morphology, cytochemistry and immunophenotypic studies.

Morphology and Cytochemistry

Routine Leishman's stain was done first and the morphology was studied followed by cytochemistry using periodic acid Schiff (PAS), myeloperoxidase and Sudan Black B [6] staining procedures. The morphological diagnosis was based on FAB criteria [7]. However, immunophenotyping and cytogenetic analysis enabled the application of the WHO classification [8].

Immunophenotyping

Immunophenotyping was performed according to Caldwell et al. [9], using an acute leukemia panel of monoclonal antibodies consisting of CD3, CD10 (CALLA) and CD19 (Dako, Denmark). For CD10 (CALLA) monoclonal antibodies, immunophenotyping was carried out employing the

alkaline phosphatase anti alkaline phosphatase (APAAP) technique (APAAP kit, Dako, Denmark).

Cytogenetic Analysis

Heparinized bone marrow aspiration samples were cultured in RPMI 1640 with sodium bicarbonate for 24 and 48 h with mitogen phytohemagglutinin (PHA). Metaphase chromosomes were banded by the conventional GTG-banding technique [10]. Karyotypes were prepared and chromosome abnormalities were described according to ISCN [11].

RT-PCR Analysis

RT-PCR was used to detect the expression of the *BCR-ABL* fusion gene (b3a2, b2a2, and e1a2). Total RNA was extracted using TRIZOL reagent from blood. First strand cDNA was synthesized using 5 µg total RNA by RT-PCR kit (Genei, Bangalore, India). The related PCR primers listed in Table 1 were used (as per the protocol of the M.D. Anderson cancer institute) to produce the respective correlated products. The PCR for *BCR-ABL* and *RAR-α* (internal control) cDNAs were performed in an Eppendorf mastercycler gradient (Eppendorf, Westbury, NY). The PCR products were then run on 2% agarose gel and visualized by ethidium bromide staining under UV illuminator.

Results

Patient Data

Of the 24 patients, 15 were male (62.5%) and nine were female (37.5%), with a median age of 10 years (range, 6 months to 12 years). The male-to-female ratio was 5:3 (62.5:37.5%).

Morphological Analysis

The FAB subtypes and results of immunophenotyping are indicated in Table 2.

Table 2 Acute lymphoblastic leukemia: distribution of morphological and immunophenotypic subtypes

FAB category	No. of cases	No. of T cell positive cases (CD3)	No. of B cell positive cases (CD19)	Negative for B and T cell	Positive for CALLA (CD10)
L ₁	12 (50%)	1 (8.33%)	11 (91.6%)	0	12 (100%)
L ₂	12 (50%)	5 (41.6%)	3 (25%)	4 (33.3%)	2 (16.6%)
L ₃	0	0	0	0	0
Total	24	6 (25%)	14 (58.3%)	4 (16.6%)	14 (58.3%)

Cytogenetic Analysis

The results are indicated in Table 3.

RT-PCR Analysis

In our study two of 24 cases of ALL showed *BCR-ABL* translocation by RT-PCR (8.3%) (Table 3). Both these patients were males, belonged to ALL-L2 subtype and were CALLA negative. Figure 1 depicts the mRNA expression of *BCR-ABL* fusion gene (e1a2) as 485 bp and the internal control, *RAR-α* as 163 bp.

Discussion

The geographic heterogeneity of chromosomal aberrations and molecular abnormalities is well known in hematological malignancies, but data are lacking in India. The present study is, to our knowledge, one of the first such for Southern India in pediatric ALL. Large clinical studies of both AML and ALL have demonstrated that the pretreatment diagnostic cytogenetics is one of the most valuable prognostic indicators for acute leukemias. Both chromosome number (ploidy) and chromosomal structural alterations have prognostic significance. Ph is present in approximately 20% of the adults and 5% of children with ALL. It is an adverse prognostic factor in children [4].

The numbers of FAB L1 and L2 cases were equal in our study. Out of 24 cases that we used for immunophenotyping, 14 cases (58.3%) were 'B' cell type (positive for CD19) and four cases (16.6%) were 'T' cell positive

Table 3 Pediatric ALL—Results of Karyotyping and RT-PCR for *BCR-ABL*

Karyotype	No. of cases	No. of cases positive for <i>BCR-ABL</i>
Hyperdiploidy	1	0
Normal (46 XY/46XX)	21	1 ^a
46 XY-21 + mar	1	1 ^a
t (12; 21)	1	0

^a 8.3% cases positive for *BCR-ABL*

Fig. 1 mRNA expression of the *Bcr-Abl* fusion gene in peripheral blood of acute lymphoblastic leukemia in children. Lane 1 show 100 bp DNA ladder. Lane 2 shows the mRNA expression of *BCR-ABL* fusion gene (485 bp) a positive control. Lanes 4 and 5 depict the mRNA expression of 485 bp show *BCR-ABL* positivity. Lane 3 shows absence of *BCR-ABL* fusion gene and presence of internal control (163 bp) *RAR-α*

(positive for CD3). Our results for immunophenotyping for B cell are similar to that of a study conducted by Dakka et al. [12], where out of 100 samples that were analyzed B-ALL was 63% and T-ALL was 37%. Early reports also [13] show the proportion of TALL as compared to precursor B-cell ALL was lower in younger children, due to an incidence peak of precursor B-ALL in toddlers and at pre-school age compared to a constant incidence of T-ALL. All the B cell cases in our study were of the precursor B immunophenotype. We could not classify the T cell cases further because of the limited antibody panel used by us. CD10 was positive in 14 cases (58.3%). It was positive in 78.5% of 'B' cell positive cases and 21.5% of the 'T' cell

positive cases. Consolini et al. [14] reported, CD10 was detected in blast cells from 1,706 of 1,784 (95.6%) patients with B-lineage ALL and 46 of 254 (18.1%) with T-cell ALL. In our study, all the cases of ALL L1 were positive for CD10, only two of 12 cases of ALL L2 was positive for CD10. Hann et al. [15] observed that CD10 was associated more commonly with FAB L1 when compare to L2. Chromosome abnormalities were observed in three of the 24 patients (12.5%) (Table 3). Chromosomal anomalies were more common in male patients (2 of 24; 8.3%) than in female patients (1 of 24; 4.13%). Among gains of chromosomal material, the most frequent was normal chromosome 46, XX/XY observed in 21 of 24 cases (87.5%). Balanced translocations, *t* (12; 21), was seen in one of 24 cases (4.13%) and 46, XY-21 with marker was seen in one of 24 cases (4.13%). Hyperdiploidy was seen in one of 24 cases (4.13%). In our study, 16.6% of the patients showed abnormal karyotypes. Although our karyotypic findings and frequencies of specific chromosomal aberrations were in accord with most other reports. Hyperdiploidy in association with trisomies of chromosomes 4, 10, and/or 17 is associated with a particularly favorable prognosis [16]. Near hyperdiploid (47–50 chromosomes), earlier known to have adverse prognosis, has improved with the use of more effective therapy. Children whose blasts demonstrate a hyperdiploid karyotype, experience longer remissions and better survival rates than other cytogenetic groups [17]. RT-PCR analysis of the *BCR-ABL* gene in peripheral blood indicated the expression of p190 kDa Bcr-Abl tyrosine kinase in two cases. Both the positive cases were negative for *CALLA* (CD 10). In one of the cases of *BCR-ABL* positivity, the Ph was negative on karyotyping, indicating a cryptic translocation. Five percent of chronic myeloid leukemia cases carry a cryptic *BCR-ABL* gene which is not detectable cytogenetically as Ph. RT-PCR has proved to be an effective, sensitive, accurate and cost-effective diagnostic tool for this gene rearrangement [3]. Ph 1 is the classic defining cytogenetic abnormality *t* (9; 22) in chronic myeloid leukemia and is identified in 20–25% of adult B-phenotype ALL and 5% of Pediatric ALL and rarely in AML. Udaykumar et al. [18] reported that 6.4% of the patients showed a *BCR-ABL* translocation and another study by Bhutani et al. [19] detected Ph' chromosome in 4/17 (24%) childhood cases of ALL. In our study 8.3% of ALL showed *BCR-ABL* translocation by RT-PCR. Many of the children with ALL cured by current therapies are over treated and thus unnecessarily exposed to the risk of short and longterm adverse effects. Subgroups of children with unfavorable genotypes such as Philadelphia positive ALL have an extremely poor prognosis. *BCR-ABL* can be detected by RT-PCR, which is of utmost importance to initiate more aggressive therapy or even to suggest early bone marrow transplantation. Low remission rate is

expected if there is lack of suppression of Ph 1 positive metaphases with intensive chemotherapy or α interferon therapy. The worse outcome of treatment in Indian patients compared with figures from the West was probably due to the higher rate of toxic deaths in the Indian patients, and possibly also due to their more extensive disease—which is, at least partly, a consequence of delay in diagnosis [1]. Therefore, identification of additional prognostic variables that can be used to tailor therapy more precisely and discovery of drugs that can modify pathways involved in transformation and resistance to therapy are top priorities.

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