Original Article

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Prevalence of ETV6/RUNX1 Fusion Gene in Pediatric Patients with Acute Lymphoblastic Leukemia in Iran

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Abstract

Objective: ETV6/RUNX1 (also known as TEL/AML_1) is the most frequent gene fusion in childhood acute lymphoblastic leukemia (ALL). Sixty-three patients were enrolled in this study to explore the distribution of this gene in Iranian population.

Methods: This study used 63 peripheral blood and bone marrow (PB/BM) samples from children with ALL. Immunophenotyping of PB and BM samples were performed using flow cytometry to illustrate the lineage. Moreover, reverse transcriptase polymerase chain reaction (RT-PCR) technique was used to amplify transcripts of leukemia-specific chromosome fusion gene ETV6/RUNX1 and to monitor the expression levels of the ETV6/RUNX1 in patients according to Van Dongen et al protocol.

Findings: On the basis of French-American-British (FAB) classification, 47 individuals had ALL-L1. The incidence of ETV6/RUNX1 fusion gene in this study was 34.9%. The laboratory and clinical features of twenty two ETV6/RUNX1 positive ALL cases were similar to those of other studies. The most positive cases of ETV6/RUNX1 fusion gene had the early pre B ALL and pre B ALL immunophenotypes.

Conclusion: The ETV6/RUNX1 fusion gene is a common genetic anomaly in Iranian childhood ALL patients and the prevalence of the ETV6/RUNX1 fusion gene is similar to that of ALL patients in other countries. However early pre B cells were the most common type in studied patients.

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Key Words: Acute Lymphoblastic Leukemia; Reverse Transcriptase; ETV6/RUNX1 Fusion; Polymerase Chain Reaction

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. The precise diagnosis and classification of ALL is based on morphology, cytochemistry, immunophenotype, and molecular analyses of bone marrow cells. In pediatric B-lineage ALL, the t(12;21) (p13;q22) chromosomal translocation is very common and usually found in a bout 25% of all cases. The t(12; 21) (p13; q22) was first described in 1994[1] and is not detectable by conventional cytogenetic methods. It leads to the fusion of two genes, RUNX1 (AML_1) on chromosome 21 and ETV6 (TEL) on chromosome12[2,3]. The RUNX1 belongs to the core binding factor family of transcription

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factors[4,5] and ETV6 is involved in chromosome translocations in a wide variety of hematologic malignancies[6]. It appears to be an important transcription factor required for hematopoiesis in the bone marrow. Most affected patients are between the ages of 1 and 10 years with WBC count <50000/μL, and a B immunophonotype[2,15]. This study recruited newly diagnosed ALL children with transloction of t(12;21) producing the ETV6/RVNX$_1$ fusion gene. The correlation of fused gene with the local incidence of disease and the prognosticc factors was explored and analyzed. Nonetheless, studies on genetic alteration in leukemic cells significantly enhance the accuracy of diagnosis and allow determining treatment strategy for childhood ALL, especially when specific aberration is present. To increase the information available on patients with this abnormality, we examined 63 children with ALL. The present study emphasizes their laboratory data, outcomes and comparisons with other patients from the literature.

Subjects and Methods

The initial diagnosis of ALL was established by morphological, cytochemical and immune phenotypic assessments. The French-American-British (FAB) classification is based on morphology and cytochemical stains[7].

Immunophenotyping was determined by flow cytometry using a panel of monoclonal antibodies to define the lineage and to determine the level of differentiation[8]. The default panel established included: CD34, CD 45, HLA-DR, CD117, CD10, CD19, CD4, CD7, CD8, CD38, TdT, CD2, CD3, CD20 and CD22.

Molecular analysis: mononuclear cells were isolated from PB/BM samples by Ficoll-Hypaque density centrifugation and the target genes amplified using the specific primers as follows:

Primer code	5' Position (size)	Sequnce (5'-3')
TEL-A	845 (20)	TGCACCCTCTGATCCTGAAC
AML1-B	611 (19)	AACGCCTCGTCATCTTGC
TEL-C	928 (22)	AAGCCCATCAACCTCTCTATC
AML1-D	577 (18)	TGGAAGGCGGCGTGAAGC
TEL-E5'	692 (20)	CGCACCAGGAGAACAACCAC

For the reverse transcriptase-polymerase chain reaction (RT-PCR) assay, total RNA was extracted by a single– step method with Trizol (Invitrogen). To quantify ETV6/RUNX1 fusion gene the RT-PCR was performed according to a standardized protocol by Van Dongen and colleagues[9]. Moreover, all cases were analyzed and reevaluated using positive and negative controls.

Findings

The correlation of the hematological and clinical prognostic factors with the outcome of the disease was analyzed. Among the sixty three patients evaluated, 39 (62%) were boys and 24 (38%) girls and their age at the time of diagnosis varied between 1 year and 13 years. The results of hematological, immunological and molecular analysis are presented in Table 1.

Of 63 patients, 56 children (88.9%) developed leukemia from B-lineage and seven (11.1%) from T-lineage. the immunophenotyping of B-lineage analysis permitted the characterization of 28 cases (44.4%) as early pre B ALL, 22 (34.9%) as pre B ALL and 4 (6.3%) pro B ALL. The co-expression of lymphoid and myeloid antigens shown in Table 2 was confirmed as follows: one (1.6%) with early pre B ALL associated with CD_2 co-expression and one (1.6%) was the early pre B along with aberrant expression of CD13.

In follow up it was found that 59 patients were at complete remission stage and 4 died. Based on FAB classification of ALL in our results, 47 individuals were of type L1; in which immunologic classification was as follows: 21 early pre B, 17 pre B, 3 pro B and 6 T-ALL. The immunophenotypes of ALL patients with TEL/AML1 fusion transcripts were early pre B, pre B, pro B and T-ALL types. No ETV6/RUNx1 fusion transcripts were detected in early pre B, with CD2 but detected in early pre B along with aberrant expression of CD13.The ETV6/RUNX1 fusion gene was identified through RT-PCR among 22 (34.9%) patients in which ten had early pre B, 10 pre B ALL, one pro B and one T-ALL. The prevalence of ETV6/RUNX1 was 37.5% (21/56) in childhood B-lineage ALL. The ETV6/RUNX1$^+$ patients were studied with regard

Table 1: Hematological, immunological data of ALL patients and RT-PCR fusion gene amplification

Patient	Age of diagnosis (yr.mon/sex)	Hgb g/dL	WBC $\times 10^3$ mL	Type of ALL	Immunophenotype	t(12;21) ETV6/RUNX$_1$	Outcome
1	7.9/F	7.5	4300	L_1	Early pre B ALL	-	CR
2	2.10/M	8.9	9840	L_1	Pre B ALL	-	CR
3	3.5/M	6.7	173300	L_2	T-ALL	-	CR
4	2/M	7	29330	L_1	Early pre BALL	-	CR
5	3/F	11.8	8380	L_1	Pre B ALL	-	CR
6	8.5/M	10.8	16560	L_1	Pre B ALL	-	CR
7	4/M	10.5	12170	L_2	Early pre B ALL	-	CR
8	7/M	10.4	10400	L_1	Pro B ALL	-	CR
9	1.5F	10.2	8600	L_2	Pre B ALL	+	CR
10	7/M	8.1	29450	L_2	Early pre B ALL	-	CR
11	3/F	4.6	16000	L_2	Pre B ALL	+	Died
12	3.8/F	7.4	2130	L_1	T ALL	+	CR
13	9/F	7.6	5720	L_2	Early pre B ALL	-	CR
14	1.7/M	5.9	7620	L_1	Early pre B ALL	-	CR
15	10/M	9.8	2470	L_1	Early pre B ALL	+	CR
16	2.5/M	10.8	13490	L_1	Early pre B ALL	-	CR
17	8/M	10.8	24140	L_1	T ALL	-	CR
18	12.5/F	6.7	10600	L_1	Pre B ALL	-	Died
19	4/F	6.7	77980	L_2	Early pre B ALL	+	CR
20	3.2/F	10	6320	L_3	Early pre B ALL	+	CR
21	4.10/F	5.3	41280	L_2	Pre B ALL	+	CR
22	4.1/F	6.6	8170	L_1	Early pre B ALL	+	CR
23	9/F	8	11200	L_1	Pre B ALL	-	CR
24	3.10/F	4.9	18400	L_1	Pro B ALL	-	CR
25	3.5/M	5.9	35020	L_1	Early pre B ALL	+	CR
26	8/F	6.6	14000	L_1	Early pre B ALL associated with aberrant expression CD2	-	CR
27	5/M	7.1	9770	L_1	Pre B ALL	+	CR
28	4/M	9.1	22200	ALL	Pre B ALL	+	CR
29	4/M	6.3	22640	L_1	Early pre B ALL along with aberrant expression of CD_{13}	+	CR
30	6.10/F	8.9	70000	L_1	Pre B ALL	+	CR
31	8.2/M	9.5	2680	L_1	Early pre B ALL	-	CR
32	3.7/M	8.1	3600	L_2	Early pre B ALL	-	CR
33	2/M	10.4	1540	L_3	Early pre B ALL	-	Died
34	3.9/M	6.6	26400	L_3	Early pre B ALL	+	CR
35	11/F	11.9	16600	L_1	Pre B ALL	+	CR
36	12/M	8.3	803370	L_1	T-ALL	-	Died
37	7/F	9.3	5700	L_1	Pre B ALL	-	CR
38	3/M	6.2	39700	L_1	Early pre B ALL	+	CR
39	11/M	7.9	2100	L_1	Pre B ALL	-	CR
40	13/M	4.6	11970	L_1	Pro B ALL	-	CR
41	1.8/F	-	5470	L_1	Pre B ALL	-	CR
42	11/M	5.2	14300	L_1	Pre B ALL	-	CR
43	4.5/M	10.1	5100	L_1	Early pre B ALL	-	CR
44	9/M	9.9	3900	ALL	Pro B ALL	+	CR
45	12/M	5.1	16930	L_1	Early pre B ALL	-	CR
46	2/F	7.6	79600	L_1	Early pre B ALL	+	CR
47	5/M	6.4	20700	L_1	Early pre B ALL	-	CR
48	1.5/M	7.9	10500	L_1	Early pre B ALL	-	CR
49	4.5/F	3.2	12500	L_2	Pre B ALL	-	CR
50	2/M	10.5	11310	L_1	Pre B ALL	+	CR
51	8/F	6.2	27420	L_1	Early pre B ALL	-	CR
52	2/F	8.7	15750	L_1	Early pre B ALL	-	CR
53	7/M	8.8	15560	L_1	T-ALL	-	CR
54	4/M	5.3	1470	L_1	Pre B ALL	-	CR
55	2/M	11	2530	L_1	Early pre B ALL	-	CR
56	1/F	13.1	7150	L_1	Pre B ALL	+	CR
57	12/F	4.2	14210	ALL	Early pre B ALL	+	CR
58	2/M	7.9	5790	L_1	Early pre B ALL	-	CR
59	6/M	10.8	113180	L_1	T-ALL	-	CR
60	3/M	7.9	11150	L_1	Early pre B ALL	-	CR
61	5/M	5.6	19710	L_1	Pre B ALL	+	CR
62	3/M	10.8	6680	L_1	T-ALL	-	CR
63	2/M	7.5	3260	L_1	Pre B ALL	-	CR

CR: Complete Remission; ALL: Acute Lymphoblastic Leukemia; WBC: White Blood cell; Hgb: Hemoglobin; M: Male; F: Female

Table 2: Fusion gene analysis as well as French-American-British classification and comparison with different immunophenotypes in ALL patients

Immunophenotype	Patients	TEL/AML$_1$ positive	L$_1$	L$_2$	L$_3$	ALL
Pro-B	4	1	3			1
Early pre B	28	9	19	5	3	1
Early pre B with CD13	1	1	1			
Early pre B with CD$_2$	1		1			
Pre B	22	10	17	4		1
T cell	7	1	6	1		
Total	63	22	47	10	3	3

ALL: Acute Lymphoblastic Leukemia

to their gender and it revealed that 12 were females and 10 males (Table 3). Based on FAB classification it must be stated that 13 individuals were type L1; 4 were L2; 2, L3 and 3 were assumed as ALL.

In the present study among the 58 patients with WBC count ≤50×10^3/μL, 20 were TEL/AML1 positive. The patients with WBC count between 50×10^3/μL and 100×10^3/μL, 2 were ETV6/RUNX1 positive. However, none of the three patients whose WBC counts were greater than 100×10^3/μL was ETV6/RUNX1 positive. The immunologic markers in our cases with regard to ETV6/RUNX1$^+$ were as follows: 10 children had early pre B, 10 pre B, 1pro B and 1 T-ALL.

Discussion

The ETV6/RUNX1 fusion gene is thought to be the most common leukemia-specific fusion gene in children with ALL. The frequency of 34.9% referring to the ETV6/RUNX1 rearrangement, is the upper 25% average reported in the literature[16,17]. It is worth noting that the lower frequency of this fusion gene has also been observed in countries such as India (6%)[10], Mexico (9.6%)[11], Argentina (11.6%)[12], Thailand (12%)[13], China (17.9%)[14] and Taiwan (19%)[15] which indicates a significant difference among them but this difference was not significant in other studies[16-18].

Table 3: Fusion gene analyses and associations with age, white blood cell count and hemoglobin in ALL patients

Variable		TEL/AML1 Positive	TEL/AML1 Negative	Total
Age (yr)	1-10	20	35	55
	>10	2	6	8
WBC count (×10^3/μL)	<50	20	38	58
	50-100	2	0	2
	>100	0	3	3
Hemoglobin (g/dL)	<6	5	7	12
	6-10	13	24	37
	>10	4	10	14
Gender	Male	10	29	39
	Female	12	12	24
French-American-British classification	L$_1$	13	34	47
	L$_2$	4	6	10
	L$_3$	2	1	3
	ALL	3		3

ALL: Acute Lymphoblastic Leukemia; WBC: White Blood Cell

The improvement of medical assessment in Iran has resulted in a significant decrease in infant mortality rates caused by ALL. In relation to the immunophenotypes of ALL patients with ETV6/RUNX1 fusion transcripts, P. Tiensiwakul[24] found in 35 ALL patients, an incidence of 8.6% of ETV6/RUNX1 translocation (12% of B-lineage ALL), which is lower than that reported in caucasians but is similar to that reported in Japanese and Koreans[25], which indicates a significant difference with our study. In the report of Zuo YX et al, FAB-L2 morphology was commonly observed, but t[12,21] was often absent in those children[26] which indicates a significant difference with our results.

Moreover, for the newly diagnosed B-ALL cases with ETV6/RUNX1 rearrangement, several studies pointed a favorable prognosis[27-30] and some authors have suggested more comprehensive assessment whereas other studies did not identify any significant difference between the prognosis of patients with or without ETV6/RUNX1 rearrangement[22,23]. Other known clinical and hematological prognostic factors including age, WBC, and the presence of early hematological response, play an important role in ALL. In a study, the patients were grouped according to their WBC count at the time of diagnosis: the groups consisted of 21 patients with, $<50\times10^3$/μL one patient with 50-100×10^3/μL, and three patients with $>100\times10^3$/μL. Among the 21 patients with WBC count $<50\times10^3$/μL seven were ETV6/RUNX1 positive. The patient with WBC count between 50 and 100×10^3/μL was also ETV6/RUNX1 positive. However, none of the three patients whose WBC count was greater than 100×10^3/μL was ETV6/RUNX1 positive[18]. So our findings are consistent with those reported in previous literature[18-21]. Among the ETV6/RUNX1 fusion gene positive patients 90.9% (20/22) had WBC count $\leq50\times10^3$/μL, anemia 90.9% (20/22), 95.4% (21/22) with B-lineage immunophenotype, died 4.5% (1/22) and most (20/22) patients were between 1 and 10 years old. In our study, patients aged 1 to 10 years had a better outcome and were similar to the findings in other studies[17,18]. More cases will be required for future research to confirm the efficacy of our quantization method using ETV6/RUNX1 fusion transcripts as the target gene for the estimation of disease progression.

Conclusion

The molecular analysis by RT-PCR was shown to be an ideal tool for detecting hybrid transcripts. So, molecular analysis was carried out in every sample, including those that were unsuitable for cytogenetic analysis, the cryopreserved ones and those with little cellularity. Furthermore, molecular analysis is more sensitive and more specific than cytogenetic as it identifies the presence of genetic rearrangements in samples where the cytogenetic result was negative, as well as the absence of important genetic rearrangements in patients with cytogenetically identical translocations. It is known that comprehensive diagnosis of childhood malignancies using molecular assessment is now achievable in Iran. Thus, application of complementary methods to detect clinically relevant specific abnormalities (e.g., ALL with fused gene) is of fundamental importance.

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Conflict of Interest: None

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