Original Article

Cytogenetic and Molecular Assessment of Childhood Acute Lymphoblastic Leukemia Patients from 2014 to 2017 in Ahvaz

Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common hematological malignancy in children that is caused by gene mutations and chromosomal rearrangements in lymphoid cells. **Aim:** In this study, for the first time, the prevalence of cytogenetic and molecular genetic abnormalities was discussed in children with ALL from 2014 to 2017 in Ahvaz. **Materials and Methods:** A total of 72 children were diagnosed as ALL patients by morphology, clinical examinations, and flow cytometry assays. Cytogenetic and molecular genetic analysis was done on bone marrow (BM) samples by BM culture and reverse transcription-polymerase chain reaction technique, respectively. Descriptive data analysis was done using SPSS software. Chi-square and independent samples *t*-test was used to assess the correlation between variables. **Results:** Sixty-five cases (90.3%) were preB lineage and 7 cases (9.7%) were T-lineage out of 72 ALL patients, t(9,22) BCR-ABL (p190) is the most frequent cytogenetic and molecular genetic abnormality in preB ALL (7%) and T-ALL patients (28.6%), respectively. t(4,3) inv (16) and t(2,8) were introduced as novel cytogenetic abnormalities in preB ALL cells. No significant correlation was found between gender, molecular genetic abnormalities, and white blood cell count in patients. **Conclusion:** For the first time in this study, the highest percentage of cytogenetic and molecular genetic abnormalities was related to t(9,22) BCR-ABL in both ALL subtypes in children. The evaluation of cytogenetic and molecular genetic abnormalities in children with ALL is essential in estimating the prognosis in both preB and T-ALL subtypes, which will be a great contribution to achieve a better diagnosis and develop appropriate therapeutic approaches.

Keywords: *Acute lymphoblastic leukemia, childhood, cytogenetic, molecular*

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Introduction

Acute lymphoblastic leukemia (ALL) is a hematological malignancy with high prevalence among children and is characterized by genetic changes such as mutations and chromosomal translocations.[1] These malignancies are responsible for approximately 80% and 20% of cases of cancers in children and adults, respectively. The ALL outbreak in men and women is 1.7/100,000 per year.[2] The main cause of the disease has not been determined yet, but it has been shown that various factors, including environmental factors, viral infections, and genetic changes, and some syndromes such as Down, Klinefelter, and Bloom have been shown to occur.[2-5] However, chromosomal translocations and related molecular variations have been shown to play a major role in pathogenesis and therapeutic response in patients. Most of these changes occur in genes that play essential roles in lymphoid development, cell cycle, or as tumor suppressors or oncogene. Genetic changes and clinical symptoms can be useful in the classification of ALL to subtypes as well as diagnostic and prognostic factors for patients monitoring.[1,6,7] Identifying chromosomal translocations and related molecular changes not only identify leukemia cells pathogenicity, but also optimize therapeutic approach to increase patient survival.[8] Therapeutic approaches are usually based on the prognostic characteristics of chromosomal translocations and their classification based on high-, moderate-, and low-risk groups. For example, recent studies have shown t(1; 19) (q23; p13) associated with the formation of TCF3-PBX1 fusion was associated with a poor prognosis in childhood B-ALL, although new

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antimetabolic drugs used in chemotherapy increased patients survival due to appropriate therapeutic response.[8,9] Several chromosomal translocations decrease therapeutic responses and increase mortality in patients. Studies showed t(9; 22) (q34, q11.2) translocation leads to BCR and ABL1 genes fusion, and ultimately leads to the formation of the Philadelphia chromosome (Ph).[10] Ph^+-ALL patients have a poor prognosis and are resistant to tyrosine kinase inhibitors. Therefore, the detection of t(9; 22) (q34, q11.2) translocation in ALL patients can increase the survival of patients before developing the disease by employing optimal therapeutic strategies.[11,12] Genetic changes associated with blood cell counting can be effective in determining the survival of the patients, and in applying therapeutic strategies that prevent the disease progression.[13] Therefore, in this study, we monitored the incidence of cytogenetic and molecular genetic abnormalities in children with ALL, as well as the relationship of genetic abnormalities with laboratory parameters such as white blood cell (WBC) from 2014 to 2017 in Ahvaz.

Materials and Methods

Study group: Patients and samples

ALL was diagnosed through bone marrow (BM) aspirate containing at least 30% blast cells based on the French–American–British classification during 2014 and 2017 in Ahvaz. After morphologic and clinical examinations, as well as flow cytometry assays on 72 ALL patients, they were enrolled in this study. ALL patients included 46 males (63.9%) and 26 females (36.1%) (5–14-year-old; median age: 5.38 years). Sixty-five cases (90.3%) were preB lineage and 7 (9.7%) were T-lineage ALL. Patients were treated based on ALL protocol in Shifa Hospital and received induction therapy with a combination of drugs, including vincristine, prednisone, cyclophosphamide, doxorubicin, and L-asparaginase. In addition, BM samples were obtained on the 7th day of treatment. A volume of 5 mL of BM sample was collected from each patient in falcon tubes containing heparin and ethylenediaminetetraacetic acid anticoagulants for performing cytogenetic and molecular genetic analyses, respectively. All BM samples were taken within 4 months with written informed consent from the patients. This study was approved by the Local Ethics Committee (IR. AJUMS. REC.1395.485) and was conducted within 8 months.

Cytogenetic and molecular genetic analyses

Cytogenetic analysis was done by BM culture on the slide (known as karyotyping technique) for each patient. BM samples were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 2 mmol/l glutamine, 25 mmol/l HEPES, 1.5 g/l sodium bicarbonate, 10% fetal calf serum, 50 U/ml penicillin, and 50 g/ml streptomycin at 37°C in 5% CO_2. The chromosomes obtained were then stained by Giemsa stain and later denaturalized by trypsin. Then, mitosis analysis was done for detecting the types of numerical and structural chromosome aberrations by microscopic examination.

Molecular genetic analysis was done using reverse transcription-polymerase chain reaction technique. In this regard, RNA extraction, cDNA synthesis, and PCR amplification were performed on BM samples according to the guidelines of kit manufacturer for each patient.

Statistical analysis

This study was an epidemiological analysis. Descriptive data analysis was conducted using SPSS software, version 22. Chi-square and independent samples *t*-test were used to assess the correlation between variables, and $P < 0.05$ was considered statistically significant.

Results

Sixty-five cases (90.3%) out of ALL patients, were preB lineage (42 males (64.4%) and 23 females (35.4%)). Seven cases (9.7%) were T-lineage (four males [57.1%] and three females [42.9%]).

Cytogenetic and molecular analysis

Forty-four (625) and 5 (71.4%) of patients were normal karyotype in preB and T-ALL, respectively. Translocation t(9.22) BCR-ABL (p 190) (28.6%) was seen in the remained patients of T-ALL [Tables 1 and 2]. T(9,22) BCR-ABL (p190) (7%) showed the highest frequency of cytogenetic and molecular genetic abnormalities in patients with preB-ALL. Three patients showed translocation of t(1.11) (4.2%) and three showed translocation of t(4.11) (4.2%). Several translocations were also observed in preB ALL patients [Table 3]. Several new genetic changes such as t(4.3) (1.4%) and inv(16) (1.4%) were observed in preB-ALL patients [Tables 3 and 4].

Table 1: Frequency of cytogenetic abnormalities in seven T-acute lymphoblastic leukemia patients

	Frequency (%)	Cumulative percent
Cytogenetic		
Normal	5 (71.4)	71.4
t(9, 22)	2 (28.6)	100.0

Table 2: Frequency of cytogenetic abnormalities in seven T-acute lymphoblastic leukemia patients

	Frequency (%)	Cumulative percent
Molecular genetic		
BCR-ABL (p190)	2 (28.6)	28.6
Negative	5 (71.4)	100.0

Association between age and white blood cell with cytogenetic analysis

Translocations such as BCL11A-MYC, KMT2A-MLLT1, and TEL-AML1 seem to increase WBC count, they are not statistically significant due to the low frequency of these translocations in patients. However, there was no significant correlation between WBC count and molecular genetic disorders in patients [Tables 5 and 6]. Furthermore, no significant relationship was found between gender and molecular genetic abnormalities in patients [Table 7].

Discussion

ALL is a hematological malignancy develops as a result of the increase of the proliferation of lymphoid precursors and impairment of their differentiation. A series of genetic and molecular changes that are associated with certain clinical features cause this impairment.[14-16] Studies showed the frequency of genetic and molecular changes in different parts of the world is different.[17,18] Forestier *et al*. in 2000, showed that translocations of 11q23 (3.7%) and t(9; 22) (q34; q11) (2.2%) had the highest frequency in their study population.[19] However, Andreasson *et al*. in 2000 showed that del (9p) was the most common chromosomal translocation in children with preB ALL.[20] In this study, abnormal BCR-ABL (p 190) showed the highest percentage of molecular genetic abnormalities in patients with preB ALL and T-ALL [Tables 2 and 3]. These results are similar to the results of De Braekeleer *et al*. study, which showed that t (9; 22) was the most common chromosomal translocation in preB and B-ALL.[21] Chopra *et al*. study also showed that t(9; 22) had the highest frequency in pediatric and adult B-ALL.[22] In the second finding of this study is the low frequency of translocations of t(1.19), t(11.19), and t(12.21) among ALL-B ALL patients [Table 4]. This finding is in accordance with the study of Chebihi *et al*. the frequency of translocations of t(1.19), t(11.19), and t(12.21) was very low in B-ALL patients in this study.[23]

Table 3: Frequency of molecular cytogenetic abnormalities in 65 preB acute lymphoblastic leukemia patients

	Frequency (%)	Cumulative percent
Molecular genetic		
Negative	47 (69.1)	97.1
BCL11A-MYC	1 (1.5)	1.5
BCL9-IGH	1 (1.5)	2.9
BCR-ABL (p 190)	5 (7)	10.3
CBFB-MYH11	1 (1.5)	11.8
E2A-PBX1	1 (1.5)	13.2
IGH-IGL	1 (1.5)	14.7
KMT2A-AFF1	3 (4.4)	19.1
KMT2A-EPS15	3 (4.4)	23.5
KMT2A-MLLT1	2 (2.9)	26.5
KMT2A-MLLT3	1 (1.5)	27.9
TEL-AML1	2 (2.9)	100

Table 4: Frequency of cytogenetic abnormalities in 65 preB acute lymphoblastic leukemia patients

	Frequency (%)	Cumulative percent
Cytogenetic		
46 xy and 46 xx (normal)	44 (62)	70.4
del (15)	1 (1.4)	1.4
del (18)	1 (1.4)	2.8
del (5 p)	1 (1.4)	4.2
del (2)	1 (1.4)	5.6
der (3)	1 (1.4)	7
Inv (16)	1 (1.4)	8.5
t(1, 11)	3 (4.2)	74.6
t(1, 14)	1 (1.4)	76.1
t(1, 19)	1 (1.4)	77.5
t(11, 19)	2 (2.8)	80.3
t(12, 21)	2 (2.8)	83.1
t(14, 22)	1 (1.4)	84.5
t(2, 8)	1 (1.4)	85.9
t(4, 11)	3 (4.2)	90.1
t(4, 3)	1 (1.4)	91.5
t(9, 11)	1 (1.4)	93
t(9, 22)	5 (7)	100

Schneider *et al*. assessed cytogenetic abnormalities in children with T-ALL, and the relationship with the prognosis in patients and indicated t(11; 14) (7%) as the most common disorder in these patients, which was associated with a favorable prognosis.[24] Furthermore, Chang *et al*. assessed cytogenetic abnormalities in children with ALL and found that t(7;14) was the most common disorder in T-ALL patients.[25] However, in the present study, t(9,22) and BCR-ABL had the highest rate of cytogenetic abnormalities in T-ALL. In our study, several new disorders, including t(4,3) (1.4%) and inv (16) (1.4%), were introduced in addition to common cytogenetic abnormalities in preB ALL patients, which were not reported in previous studies.

Studies have shown that several characteristics such as cell count, including WBC, age, along with the identification of genetic and molecular changes, can be useful in estimating therapeutic response survival time.[26,27] Awan *et al*., showed the increase in age, enhance the incidence of BCR/ABL in ALL patients. Patients with BCR/ABL translocation increased the count of WBC, which was associated with therapeutic resistance.[28] Soszynska *et al*. found that patients in younger age had better survival and prognosis and patients with high WBC and BCR/ABL translocation were more resistant to conventional treatments and had a poor prognosis.[29] However, in our study, no significant relationship was found between molecular genetic abnormalities and gender, and WBC counts in both ALL subtypes [Tables 5-7].

Table 5: Frequency of white blood cell and molecular genetic abnormalities in 72 acute lymphoblastic leukemia patients

Molecular	*n*	WBC			
		Mean	SD	Minimum	Maximum
Negative	52	19.0398	16.67566	1.00	87.50
BCL11A-MYC	1	49.3000	.	49.30	49.30
BCL9-IGH	1	74.9000	.	74.90	74.90
BCR-ABL (p 190)	7	4.9200	3.22365	0.10	12.76
KMT2A-AFF1	3	3.7000	1.55563	2.60	4.80
KMT2A-EPS15	3	8.9000	8.34386	3.00	14.80
IGH-IGL	1	1.9000	.	1.90	1.90
CBFB-MYH11	1	5.3000	.	5.30	5.30
KMT2A-MLLT1	2	15.5000	.	15.50	15.50
E2A-PBX1	1	6.0000	.	6.00	6.00
TEL-AML1	2	15.9000	18.38478	2.90	28.90
KMT2A-MLLT3	1	4.5000	.	4.50	4.50
Total	72	15.0499	16.68295	0.10	87.50

WBC: White blood cell, SD: Standard deviation

Table 6: Correlation between white blood cell and molecular genetic abnormalities in 72 acute lymphoblastic leukemia patients

	Levene's test for equality of variances		*t*-test for equality of means						
	F	Significance	*t*	df	Significance (two-tailed)	Mean difference	SE difference	95% CI of the difference	
								Lower	Upper
WBC									
Equal variances assumed	0.976	0.327	0.903	70	0.370	3.70152	4.09862	−4.47293	11.87597
Equal variances not assumed			0.933	57.210	0.355	3.70152	3.96712	−4.24187	11.64492

Df: Difference, WBC: White blood cell, SE: Standard error, CI: Confidence interval

Table 7: Correlation between gender and molecular genetic abnormalities in 72 acute lymphoblastic leukemia patients

	Value
Pearson χ^2	10.607[a]
Likelihood ratio	14.037
Linear-by-linear association	0.362
Number of valid cases	72

[a]This value calculated for association between gender and molecular genetic according to static analysis. df: Difference, Sig: Significance

Conclusion

Finally, we can say since genetic and molecular variations were different in compare to other studies, we concluded environmental, geographic, and population factors caused these differences among different populations. On the other hand, the evaluation of these molecular changes can be used as diagnostic and prognostic factors along with other clinical parameters in monitoring the patient and this hypothesis requires further studies in the future.

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Conflicts of interest

There are no conflicts of interest.

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