Acute Lymphocytic Leukemia (ALL) is a genetic abnormality with defective maturation and differentiation of lymphoid cells, which is observed in 3%, and 30% of children and adults respectively\(^\text{[1, 2]}\) with inappropriate prognosis which can be recovered with Imatinib (Tyrosine kinase inhibitors-TKIs) as a chemotherapy drug.\(^\text{[3]}\) Although, ALL is curable in more than 80% of children but relapsed ALL is accounts as the main cause to die during the childhood.\(^\text{[3]}\) Besides age, karyotype abnormality is more related to prognosis and can be used for patient's classification in standard risk and high risk \(^e\) groups.\(^\text{[5, 6]}\) Presence of positive Philadelphia (ph) chromosome in ALL leukemia cells is with reserve movement which is called t (9;22) (q34, q11) and causing a BCR-ABL chimeric attaching gene (fraction point e1a2) and creates a 190kD protein with kinase tyrosine activation that is able to change the various signaling pathways with participation in tumor growth and proliferation.\(^\text{[7]}\) In adults who were suffering from ALL, ph chromosome is the most common cytogenetic abnormality that reports in 20%-30% of ALL cases with >50 years old.\(^\text{[8, 9]}\) Ph chromosome is a result of a reserve and bipartite movement that conducing to transcription with a new joining that called fraction point of region V-Abelson (ABL1) on chromosome number 9 and creates murine leukemia viral oncogene ho-
mologue joining gene which this movement finally conclude to an active tyrosine kinase protein composition.\cite{10} Chromosomal rearrangements which involved ABL 1 gene and conclude to BCR-ABL 1 joining gene, generally are related to Chronic Myeloid Leukemia (CML) and B-ALL.\cite{11} At the moment, another 6 genes that will pair with ABL 1: indicated BCR-ABL 1,\cite{10} TETV6-ABL 1,\cite{12} ZMTZ1-ABL 1,\cite{13} EML 1-ABL 1,\cite{14} NUP 214-ABL 1,\cite{15} RCSD 1-ABL 1,\cite{16} SFPQ-ABL 1,\cite{17} ABL 1 kinase dominate conserve in ALL chimeric proteins which are involved from N-terminal part of the protein, and it often includes a coil-coiled or helix-loop-helix dominant.\cite{22} Screening for ABL 1 chimeric genes can perfume in ALL patients especially in some of them with T-ALL because ABL 1 regulates T-cells maturation and play a primary role in the process of cellular-skeleton deforming in T cells.\cite{23} Mostly, 75% of ALL cases in children are chromosomal relapse changes which are indicatable by karyotyping, FISH and molecular techniques. With increasing age, the prevalence of genetically changes are reduced with proper result and variations are common with the inappropriate result like BCR-ABL 1.\cite{14} According to sensitivity and evaluating feature of P190 fraction point, it seems that P190 can mention as a proper evaluating marker in the diagnosis and prognosis of ALL. This study was done with the aim of determination of the P190 BCR-ABL 1 transcript prevalence in different ethnics of Iranian population.

**Methods**

**Study Group: Patients and Samples**

In this cross-sectional study, after morphologic examinations, clinical and Flow cytometric immunophenotyping on ALL patients, 50 were diagnosed as ALL based on FAB (French, American, British) classification. ALL patients included 28 males (56 %) and 22 females (44 %) (3 month-15 years old; median age: 5.56 years). Five ml, peripheral blood (PB) sample was collected from each patient in tubes containing EDTA anticoagulant for detection of P190 BCR-ABL1 transcript. All demographic, clinical and laboratory features of the patients, including age, gender, and ethnicity were listed in Table 2. All PB samples were obtained from the Shafa Hospital in Research Center of Thalassemia & Hemoglobinopathy of Ahvaz Jundishapur University of Medical Sciences during 2 years with written informed consent from the subjects. This study was approved by the local ethics committee of the Ahvaz Jundishapur University of Medical Sciences (Ajums.REC.1393.83) and was conducted within six months.

**RNA Extraction, CDNA Synthesis and, Nested-PCR Assays**

First 5 cc of blood in a tube which involved EDTA in Falcone that previously add 2 cc Lymphodex, centrifuged which included: plasma, WBC, Lymphodex and RBC. Mononuclear cells separated from 2-5 ml peripheral blood by centrifugation with high concentration gradient on Hypaque-Ficol. The total RNA was extracted from approximately 10^4 mononuclear cells by the RIBO-Prep kit (Central Research Institute for Epidemiology, Moscow). The whole RNA was determined by gel electrophoresis before reverse transcription. For the preparation of CDNA, the concentration of RNA, first by The spectrophotometric method was measured (suitable concentration is 80-160 μl/ng). cDNA synthesis was done with BIONEER (made in South of Korea) as follows: first, 1.5 μL of specific primer, 2 μL RNA and 18 μL distilled water were mixed. Then, the mixture was placed in ABI step one plus PCR instrument for 5 minutes in 95 °C, 30 seconds in 50 °C, 4 minutes in 42 °C and 30 seconds in 20 °C. The presence of P190 BCR-ABL1 fusion gene was confirmed by Nested polymerase chain reaction (PCR) in 2 steps with 10 μL PCR 2X Mastermix (Fermentas Life Sciences, St Leon-Rot, Germany) in a Rotor-Gene 6000 system (Corbett, Concorde, NSW, Australia) according to manufacturer's instructions. TaqMan® Reverse Transcription Kit (Applied Biosystems, Grand Island, NY, USA) was used for reverse transcription with 0.5 μM of 4 primers: BCR e1 F, ABL a2 R, BCR e1 F and ABL a3 R provided in the TaqMan® assays. Then, 1 μL of template and 8 μL of distilled water were added. PCR reaction was performed as follows: initial polymerase activation at 95 °C for 3min, 35 amplification cycles at 95 °C for 30sec, 60 °C for 30sec, 72 °C for 30sec and final amplification at 72 °C for 4min. The data were normalized using Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) endogenous control. The following primers were used for cDNA synthesized from P190 BCR-ABL1 were listed in (Table 1). Final PCR products analyzed on 1.5% agarose gel and presented by ethidium bromide staining (Figs. 1, 2).

**Statistical Analysis**

Statistical Package for Social Sciences (SPSS) version 23 were used for data analysis. K2 test was used for calculating the prevalence of P190 BCR-ABL1 transcript in different ethnicity. Independence T-test was applied for surveying of the age and Fisher-test was used for gender of patients. One-way Variance analysis test was applied for the comparison of age and gender with different ethnic groups. P value <0.05 was considered statistically significant.

**Results**

**Demographic Features of the ALL Patients**

The median age of the mentioned patients was 5.65 (3 months-15 years old), which 26 (52%) were <5, 12 (24%)
were 5-9 and 12 (24%) were >10 years old. From ALL patients, 28 (56%) were males (median age: 4.57 years old) and 22 (44%) were females (median age: 7 years old). There was not a significance difference between age and gender groups of the patients (p=0.38) and (p=0.14) respectively (Table 2).

According to obtained results, from All patients, 7 (14%) were Fars, 13 (26%) were Lur, 27 (54%) were Arab and 3 (6%) were Kurd ethnicity (Table 2). The median age of Fars, Lur, and Arab were 8 years, 2 years, and 5 years, respectively. There was no significance difference between these ethnicities (p=0.97) (Table 2).

**Table 1.** Primers used in 2 steps of Nested-PCR assays in ALL patients

<table>
<thead>
<tr>
<th>Step</th>
<th>Gene</th>
<th>Primer sequence</th>
<th>Primer size (NT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>BCR e1 A F</td>
<td>5’-GACTGCACTCCAATGAGAAC-3’</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>ABL a3 B R</td>
<td>5’-GTTTGGGCTTCACACCATCC-3’</td>
<td>21</td>
</tr>
<tr>
<td>Second</td>
<td>BCR e1 C F</td>
<td>5’-CAGAACCACAGTCCTTC-3’</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>ABL a2 D R</td>
<td>5’-TCTCCCTGTGATTAGGCC-3’</td>
<td>21</td>
</tr>
<tr>
<td>Control</td>
<td>GAPDH F</td>
<td>5’-CCTGAGCTCAGTGTAGTG-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GAPDH R</td>
<td>5’-TCAGTCTGTCCATAATTAGTCC-3’</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** The distribution abundance of P190 BCR-ABL1 Philadelphia chromosome based on demographic features

<table>
<thead>
<tr>
<th>Variable</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 5 years</td>
<td>50% 24</td>
<td>100% 2</td>
</tr>
<tr>
<td>5-9 years</td>
<td>25% 12</td>
<td>0% 0</td>
</tr>
<tr>
<td>10 and more than 10</td>
<td>25% 12</td>
<td>0% 0</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56.3% 27</td>
<td>50% 1</td>
</tr>
<tr>
<td>Female</td>
<td>43.8% 21</td>
<td>50% 1</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fars</td>
<td>14.6% 7</td>
<td>0% 0</td>
</tr>
<tr>
<td>Lur</td>
<td>27.1% 13</td>
<td>0% 0</td>
</tr>
<tr>
<td>Arab</td>
<td>52.1% 25</td>
<td>100% 2</td>
</tr>
<tr>
<td>Kurd</td>
<td>6.3% 3</td>
<td>0% 0</td>
</tr>
</tbody>
</table>

**Figure 1.** PCR products on 1.5% agarose gel for the first step of Nested-PCR in ALL patients with P190 BCR-ABL e1-a3 fusion gene. Line 1: Positive control (P190 BCR-ABL), Lines 2 and 3: Negative bands for ALL patients, Line 4: Negative control, Line 5: Deionized water.

**Figure 2.** PCR products on 1.5% agarose gel for the second step of Nested-PCR in ALL patients with P190 BCR-ABL e1-a2 fusion gene. Line 1: Positive control (P190 BCR-ABL), Lines 2 and 3: 381 bp ladder related to ALL patients with positive translocation, Line 4: Negative control, Line 5: Deionized water.
Arab and Kurd patients were 5.28, 3.8, 6.3 and 8.7 respectively. But there was not a significance difference between ethnic groups of the patients (P=0.97) (Table 2). In addition, there was not a significant difference between the age of patients and ethnicity (P=0.24). Also, with gender distribution, in the Fars, Lur, Arab and Kurd, 4 (57.1%), 8 (61.5%), 14 (51.9%) and 2 (66.7%) were males and 3 (42.9%), 5 (38.5%), 11 (48.1%) and 1 (33.3%) were females respectively. But there was not a significant difference between the gender of patients and ethnicity (P=0.12).

P190 BCR-ABL1 Transcript Prevalence in ALL Patients and Demographic Features

This study was done on 2 step Nested-PCR for P190 BCR-ABL1 (e1-a3 and e1-a2) fusion genes. There wasn't any band for e1-a3 fusion gene in the first step of Nested-PCR (Fig. 1) in all patients. But the main band for e1-a2 fusion gene in second step of Nested-PCR was observed (Fig. 2). Among 50 patients with ALL, 2 (4%) were positive for P190 BCR-ABL1 transcript (e1-a2 fusion gene). The median age of patients with positive and negative P190 BCR-ABL1 transcript was 2.5 and 5.78 years old. Also, both positive P190 BCR-ABL1 patients were <5 years old, one (50%) and one female (50%). Also with ethnicity, both patients were Arab (100%) (Table 2) (Fig. 3).

Discussion

The Nested-PCR assay is a useful device for indicating attachment transcripts in the result of special chromosomal movement in leukemia cells. Primer’s compounds that were used in Nested-PCR has a $10^{-3}$–$10^{-4}$ sensitivity, and it can indicate different types of attachment transcripts in PCR reaction. This method shows trustable discovery of BCR-ABL transcripts as a result of m-bcr fraction points like e1 a2 or e1 a3. Researchers try to using the effects of BCR-ABL attachment transcripts for inappropriate prognosis prediction in adults and children with ALL. The prevalence of P190 BCR-ABL1 attachment transcripts is less studied especially in children. It seems that the fraction point of BCR-ABL1 attachment transcripts is different between the various populations with age, gender, and ethnicity. For instance, in a study that was done in Pakistan by Faiz et al in 2011, the median age in BCR-ABL1 positive group was significantly higher than BCR-ABL1 negative group.[23] There is a few evidence about the P190 BCR-ABL1 prevalence and its relation with gender, age, and ethnicity with in Ph+ ALL in Iranian population. In this study, P190 BCR-ABL1 transcript in patients who referred to Shafa hospital in 2 cases (4%) with <5 years old were positive for P190 BCR-ABL1 with e1 a2 fusion gene. This prevalence compacts with Gutierrez et al. study with the 3.1% prevalence in Indian population.[24] Also, Jaso et al. and Gleissner et al. reported that the prevalence of P190 BCR-ABL attachment gene was 77% in the US and Germany, which showed that, children are faced to the acquired genetically features equal to adults in relation with unsuitable consequences of P190 BCR-ABL transcript.[10, 25] Therefore, according to the results of our study and other related studies, P190 BCR-ABL transcript prevalence seems to be variable in different regions and ethnicities and also, it can be presented in both children and adults. But more studies and more populations in different regions are needed to answer these challenges.

The present study could not find a significant difference between prevalence of P190 BCR-ABL and age, gender, and ethnicity due to low positive population. But Faiz et al. in 2011, showed a relationship between the prevalence of BCR-ABL1 and WBC count and also complete recovery (CR) in BCR-ABL negative patients equal to BCR-ABL positive.[23] Therefore, according to the results of our study and other related studies, P190 BCR-ABL transcript can be used as a marker for determining the diagnosis and prognosis of ALL patients but it needs still more studies in this field.

The limitations of the current study were the low population of the positive cases with P190 BCR-ABL and more populations are needed to confirm this prevalence in different ethnicities of Iranian populations. Also different age groups of children and adults should be evaluated for better understanding the relationships between P190 BCR-ABL transcript and age in these patients. In addition, in this study, 2 step Nested-PCR for P190 BCR-ABL1 (e1-a3 and e1-a2) fusion genes was done. But e1-a2 fusion gene band was just observed in 2 cases (4%) in second step of Nested-PCR in Arab ethnicity of our patient’s population. So, according to our findings, more studies are still required to investigate the relationships between different populations and P190 BCR-ABL1 fusion genes.
Conclusion

In this study, P190 BCR-ABL1 transcript prevalence for e1-a2 fusion gene was 2 (4%). According to different prevalence of P190 BCR-ABL1 transcript in different populations and also in different age groups, more studies are still required to better understanding of this transcript prevalence and its correlations with age and ethnicity of the patients. Also, P190 BCR-ABL1 transcript prevalence relationship with diagnosis and prognosis of the patients should also be evaluated.

Disclosures

Ethics Committee Approval: All the procedures performed in the studies involving human participants were in accordance with the ethical standards of local ethics committee of the Ahvaz Jundishapur University of Medical Sciences (Ajums.REC.1393.83), as well as 1964 Helsinki declaration.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Authors declare that they have no conflict of interest.

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Authorship Contributions: Concept – S.A.T; Design – Z.T.A; Supervision – Z.T.A; Materials – A.A.A; Data collection &/or processing – F.T; Analysis and/or interpretation – M.A.J; Literature search – S.A.T; Writing – S.A.T; Critical review – Z.T.A.

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