P190 BCR-ABL1 Transcript Prevalence in Iranian Children with Acute Lymphoblastic Leukemia

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Abstract

Objectives: Acute lymphoblastic leukemia (ALL) occurs due to the defective maturation of lymphoid cells and lack of differentiation. The present study was aimed to determine the prevalence of P190 BCR-ABL1 transcript in ALL patient and their relation with age, gender, and ethnicity in Iranian population.

Methods: This cross-sectional research was done on 50 children with ALL including 28 (56%) males and 22 (44%) females. The presence of P190 BCR-ABL1 transcript was assessed by Nested-PCR technique.

Results: From 50 ALL patients, P190 BCR-ABL1 transcript with e1-a2 fusion gene was positive in 2 (4%) cases, one patient (50%) male, and one female (50%) with <5 years old age range and Arab ethnicity.

Conclusion: It seems that P190 BCR-ABL1 transcript prevalence and its relations with diagnosis and prognosis, age and ethnicity of ALL patients, need a higher population of the patients to better achievement in this field.

Keywords: Acute lymphocytic leukemia, BCR-ABL1, Nested-PCR

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,[1, 2] with inappropriate prognosis which can be recovered with Imatinib (Tyrosine kinase inhibitors-TKIs) as a chemotherapy drug.[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.[4] 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.[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.[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.[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-
molecule joining gene which this movement finally conclude to an active tyrosine kinase protein composition.10 Chromosomal rearrangements which involved ABL1 gene and conclude to BCR-ABL1 joining gene, generally are related to Chronic Myeloid Leukemia (CML) and B-ALL.11 At the moment, another 6 genes that will pair with ABL1: indicated BCR-ABL1,10 TETV6-ABL1,12 ZMT1Z1-ABL1,13,14 EML1-ABL1,15 NUP214-ABL1,15 RCSD1-ABL1,16-18 SFQ-ABL1.19,20 ABL1 kinase dominate conserve in ALL chimeric proteins which are involved in N-terminal part of the protein, and it often includes a coil-coiled or helix-loop-helix dominant.21 Screening for ABL1 chimeric genes can perfume in ALL patients especially in some of them with T-ALL because ABL1 regulates T-cells maturation and play a primary role in the process of cellular-skeleton deforming in T cells.22 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-ABL1.4 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-ABL1 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 prevalence in different ethnics of Iranian population. 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 prevalence in different ethnics of Iranian population.

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,

| Step   | Gene   | Primer sequence | Primer size (NT) |
|--------|--------|-----------------|------------------|
| First  | BCR e1 A F | 5’-GACTGCAGCTCCAATGAGAAC-3’ | 21               |
|        | ABL a3 B R | 5’-GTTTGGGCTTCACACCATTCC-3’ | 21               |
| Second | BCR e1 C F | 5’-CAGAACTCGCAACAGTCTCTC-3’ | 21               |
|        | ABL a2 D R | 5’-TTCCCCATTGTGATTAGGCC-3’ | 21               |
| Control| GAPDH F   | 5’-CCTGGCGGTGCGATTAGTG-3’; |                  |
|        | GAPDH R   | 5’-TCAGTCCGTCATGTCGTTCC-3’ |                  |

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

| Variable         | Percent | Number | Percent | Number | p     |
|------------------|---------|--------|---------|--------|-------|
| Age              |         |        |         |        |       |
| Less than 5 years| 50      | 24     | 100     | 2      | 0.38  |
| 5-9 years        | 25      | 12     | 0       | 0      |       |
| 10 and more than 10 | 25   | 12     | 0       | 0      |       |
| Gender           |         |        |         |        |       |
| Male             | 56.3    | 27     | 50      | 1      | 0.14  |
| Female           | 43.8    | 21     | 50      | 1      |       |
| Ethnicity        |         |        |         |        |       |
| Fars             | 14.6    | 7      | 0       | 0      | 0.97  |
| Lur              | 27.1    | 13     | 0       | 0      |       |
| Arab             | 52.1    | 25     | 100     | 2      |       |
| Kurd             | 6.3     | 3      | 0       | 0      |       |

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

Figure 1. PCR products on 1.5% agarose gel for the first step of Nested-PCR in ALL patients with P190 BCR-ABL1 e1-a3 fusion gene. Line 1: Positive control (P190 BCR-ABL1), 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-ABL1 e1-a2 fusion gene. Line 1: Positive control (P190 BCR-ABL1), Lines 2 and 3: 381 bp ladder related to ALL patients with positive translocation, Line 4: Negative control, Line 5: Deionized water.
were used in Nested-PCR has a 10\(^{-3}\) – 10\(^{-4}\) sensitivity, and movement in leukemia cells. Primer's compounds that attach transcripts in the result of special chromosomal attachment genes was done. But e1-a2 fusion gene band was just observed in 2 cases (4%) in second step of Nested-PCR in 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. 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.

Therefore, according to the results of our study and other related studies, P190 BCR-ABL1 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. There is a few evidence about the prevalence of 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. 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. Therefore, according to the results of our study and other related studies, P190 BCR-ABL1 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 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.

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