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Frequency of BCR-ABL Fusion Transcripts in Iranian Azeri Turkish patients with Chronic Myeloid Leukemia

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ABSTRACT

Background: The Philadelphia chromosome (Ph) characterized by t (9; 22) (q34; q11.2) is a reciprocal translocation giving rise to a chimeric BCR-ABL fusion gene. Incidence of Ph chromosome is over 98% in Patients with Chronic Myeloid Leukemia (CML) and around 20% in acute lymphoblastic leukemia (ALL). The finding of this fusion gene is essential for diagnosis of CML by detection of various fusion transcripts such as b2a2 and b3a2 transcripts and Ph positive ALL by detection of e1a2 (p190) transcripts. We conducted this study to determine the frequency of various BCR-ABL fusion transcripts in the west Azerbaijani patients with CML.
Methods: RNA was isolated from peripheral blood samples by standard protocols. BCR-ABL fusion gene detection was carried out with one-step multiplex RT-PCR in 41 west Azerbaijani patients with CML.
Results: Among patients with CML, the frequencies of b2a2 and b3a2 transcripts were 52.5% and 12.5%, respectively. Co-expression of b3a2 and b2a2 transcripts was found in 12.5% of the patients.
Conclusion: The findings of this study showed that multiplex RT-PCR is a suitable technique to identify the typical BCR-ABL fusion transcripts in the west Azerbaijani patients with CML. Atypical transcripts possibly run away while using multiplex PCR.

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Introduction

Chronic myeloid leukemia (CML) as a myeloproliferative disorder is characterized by increased in myeloid, erythroid, and megakaryocytic lineage cells in peripheral blood as well as myeloid hyperplasia in the bone marrow.[1] CML generally occurs in adults about 60-65 years of age, but rarely occurs in children. Worldwide annual incidence of CML is 1-2 cases per 100,000 individuals (males and females).[2] The typical symptoms of CML are fatigue, pain, weakness, weight loss, night sweats or fever, bone or joint pain and splenomegaly. About 40 percent of patients with CML are asymptomatic.[3, 4] CML is often discovered by a complete blood count (CBC). The CBC may show abnormally high or low white blood cell counts. Also, there may be abnormalities of the red blood cells or platelets. Immature white blood cells (blasts) may be present in the CBC.[5] The presence of Philadelphia (Ph) chromosome confirms diagnosis of CML. 98% of the patients with CML and 20- 40% of adult patients with ALL, 5% of childhood ALL, and 2% of AML patients have Ph chromosome.[6] Philadelphia chromosome results from a reciprocal translocation between chromosome 9q34.1 and 22q11.21. The fusion of the proto-oncogene ABL (9q34) and the breakpoint cluster BCR (22q11.21) leads to production of a chimer gene. Due to this rearrangement, the 5' part of the BCR gene is fused to the 3' part of the ABL gene, resulting in the BCR-ABL fusion gene (OMIM 151410), which encodes a fusion protein

with constitutive tyrosine kinase activity.[6-8] There are two forms of the BCR-ABL chimeric gene arising from exon 2 of the ABL gene and different exons of the BCR gene. Three breakpoint cluster regions including Major (M), minor (m) and micro (μ) BCR are located in the *BCR* gene.[9, 10] In CML patients, breakpoint occurs in BCR (exon 13 [b2] or exon 14[b3]) and ABL (exon 2) genes (a2) and leads to b2a2 or b3a2 fusions, respectively. These fusions are translated into a chimer protein of p210 (210 kDa). Alternative splicing of transcripts could result in co-expression of b3a2 and b2a2 fusions. The breakpoint in the m-BCR results in e1a2 transcript that is due to fusion between BCR exon 1 (e1) and ABL exon 2 (a2) that encodes smaller p190 protein (190 kDa).[11, 12] In rare cases, the breakpoint between BCR exon 19 (c3) and ABL exon 2 (c3a2) in μ-bcr induces a larger p230 $^{BCR-ABL}$ protein.[13] Numerous reports claim that the frequency of different BCR-ABL fusion transcripts vary in different ethnic groups which can have a significant influence on the management and therapeutic implication of the related disorders.[14-16] Most of CML patients have transcripts with b2a2 and b3a2 junctions while e1a2 fusion transcript is seen primarily in Philadelphia ALL. The e19a2 fusion is associated with prominent neutrophilic maturation in CML.[17] Different molecular methods have been used for rapid and sensitive detection of the BCR-ABL fusion genes in clinical diagnosis of hematological disorders.[18] Cross et al. reported a multiplex polymerase chain reaction (PCR) assay for detection of BCR-ABL fusion transcripts that is a useful method for determining of presence or absence of two or more chimeric BCR-ABL genes.[19] In this study, we used multiplex reverse transcriptase polymerase chain reaction (RT-PCR) assay for determining the frequency of various BCR-ABL fusion transcripts in Iranian Azeri Turkish patients with CML.

Materials and Methods

After approval by the ethics committee of Urmia University of Medical Sciences (UMSU), Urmia, Iran, 41 patients with CML were diagnosed and sequentially selected among patients referred to Imam Khomeini Hospital, UMSU. The diagnosis of CML was established according to WHO criteria of CML.[20] Peripheral blood samples were collected in 3 ml vacutainer tubes with EDTA as anticoagulant. All the samples were stored at 4°C prior to RNA extraction. RNA was extracted from peripheral blood leukocytes using RNX- Plus Solution (Cat. No. : RN7713C) (Cinnagen, Iran). The quality of extracted RNA samples was evaluated by measuring the absorption at 260 nm and 280 nm in a biophotometer (Eppendorf AG, Germany). The samples with 260A/280A ratio more than 1.8 were considered as qualified. Complementary DNA (cDNA) was synthesized using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit from 2μl of a sample (Thermo Fisher Scientific Inc.). In 25 μl reaction, 100 ng product was amplified using 30 U/mL of Taq DNA polymerase, 1x reaction buffer, 200 μM dNTP, 1.5 mM MgCl2, and 10 pM of the four primers (Genefanavaran, Tehran, Iran) (Table 1). Multiplex RT-PCR assay was performed on a PCR machine with the following program: 30 s at 96°C, 50 s at 60°C, 1 min at 72°C (35 cycles), followed by 7 min at 72°C.[15] The PCR products were analyzed on 2.5% Agarose gel.

Table 1: Sequence of primers used in our study for detection of BCR-ABL fusion transcripts as well as BCR transcripts (internal control)[22]

Primers (5'→3')	
C5e	5'-ataggatcctttgcaaccgggtctgaa-3'
B2B	5'-acagaattccgctgaccatcaataag-3'
BCR-C	5'-accgcatgttccgggacaaaag-3'
CA3	5'-tgttgactggcgtgatgtagttgcttg-3'

Results

The b2a2 and b3a2 transcripts were the only fusion transcripts found in this study. The frequencies of b2a2 and b3a2 transcripts were 52.5% and 12.5%, respectively. Co-expression of the b3a2 and b2a2 transcripts was found in 12.5% of patients. In the remaining cases, typical transcripts were not found. Figure 1 shows the frequency of b2a2, b3a2 and b2a2/b3a2 transcripts in this study. Figure 2 shows a representative gel image of our findings.

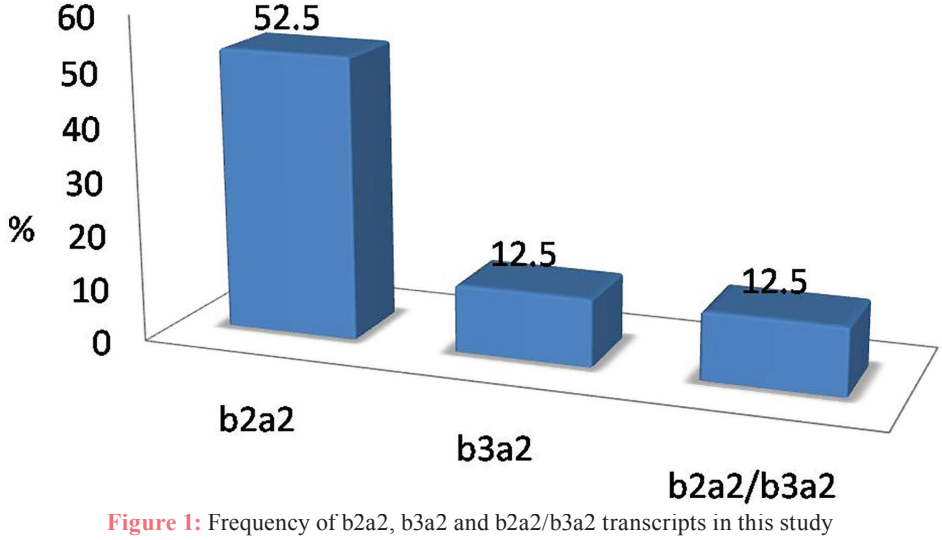

Figure 1: Frequency of b2a2, b3a2 and b2a2/b3a2 transcripts in this study

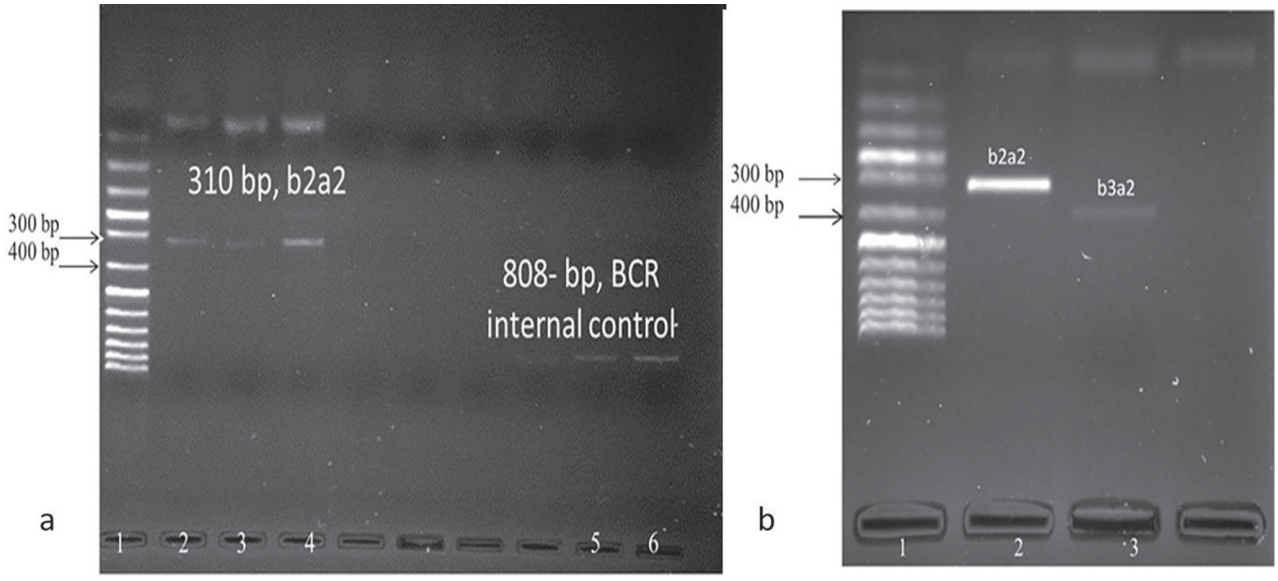

Figure 2: One-step multiplex RT-PCR results for BCR-ABL fusion transcript in Philadelphia chromosome t(9:22) with b2a2 (a) and b2a2/b3a2(b) junction. a) Lane 1: 50 bp marker; lanes 2-4: b2a2 transcript; lanes 5 and 6: BCR as internal control, b) Lane 1: 50 bp marker; lane 2: b2a2 transcript; lane 3: b3a2 transcript (b2a2/b3a2(b) junction)

Discussion

Different various types of BCR-ABL fusion transcripts can be detected by molecular techniques such as southern blot, FISH and conventional RT-PCR. RT-PCR is one of the most useful techniques to detect BCR-ABL fusion transcripts in patients with hematological disorders. This method is specific and highly sensitive. Multiplex RT-PCR is carried out with more than a set of primers compared with the conventional RT-PCR, so that it can identify different types of chimeric RNAs in a single sample.[21] In this study, we used Multiplex RT-PCR method to study the most frequent type of BCR-ABL fusion transcripts in our patients. In our study, more than half of the patients (52.5%) showed b2a2 transcript, while 12.5% showed b3a2 transcript. Co-expression of both types of transcripts (b3a2 and b2a2) was found in 12.5% of cases. Yaghmaie et al. found the frequency of b3a2 and b2a2 transcripts in 75 Iranian patients with CML to be 63% and 20%, respectively. In his study, the remaining of patients showed one of the b3a3/b2a2, e1a2, b3a3/b2a3, and e19a2 variants.[15] A study on Korean patients by Goh et al. showed that b3a2 and b2a2 were more frequent transcripts; but c3a2, e1a2, b2a3, b1a1, and e1a3, b2a2/b3a2 were less common (2%).[20] A group of 200 patients in North Indian displayed predominance of the b3a2 transcript (68%), but b2a2 was detected in 24% of the cases, while 8% of the patients showed b3a2/b2a2 variants.[22] In Pakistani CML patients, frequency of b3a2 and b2a2 transcripts were 64% and 36%, respectively.[23] Paz-y-Miño et al. reported different distribution in Ecuadorian Mestizos CML patients which indicated frequency of 94.6% and 5.4% for b2a2 and b3a2 transcripts, respectively.[24] A study carried out by Osman and co-authors implied that frequency of b3a2 and b2a2 transcripts were 41.6% and 53.5%, and 4.6% of the cases had b3a2/b2a2 and b3a2/b2a2/e19a2 variants in Sudanese group.[25] In most reports, frequency of b3a2 transcript was more than b2a2 transcript in CML patients.

In our study the pattern of BCR-ABL fusion transcripts demonstrated significant differences when compared to others reported from Iran and other geographical areas. Geographical and ethnic differences could explain theses contradictory findings. It has been demonstrated that the type of the fusion transcript may have clinical significance. For example, a study on CML patients by Perego et al. showed that patients with b3a2 transcripts had higher survival compared to patients with b2a2 transcripts.[26] Therefore, it is recommended that a comprehensive survey on Azeri patients with CML should be carried out to determine the prognostic importance of each fusion transcript.

Conclusion

The findings of this study showed that multiplex RT-PCR is a suitable technique to identify the typical BCR-ABL fusion transcripts in patients with CML. Atypical transcripts possibly run away while using multiplex PCR that allows detection of the typical BCR-ABL transcripts.

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Ethics approval and consent to participate

All stage of this study was permitted by ethics committee of Urmia University of Medical Sciences (permit number ir.umsu.rec.1395.190). Each person was informed about the contents and aims of the study. Written informed consent was obtained from all patients.

Conflict of Interest: None declared.

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