Frequency of different BCR-ABL Fusion Transcripts in Chronic Myelogenous Leukemia Patients in Pakistan

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Abstract

Bcr-abl translocation has been found to be the hallmark of chronic myelogenous leukemia. It is present in 90-95% cases of chronic myelogenous leukemia. The present study was performed to check the frequency of Bcr-abl fusion transcripts in Pakistani chronic myelogenous leukemia patients. The frequency of Bcr-abl fusion transcript was determined by using RT-PCR. RNA was isolated from the peripheral blood of 23 CML patients using TRizol reagent. It was reverse transcribed by using MMLV reverse transcriptase enzyme followed by RT-PCR. RT-PCR was done by using two combinations of primers (BCR-C, CA3) and (B2B, CA3). The PCR product was analysed on 3% Agarose gel. Results: All 23 samples were positive for Bcr-abl translocation. Out of 23 samples 13 (56.5%) were positive for b2a2 type transcript. B3a2 transcript was observed in 6 (26%) out of 23 samples. Other samples gave unusual bands of around 209 bp referring to fusion fragments other than b2a2 or b3a2. None of 23 samples was positive for e1a2 type transcript. In Pakistani population b2a2 type transcript is most frequent in contrast to the studies conducted on other populations. That is perhaps due to the ethnic differences.

Key word: CML, Bcr-abl translocation, frequency, RT-PCR.

Introduction:

Bcr-abl translocation has been found to be closely associated with chronic myelogenous leukemia. In this translocation, the 30 segment of the c-abl proto-oncogene on chromosome 9 is Juxtaposed with the 50 segment of the bcr gene on chromosome 22 1-3. It is considered as a diagnostic marker in CML cases 4. Bcr-abl translocation results in the creation of different types of fusion transcripts depending upon the breaks in bcr gene. Breaks in Bcr gene occur usually in one of the three regions: the major breakpoint cluster region, minor break point cluster region and micro breakpoint cluster region 5. Breaks in c-abl gene usually occur in the first intron. The breaks in M-Bcr region join exon b2 and b3 with a2 exon of abl gene resulting in the formation of b2a2 and b3a2 transcripts. These transcripts code for 210 kD chimeric protein 6-7. Break in m-bcr region joins first exon e1 with a2 resulting in the formation of e1a2 transcript which code for 190 kD chimeric protein 8. Rarely break occurs in micro-bcr region joining e19 and a2 which creates e19a2 transcript coding for 230 kD protein 9. B2a2 and b3a2 transcripts are found in majority of CML cases while e1a2 transcripts are associated with Philadelphia positive acute lymphoblastic leukemia. The presence of rare e19a2 type fusion transcript has been reported in cases of CML with prominent neutrophilic maturation 10. The fusion of BCR and ABL can be detected by conventional cytogenetics or FISH 11. But polymerase chain reaction is most reliable method for the detection of fusion transcripts. Goh et al showed the potential feasibility of highly sensitive PCR approaches for molecular monitoring and clinical relevance in CML.
management. Furthermore techniques have been developed for the detection of fusion proteins. Dekking et al developed a flow cytometric immunobead assay for detection of fusion proteins in lysates of leukemia cell samples. We conducted this study to set up an RT-PCR assay for diagnosis of patients with CML at various hospitals of Lahore Pakistan, and to determine the frequency of different Bcr-abl fusion transcripts among Pakistani CML population.

**Methods:**

Twenty three patients with CML were enrolled in this study. The mean age of the patients was 35 years. Out of twenty three 17 were male and other 6 were females. All twenty three patients were in chronic phase at the time of sample collection.

**RNA isolation and cDNA preparation:**

RNA was isolated from the blood cells using TRIzol reagent. The integrity of RNA was confirmed on 1.5% Agarose gel. For cDNA synthesis concentration of RNA was firstly measured using spectrophotometric method. For cDNA synthesis one microgram RNA was reverse transcribed using 5U/ul MMLV in 1x RT buffer, 0.5 uM CA3 (reverse primer), 25 uM dNTPs at 65°C for 5 min, 42°C for 1 hour and 70°C for 5 minutes.

**RT-PCR:**

For amplification of cDNA two different reactions with two different set of primers were used. cDNA was amplified using 1U/ul Taq polymerase, 240 uM dNTPs, 1.8mM MgCl$_2$, 0.6 uM of (CA3 and BCR-C) and (CA3 and B2B) primers. RT-PCR was performed on Bio-Rad PCR machine with the program of 5 min at 94°C followed by 35 cycles of 40 seconds at 94°C, 1 minute at 58.8°C and 40 seconds at 72°C and final extension of 10 minutes at 72°C. The sequence of the primers used is given in table 1. The PCR products were analyzed on 3% Agarose gel.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence 5'----3'</th>
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<tr>
<td>B2B</td>
<td>5'ACAGAATTCGCTGACCATCAATAAG3'</td>
</tr>
<tr>
<td>BCR-C</td>
<td>5' ACCGCATGTTCGGGACAAAAG3'</td>
</tr>
<tr>
<td>CA3</td>
<td>5'TGTTGACTGGCGTGATGTAGTTGCTTGG3'</td>
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**Results**

The primer pairs used in RT-PCR allowed the amplification and detection of all types of fusion fragments except normal BCR gene. The expected bands were as follows: 304 bp, b2a2; 385 bp, b3a2, 209 bp; b3a3; 481 bp, e1a2. We were able to detect most common type of fusion fragments b2a2 and b3a2 along with atypical transcripts. Majority of the patients expressed one of the p210 type transcripts. Out of twenty three samples analyzed, 13 were found to be positive for b2a2 transcript giving a band of 304 bp on amplification (Figure 1, 2).
FREQUENCY OF BCR-ABL FUSION TRANSCRIPTS IN CML PATIENTS

While six out of 23 samples were positive for b3a2 transcript giving a band of 385 bp on amplification (Figure 3), four samples gave unusual bands of less than 250 bp but greater than 200 bp referring to Bcr-abl transcripts other than the most common b2a2 and b3a2. As the size of one of the band was approximately about 209 bp which corresponds to b3a3 transcript so that patient was might be positive for b3a3 transcripts. The frequencies of different fusion fragments were 56% for b2a2, 26% for b3a2 and 4% for b3a3 (table 2).
FREQUENCY OF BCR-ABL FUSION TRANSCRIPTS IN CML PATIENTS

Discussion

RT-PCR assay is a useful and convenient method to study all type of Bcr-abl gene rearrangements. In our study we included 23 CML patients to check the most frequent type of fusion fragment in Pakistani CML population. We found the frequency of 56% and 26% for b2a2 and b3a2 transcripts respectively. In a study by Reiter et al., the incidence of b2a2 and b3a2 transcripts in CML patients with Philadelphia chromosome was 31.6% and 68.4%, respectively 14. Verschraegen et al., found that the frequency of b2a2 and b3a2 transcripts was 30.2% and 67.9%, respectively 15. Yaghmaie et al found the frequency of b2a2 and b3a2 was 63% and 20% respectively in Iranian CML population 16. Similarly frequency in Serbian patient was found to be 73.5% for b3a2 and 25% for b2a2 17. Sastre et al., found contrary results, namely b2a2 transcript was more frequent in the patients of Argentine. The frequency they found was 41.7% and 37.5% for b2a2 and b3a2 transcript respectively 18. However in Pakistani CML population the frequency of b2a2 transcript was twice as higher as that of the frequency of b3a2 transcript. The ethnic difference counts for the variations in the frequency and the type of most frequent fusion transcript among different races as Arrifins et al found the incidence of Bcr-abl translocation greater in Malay and Chinese population as compared to the Indian population 19. The knowledge of the type of fusion transcript may help in better prognosis of the disease. As studies suggest that patients expressing b3a2 transcript have higher survival as compared to b2a2 transcript 20, and further the patients expressing b2a2 have shorter latency to
develop leukemia. These transcripts can also be differentiated using Real Time PCR because all these transcripts have their unique melting profiles that allow their differentiation. Further these can be sequenced to know the exact breakpoint region. CML patients with Bcr-abl translocation are currently being treated with imatinib mesylate and hydroxyurea in Pakistan. But certain mutations in abl kinase domain are rendering imatinib ineffective in some patients. So it is important to discover other methods for treatment of CML beside imatinib therapy. Researches have shown that in vivo application of targeted nonvirally delivered synthetic Bcr-abl siRNA in imatinib resistant Philadelphia positive patients can silence the expression of Bcr-abl gene. So in future RNAi can also be used effectively for the treatment of CML patients.

References


