Frequency of T315I Mutation in Patients with Chronic Myeloid Leukemia Before and During Imatinib Treatment: A Study in North-East of Iran

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ABSTRACT

Background & Objective: Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by an aberrant BCR-ABL fusion protein. Imatinib mesylate (IM) is a tyrosine kinase inhibitor that induces clinical remissions in chronic-phase CML patients. The T315I mutation at the gatekeeper residues of BCR-ABL confers resistance to both IM and second-generation TKIs, including dasatinib and nilotinib. Our objective was to determine the prevalence of T315I mutation between two groups of CML patients before and during Imatinib treatment in North-East of Iran.

Materials & Methods: This study was conducted on 100 newly diagnosed cases of CML (before commencing IM treatment) and 25 IM-resistant CML patients. PCR-RFLP, ASO-PCR, and direct sequencing were performed to detect T315I mutations.

Results: The median age of newly-diagnosed and IM-resistant patients was 48±14 and 50±12.3 years, respectively. Males/Females ratio was 1 and 1.08 for newly diagnosed and IM-resistant patients, respectively. There was no significant difference regarding the age and sex between the two groups. During the study, T315I mutational analysis was performed for all 125 patients. The prevalence of T315I mutation was 0% and 4% for newly-diagnosed and IM-resistant patients, respectively. T315I mutation was not detected before IM administration, although it was detected in 1(4%) among resistant patients who were at least 6-months on IM treatment.

Conclusion: These observations suggest that T315I mutation may be categorized as secondary resistance and induce clonal expansion due to BCR/ABL instability. Hence, BCR-ABL mutations are less likely to appear before the onset of treatment, as presented in our study.

Keywords: T315I Mutation, Chronic Myeloid Leukemia (CML), Imatinib Resistance

Introduction

Chronic myeloid leukemia (CML) accounts for nearly 20% of all diagnosed leukemia in adults, with similar frequency rates worldwide (1). CML is considered a myeloproliferative disorder which characterized by extensive proliferation and expansion of myeloid cells at various stages of maturation and differentiation (2-6). BCR-ABL fusion gene is the hallmark of CML, which results from reciprocal translocation t(9; 22) (q34;q11), creating Philadelphia (Ph) chromosome (6). Ph chromosome is observed in more than 95% of CML patients (7, 8).

Substantial evidence indicates that BCR-ABL alterations lead to high levels of tyrosine kinase (TK) activities and deregulations in leukemic cell growth (2-5, 9, 10). BCR-ABL tyrosine kinase inhibitors (TKIs) are the first-line treatment option for CML patients (1, 7, 11). Imatinib mesylate (IM) is the first-generation of TKIs currently used as the frontline therapy for CML patients.
Since its introduction into clinical practice, this inhibitor has become a standard treatment option for CML patients worldwide. However, some patients treated with imatinib fail to respond, respond sub optimally, or experience relapse because of primary or acquired resistance or intolerance (7). BCR-ABL mutations interfere with IM binding to the ABL kinase domain. Hence, they may lead to various levels of resistance. These mutations account for up to 33% of patients’ suboptimal response to IM therapy (3, 4, 12, 13).

Among these mutations, the T315I mutation at the gatekeeper residues of BCR-ABL confers resistance to both IM and second-generation TKIs, including dasatinib and nilotinib (5, 14).

T315I mutation represents the replacement of threonine by isoleucine at position 315 in the ABL component of BCR-ABL (15, 16). In contrast, Ponatinib is a multi-targeted oral TKI that overcomes the T315I mutation (17). Due to its serious cardiovascular side effects, it is prescribed only for special patients. Therefore, the therapy of the BCR-ABL1T315I-mutated CML represents a serious clinical challenge (18). In addition to resistance, the TKIs are disabled to eradicate leukemic stem cells. Hence, the disease relapse often occurs when the treatment is stopped (19).

Thus, the despite development in treatment, we need to investigate the interventional factors of the treatment. Screening of T13I mutation is currently suggested for all CML patients, undergoing TKI treatment. Early detection of this mutation is paramount for modifying therapeutic regimens at the lowest levels of mutant clones (5, 14, 20). Otherwise, disease progression towards a fatal blast crisis is inevitable (21, 22).

In this study, we aimed to develop an allele-specific oligonucleotide (ASO) polymerase chain reaction (PCR) assay to determine the prevalence of T315I mutation in CML patients in North-East of Iran. Also, we analyzed and compared the clinical findings of newly-diagnosed and IM-resistant patients.

**Materials and Methods**

**Study Population**

This retrospective study was conducted on 100 newly-diagnosed (before the onset of treatment) and 25 IM-resistant CML patients from February 2012-December 2014. BCR-ABL translocation was detected in all patients, and the two expert pathologists confirmed the disease at the Molecular Pathology Department of Ghaem Hospital, Mashhad, Iran. Chronic CML was defined by the presence of BCR-ABL translocation as well and < 10% blasts in peripheral blood or bone marrow, < 20% basophils in peripheral blood, and < 30% blasts plus promyelocytes in peripheral blood or bone marrow. Demographic, clinical, and treatment-related data were collected from patients’ archived medical records. The ethical committee of Mashhad University of Medical Sciences (IRMUMS IMREC 628493) reviewed and approved the study protocol.

**Quantitative qRT-PCR for BCR-ABL**

Total RNA was extracted from the aliquots of 125 peripheral blood leukocytes, using TRizol reagent (Invitrogen, CA, USA). Also, cDNA was synthesized by a superscript III cDNA synthesis kit (Invitrogen, CA, USA), according to the manufacturer's instructions. Next, RNA and cDNA qualities were assessed by a NanoDrop (Thermo Scientific NanoDrop2000, Finland). Real-time quantitative PCR (RQ-PCR) was performed, and BCR/ABL transcripts and ABL control gene were quantified according to Ipsogen BCR-ABL Mbcr IS-MMR Kits (QIAGEN, Germany).

**ASO-RT-PCR Assay for the Detection of T315I Mutation**

ASO-PCR was implemented using three primers including a forward primer (5’-CGC AAC AAG GCC ATC GTG-3’), a reverse primer (5’-TCC ACT TCG TCT GAG ATA Ctg GT-3’) and a reverse mutant primer (5’-CGT AGG TCA TGA ACT CAA-3’), all primers were optimized by adjusting the annealing temperature (Ta), MgCl2 concentration, and primer concentrations. Each PCR reaction was prepared at a final volume of 25 µL, using 2 µL cDNA, 0.5 µL of F1 and R1 primers, 1 µL of Rm primer, and 12.5 µL of Taq DNA Polymerase Master Mix Red Kit (Ambicon, Denmark).

Finally, PCR products were assessed on 2% Agarose gel after ethidium bromide staining. The PCR condition was as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 10 sec, optimized primer annealing at 59 °C for 20 sec, extension at 72 ° C for 40 sec, and the final extension at 72 °C for 5 min, using a thermal cycler (Applied Biosystems Veriti, USA). The mutant T315I allele gave a band of 304bp and an 863-bp for the entire ABL domain. However, the wild-type ABL allele gave a band of 863 bp.

**Restriction Fragment Length Polymorphism (RFLP) Assay**

The PCR profile used for amplification was as follows: initial denaturation at 94 °C for 12 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, extension at 72 ° C for 1 min, and a final extension at 72 °C for 5 min. Next, primer sequences were Primer forward 5’-GAGGGCGTGTGGAAGAAATA-3’ and Primer reverse 5’-GCTGTGTAGGTGTCCCCT-GT-3’.

The PCR product was digested with 5 U of Ddel restriction enzyme (Fermentase, Canada) and electrophoresed on 8% Acrylamide gel. RFLP products were as follows: wild-type bands (36 and 171 bp), a mutant band (207bp), and an undigested band (412 bp). Finally, Direct Sequencing was performed to confirm positive results (Figure 1).
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Figure 1. RFLP-PCR electrophoresis gel (Acrylamide)1-6 and 10,12,13 were wide type,11 was positive, 8 was cut band, and 7 was uncut PCR product.

Statistical Analysis

The statistical analysis was performed using the software SPSS version 22 (version 22, SPSS Inc). Continuous data were analyzed by independent sample t-test and Mann–Whitney test. Also, the chi-square test was used for categorical data. The statistical level of significance was defined as a P-value less than 0.05.

Results

Regarding patients undergoing IM treatment, they comprised 25 CML patients who were at least 6-months under IM treatment (400 mg/day) and showed an inadequate response to IM therapy. Regarding newly diagnosed cases, they was 100 CML patients before the start of the treatment. The median age of newly-diagnosed and IM-resistant patients were 48±14 and 50±12.3, respectively. Patients aged at least 17 years were considered eligible for the study. In the newly-diagnosed group, males and females comprised 50% of the total cases. However, in IM-resistant patients, males were slightly more than females (52% versus 48%). There was no significant difference between male and female subjects regarding age or gender distribution in the two groups. In addition, the clinical characteristics of patients are summarized in Table 1.


<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Mean (±SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x 10^6/µL)</td>
<td>B</td>
<td>3.77 (0.87)</td>
<td>6.00-1.78</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>3.97 (0.79)</td>
<td>5.40-2.63</td>
</tr>
<tr>
<td>WBC (x 10^3/µL)</td>
<td>B</td>
<td>117.21 (70.45)</td>
<td>339.00-15.90</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>45.07 (65.64)</td>
<td>253.26-3.30</td>
</tr>
<tr>
<td>PLT (x 10^3/µL)</td>
<td>B</td>
<td>501.67 (101.94)</td>
<td>10100.00-62.00</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>377.28 (230.28)</td>
<td>894.00-9.00</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>B</td>
<td>33.42 (6.82)</td>
<td>49.20-15.70</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>33.99 (6.54)</td>
<td>29.80-23.50</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>B</td>
<td>10.74 (2.09)</td>
<td>15.60-6.10</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>10.96 (2.26)</td>
<td>15.00-7.40</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>B</td>
<td>87.81 (7.95)</td>
<td>103.90-59.00</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>87.09 (6.79)</td>
<td>100.00-65.00</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>B</td>
<td>28.5 (3.45)</td>
<td>38.50-17.20</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>27.76 (2.33)</td>
<td>32.30-19.00</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>B</td>
<td>32.29 (2.4)</td>
<td>43.70-28.40</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>31.78 (1.58)</td>
<td>34.60-29.10</td>
</tr>
</tbody>
</table>

Splenomegaly and hepatomegaly were reported in 89.9% and 54.4% of patients, respectively; the difference between male and female subjects was significant (P<0.04). Moreover, a correlation was detected between Hct and Hb levels in both genders (P< 0.0001). Also, White blood cell count (WBC), hemoglobin (Hb), and hematocrit (Hct) levels were significantly different between the two groups.

In both groups, T315I mutation detection was carried out by ASO-PCR and PCR-RFLP. The mutation was detected in 4% of 25 IM-resistant patients.
Discussion

In the current study, 125 CML patients (before the onset of IM therapy and during therapy) were evaluated to determine the frequency of T315I mutation in patients from the North-East of Iran. IM resistance due to BCR-ABL mutations represents a significant clinical problem that may not be overcome by increased IM dosage. In previous studies, the frequency of these mutations ranged between 2% and 24% worldwide. This variation in frequency may be attributed to varying sensitivities of different methods used and the time of mutation detection and effects of different races (23-25).

Similarly, in another study from Iran, Chahardouli and coworkers assessed T315I mutation in 60 resistant CML patients by ASO-PCR and reported this mutation in 4% of their patients (18). Also, Elias et al. evaluated 125 CML patients from Malaysia and found that 22.4% of those had resistant mutations. Of these, 7.2% were T315I. The mean age of T315I emergence was reported to be 42.1 in Malay patients (26). However, it was 48.1 in the North-East of Iran and 48 years in a study by Nicolini FE et al. on 222 CML patients with different nationalities (23).

In our study, a significant correlation was observed between the emergence of T315I mutation and disease progression, with a shift in disease severity. Also, our finding was confirmed by the study of Chahardouli et al. (14). Also, Nicolini FE et al. observed a strong association between the presence of the T315I mutation and disease progression (23). In contrast, in Goranova-Marinova V et al. study, there was no significant difference in the phase; it may be due to using the different method for detecting mutation (27).

Besides, Nicolini et al. concluded that the survival rate of patients with T315I mutation is affected by the disease phase and the time of the mutation detection (22). Moreover, in a study by Elias et al 2014, they reported that the frequency and type of BCR-ABL mutations varied among different races (26).

Overall, early detection of T315I mutation can facilitate the identification of CML patients and may help clinicians to define the mode of therapy before the emergence of resistance in the patients (14). Determination of T315I frequency before the onset of treatment for screening CML patients and predicting their IM resistance was a part of our current study. However, no positive cases were found among these patients. According to our study, the application of T315I mutation as a screening test before the onset of treatment for selecting the best therapy may not be suggested (13, 14, 28).

Generally, it has been suggested that cells affected by CML show an increased genomic instability, as it is more clearly evident in blast crises where a range of secondary chromosomal changes are frequently observed (16). Therefore, BCR-ABL mutations are less likely to appear before the onset of treatment, as presented in our study. Furthermore, the success of IM therapy for CML has led to its prevalent use. Screening for T315I mutation is now recommended for all CML patients undergoing TKI treatment and should be performed promptly to detect the lowest levels of clone (13, 16). Overall, according to previous studies, the emergence of T315I mutation may be due to the increased clonal instability and proliferation rate in the advanced phase disease (23, 29, 30).

Currently, Ponatinib is the only TKI available and is successfully used in patients with BCR-ABL1 T315I+ CML. Although it is associated with a high risk cardiovascular side effects, it is not optimal for all patients (17). Therefore, current studies aim to find founding additional therapeutic strategies to control BCR-ABL1T315I Ph+ CML. However, this mutation couldn’t be detected in any of the 100 newly diagnosed patients, suggesting the hypothesis that the emergence of T315I mutation may be due to the increase in clonal instability and proliferation rate in the advanced phase disease. One of the limitations of this report was the small sample size. Therefore, we recommend investigating the frequency of this mutation in more samples, especially in patients before treatment to prove that it can be used as a screening test for patients before treatment.

Conclusion

In conclusion, the T315I mutation was not detected before IM administration. However, it was detected in CML patients who were under IM therapy. These observations indicate that T315I mutation may be categorized as secondary resistance and induce clonal expansion due to BCR/ABL instability. Therefore, BCR-ABL mutations are less likely to emerge before the commencing of treatment, as shown in our study.

Acknowledgments

None.

Conflict of Interest

Authors declare that they have no conflict of interests.

References


