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High Expression of TWIST-1 in the Chronic Myeloid Leukemia Patients with T315I Mutation

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Abstract

Background: Among the known ABL mutations in chronic myeloid leukemia (CML), T315I is of particular importance. The T315I mutation may develop resistant cells that increase disease progression. TWIST-1 expression is impaired in patients with increased drug resistance.

Objectives: The current study aimed to measure the expression of TWIST-1 gene in CML patients to investigate its association with T315I mutation.

Methods: Peripheral blood samples were taken from 40 CML patients. The expression of *TWIST-1* and *BCR-ABL1* genes was quantified by real-time polymerase chain reaction (PCR). The gene expression was evaluated by REST software. cDNA was used for amplification refractory mutation system (ARMS)-PCR reaction.

Results: Of the 40 patients (age range: 19 - 72 years) participating in the study, 23 (57.7%) were female, and 17 (42.5%) were male. The expression of TWIST-1 gene was 43 ± 184.09-fold. The T315I mutation was detected in 3 (7.5%) patients.

Conclusions: According to our results, the TWIST-1 gene expression in patients with T315I mutation was significantly higher than patients without that mutation.

Keywords: Chronic Myelogenous Leukemia, *BCR-ABL1*, *TWIST-1*, *T315I* Mutation

1. Background

Chronic myelogenous leukemia (CML) is a clonal proliferative disorder caused by the neoplastic alteration of pluripotent stem cells, which results in the overproduction of granulocytes. The hallmark of this leukemia is the Philadelphia chromosome (9; 22) (q34; q11) (1). The BCR/ABL fusion protein plays a significant role in the blast crisis of this type of leukemia. Increased expression of BCR/ABL leads to CML cell malignancy and their resistance to antitumors and apoptosis inducers (2). If the CML cells undergo further changes, the disease becomes invasive (3). Among the known ABL mutations in CML, T315I is of particular importance. This mutation was most common in patients receiving imatinib (2). The T315I mutation can be detected using molecular techniques in the chronic CML phase, which may develop resistant cells that increase disease progression. It should be noted that the T315I mutation does not necessarily cause resistance and can also be detected in tyrosine kinase inhibitor (TKI)-sensitive patients (4).

Reports indicated that TWIST-1 expression is impaired in cancers and patients with increased drug resistance (5). This protein is also one of the prognostic factors of leukemogenesis, and its expression is increased in CML patients who have cytogenetic resistance to imatinib (6). Accordingly, since no study has examined the association between T315I mutation and TWIST-1 expression so far, the present study was designed to evaluate the relationship between TWIST-1 gene expression and T315I mutation in CML patients.

2. Methods

2.1. Patients and Samples

Peripheral blood samples were taken from 40 CML patients (by census method) referred to the laboratory for their follow-up in the period of October 2015-January 2016. Also, 10 healthy controls participated in the study. Sampling was performed after receiving an informed written consent from the patients and with the agreement of the

attending physician. The study was approved by the Ethical Committee of Ahvaz Jundishapur University of Medical Sciences (Ref. ID: IR.AJUMS.REC.1394.343). To minimize interference, patients who had a history of other disorders, including JAK2+ and P190 were excluded from the study.

2.2. Reverse Transcription-Polymerase Chain Reaction

Mononuclear peripheral blood cells were isolated by Ficoll-Paque (Lymphodex, inno-train, Germany) centrifugation. The isolation of DNA from PB was performed by Qiagen kit (Germany). DNA was spectrophotometrically evaluated at 260 nm. Then, mutational screening was conducted by direct sequencing technique using two sets of primers casing the Abl kinase domain.

RNA was extracted by Ribo-Prep kit (Russia). Then cDNA products of *TWIST-1* gene were amplified by PCR in the subsequent settings: initial denaturation for 2 min at 95°C, 10 s at 95°C, 20 s at 60°C, 40 cycles, and extension for 20 s at 72°C. Reaction system contained within 0.5 μL PCR Forward primer 10 pM, 0.5 μL PCR Reverse primer10 pM, 2 μL template cDNA 80 ng/μL, 11 μL SYBR Green (Takara, Korea), and 8 μL DEPC.

The primers were used in this way: ABL1-ex6-Forward (F): 5′-AGTCTCAGGATGCAGGTGCT-3′; ABL1-ex6-Reverse (R): 5′-AATGTGTTGCCAGCACTGAG-3′; TWIST-1 Forward primer: 5’ GGC-TCA-GCT-ACG-CCT-TCT-C 3’; TWIST-1 Reverse primer: 5’ CCTTCTCTGGAAACAATGACATCT 3’

2.3. Amplification Refractory Mutation System (ARMS)-PCR Reaction

cDNA was used for ARMS-PCR reaction. The subsequent specific primers were used in this method: ABL kinase Forward (F): 5′-CGCAACAAGCCCACTGTCT-3′; ABL Kinase Reverse (R): 5′-TCCACTTCGTCTGAGATACTGGATT-3′ and AS-T315: 5′ CGTAGGTCATGAACTCAA-3′. 25 mL PCR reaction mixture contained 5 mL 10 buffer, 2 mL dNTP, 1 mL each primer, 3 mL MgCl2 (50mM), 0.3 mL Taq polymerase Enzyme, 9.7 mL DW, and 2 mL cDNA. The thermal cycling settings were: 5 min at 95°C for 1 cycle, 30 s at 95°C, 30s at 67°C, 40 s at 72°C followed by 35 cycles and 5 min at 72°C performed at 1 cycle.

2.4. Statistical Analysis

Data of RT-PCR data was performed using REST Software (2009, QIAGEN, Valencia, USA). Spearman’s rho test was used to evaluate the relationship between quantitative data. For assessing the differences between three groups of CML patients, Kruskal-Wallis method was used. $P < 0.05$ was reflected as the significance level.

3. Results

Of the 40 patients (age range: 19 - 72 years) participating in the study, 23 (57.7%) were female, and 17 (42.5%) were male. The patients were divided into three groups: optimal (55 %), warning (12.5 %), and failure (32.5 %) of response to treatment (according to ELN criteria). The mean $\pm$ SD (min-max) of WBC, Hb, and Plt were 18 $\pm$ 37 (3 - 190) $10^3/mm^3$, 11 $\pm$ 2 (8 - 17) gr/dL, and 210 $\pm$ 62 (60 - 457)$10^3/mm^3$, respectively (Table 1).

Table 1. Patient’s Data [a]

Variable	Mean $\pm$ SD
WBC, $10^3/mm^3$	18 $\pm$ 37
Hb, gr/dL	11 $\pm$ 2
Plt, $10^3/mm^3$	210 $\pm$ 62
Treatment group	
Optimal	22 (55)
Warning	5 (12.5)
Failure	13 (32.5)
BCR-ABL1, fold	7 $\pm$ 19
TWIST-1, fold	43 $\pm$ 184.09
T315I mutation	3 (7.5)

[a] Values are expressed as mean $\pm$ SD and No. (%).

The T315I mutation was detected in 3 (7.5 %) patients. Of these, one patient was in the optimal treatment response group (4.76%) and two patients in the failure treatment response group (15.38%).

The expression of *TWIST-1* gene was 43 $\pm$ 184.09-fold with min and max of 0.006 and 1140.07, respectively. The BCR-*ABL1* fusion gene expression in CML patients was 7 $\pm$ 19-fold with min and max of 0.0001 and 100-fold, respectively. The means of *TWIST-1* gene expressions in groups of optimal, warning, and failure were 8.66 $\pm$ 14.31, 23.5 $\pm$ 50.59, and 102.19 $\pm$ 307.81-fold, respectively ($P = 0.889$). The means of BCR-ABL1 expressions in optimal, warning, and failure groups were 0.51 $\pm$ 2.07, 0.4 $\pm$ 0.13, and 20.85 $\pm$ 29.46, respectively. The *TWIST-1* gene expressions in patients with and without T315I mutation were 401.2 $\pm$ 648.55 and 14.23 $\pm$ 42.49-fold, respectively ($P = 0.02$). The *TWIST-1* gene expression in patients with T315I mutation was significantly higher than patients without that mutation.

4. Discussion

CML is a chronic myeloproliferative disease caused by specific mutations in multinucleated bone marrow stem cells (7). P210 BCR-ABL plays a key role in the pathogenesis of this disorder by interfering with several molecules

regulating cell survival, proliferation, and differentiation (8). So far, various mechanisms have been identified for resistance to imatinib, including amplification of the BCR gene, mutations at the site of kinase action, and alternative signaling pathways that functionally replace imatinib-sensitive mechanisms (9-11). TWIST-1 acts at the onset of malignancy by counteracting apoptosis and at the progression of malignancy by increasing resistance to treatment (12).

In our study, 3 (7.5%) patients had T315I mutation, of whom one was in the optimal response to treatment (4.76%), and two were in the failure response to treatment group (15.38%). A study by Chahardouli et al. reported that the frequency of this mutation in patients with CML was 7% (3). Another study conducted in Malaysia reported this rate as 5.26% (13). In Kagita et al.'s study on imatinib-resistant CML patients, the highest mutations observed in these patients were related to the T315I mutation (19.04%) (14). Therefore, T315I mutation is associated with resistance to treatment, and according to Mat Yusoff et al., the diagnosis of this mutation in CML patients is clinically useful in selecting appropriate treatment strategies to prevent disease progression (13). The same is true for acute lymphocytic leukemia (ALL). Watanabe et al. reported that the presence of T315I mutation in Ph + ALL patients is associated with a very invasive phenotype of disease and resistance to TKIs. They maintained that the identification of this mutation in advanced cases and non-response to treatment are important and indicate the need to change treatment (15).

In the present study, we studied the expression of TWIST-1 gene in CML patients. This expression increased in failure response to treatment compared to optimal and warning responses, but the difference was not significant. Cosset et al. reported that the increase in TWIST-1 expression was specific to TKI-resistant patients (6). Yuan et al. found that increased TWIST-1 expression raised resistance to treatment by increasing PI3K/AKT expression in patients with CML (16). Another study reported that TWIST-1 initiates cell growth, colony formation, and drug resistance of AML and CML cell lines (17). K562/A02 may be the cause of cell line survival with multidrug resistance (18). Therefore, further studies are required to evaluate the expression of this gene in CML patients and in different response groups.

According to the results of this study, there was a significant correlation between the expression of TWIST-1 gene expression and the presence of T315I mutation. The TWIST-1 gene expression in patients with T315I mutation was significantly higher than patients without that mutation. The present study was the first to examine this association. Further studies are needed for evaluating this relationship.

4.1. Conclusions

According to our study, the TWIST-1 gene expression in patients with T315I mutation was significantly higher than patients without that mutation.

Footnotes

Authors' Contribution: N.S. directed the project; N.H. and F.N. performed the experiments; N.H. wrote the article.

Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Approval: This study was approved by the ethics committee of the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1394.343).

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Informed Consent: A written informed consent was obtained from all patients and normal subjects.

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