İSTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences / Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2024
DOI: 10.26650/JARHS2024-1311348

PRSS57 GENE EXPRESSION PREDICTS EARLY MOLECULAR RESPONSE FAILURE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

KRONİK MİYELOİD LÖSEMİLİ HASTALARDA ERKEN MOLEKÜLER YANIT TAHMİNİ İÇİN PRSS57 GEN İFADESİ

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Citation/Atıf: Bozdağ EN, Çetin G, Karaman S, Ünüvar A, Karakaş Z, Abacı N, et al. PRSS57 gene expression predicts early molecular response failure in patients with chronic myeloid leukemia. Journal of Advanced Research in Health Sciences 2024. Advance Online Publication. https://doi.org/10.26650/JARHS2024-1311348

ABSTRACT

Objectives: The molecular response to tyrosine kinase inhibitors in chronic myeloid leukemia (CML) is monitored by quantitative detection of BCR/ABL transcripts. After the initiation of the treatment, patients are followed-up with molecular analysis at three-month intervals. Early molecular response (EMR) is considered achieved when the BCR/ABL international scale (IS) is 10% or below in the three-month follow-up after treatment. This response, which has been reported to have a strong prognostic significance in CML patients, is associated with favorable long-term outcomes. However, the three-month follow-up period may be too long in terms of disease progression and treatment management for patients who fail to achieve EMR. Therefore, additional biomarkers that can predict the prognosis are needed.

Material and Methods: This study investigated the relationship between serine protease 57 (*PRSS57*) gene expression, and EMR. The *PRSS57* gene expression in 20 CML patients was determined by the quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) method and its relationship with EMR was analyzed.

Results: The *PRSS57* gene expression was found to be significantly higher in patients who failed EMR (p=0.002) and positively correlated with BCR/ABL IS value (r=0.567, p=0.009). Our results also revealed that the *PRSS57* gene expression was decreased in the post-treatment follow-up sample when compared with the diagnostic sample (p=0.000).

Conclusion: These findings indicate that the *PRSS57* gene expression in diagnosis may be useful for predicting patients at high risk of EMR failure.

Keywords: CML, early molecular response, *PRSS57*

ÖZ

Amaç: Kronik myeloid lösemide (KML) tirozin kinaz inhibitörlerine verilen yanıtın moleküler takibi BCR/ABL transkriptlerine göre yapılmaktadır. Tedavi başlangıcından sonra hastalar üç aylık periyotlarla moleküler analizlerle takip edilmektedir. Tedavi sonrası üç aylık takipte BCR/ABL Uluslararası değeri (IS) %10 ve altına düştüğünde erken moleküler yanıt (EMY) başarılı kabul edilmektedir. KML hastalarında önemli prognostik değeri olduğu bildirilen bu yanıtın uzun vadede olumlu sonuçlarla ilişkili olduğu bulunmuştur. Ancak EMY başarısız olan hastalar için hastalığın ilerlemesi ve tedavi yönetimi için üç aylık takip geç olabilmektedir. Bu nedenle tanı sırasında prognozun tahmini sağlayabilecek ek biomarkerlara ihtiyaç duyulmaktadır.

Gereç ve Yöntemler: Çalışmamızda serin proteaz 57 (*PRSS57*) gen ifadesinin EMY ile ilişkisi araştırıldı. 20 KML hastasında *PRSS57* gen ifadesi kantitatif ters transkriptaz polimeraz zincir reaksiyonu (qRT-PZR) yöntemiyle belirlendi ve EMY ile ilişkisi analiz edildi.

Bulgular: *PRSS57* gen ifadesi EMY başarısız hastalarda anlamlı derecede yüksek olduğu (p=0,002) ve BCR/ABL IS değeri ile pozitif yönde bağlantılı olduğu bulundu. Sonuçlarımız aynı zamanda *PRSS* gen ifadesinin tedavi sonrası izlem örneklerinde azaldığını gösterdi.

Sonuç: Bu bulgular tanıda *PRSS57* ifadesinin EMY başarısızlığı riski yüksek hastaların tahmininde yararlı olabileceğini göstermektedir.

Anahtar Kelimeler: KML, erken moleküler yanıt, *PRSS57*

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Submitted/Başvuru: 08.06.2023 • **Revision Requested/Revizyon Talebi:** 06.07.2023 • **Last Revision Received/Son Revizyon:** 19.07.2023 • **Accepted/Kabul:** 11.10.2023 • **Published Online/Online Yayın:** 08.02.2024

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INTRODUCTION

Chronic myeloid leukemia (CML) accounts for 15% of all leukemia and 0.5% of all cancers. The BCR/ABL fusion gene which stimulates tyrosine kinase activity is present in 95% of patients with CML (1-4). Therefore, synthetic tyrosine kinase inhibitors (TKI) should decrease tyrosine kinase activity in CML patients. The National Comprehensive Cancer Network (NCCN) and the European Leukemia Net (ELN) are two of the main international committees, which develop evidence-based guidelines or recommendations for treating and managing CML (5, 6). Implementation of the approved first generation TKIs (imatinib) and second generation TKIs (dasatinib, nilotinib, and bosutinib) comply with the recommendations provided in their guidelines (4-6).

CML has three clinical stages (chronic, accelerated, or blast) and CML patients are mostly diagnosed in the chronic phase. However, the progression might worsen for accelerated and blast phases in untreated patients or patients who develop resistance to treatment (7).

In the clinical management of CML in the chronic phase, imatinib is the most used treatment (8-12). Subsequently, the response of patients to the treatment is monitored hematologically (normalization of peripheral blood counts), cytogenetically (reduction in the number of Ph-positive meta-phases using bone marrow cytogenetics), and molecularly. Quantitative PCR is extensively used for molecular detection (decrease in the amount of BCR/ABL transcripts) of the treatment progression in accordance with the international scale (IS) (4-6).

It is possible to identify relapse and treatment responses at an early stage with an understanding of the molecular basis of CML. The molecular analysis of the BCR/ABL transcript levels is one of the most accurate ways to determine the stage of CML disease (11). According to the NCCN, there are three stages of molecular response; (i) the BCR/ABL IS value should be 10% or less at three and six months for early molecular response (EMR), (ii) the BCR/ABL IS value should be 0.1% or less for Major Molecular response (MMR), and (iii) the BCR/ABL IS value should be 0.01% or less for MR4.0 Deep Molecular Response (DMR) or the BCR/ABL IS value should be 0.0032% or less for MR4.5 DMR (5).

Many studies for understanding the importance of molecular response have shown that achieving molecular response is important in prognosis and progression-free survival. Hence, new molecular markers will be especially helpful in identifying individuals who are most at risk for disease progression or recurrence. The earliest sign that a patient has developed imatinib resistance and/or whether a BCR/ABL mutation has taken place may be the rise of the BCR/ABL levels (11). Following the first line TKI therapy, the importance of EMR has been proven by studies as a reliable indicator of progression-free survival (PFS) and overall survival (7, 11). Consequently, decisions can be made promptly regarding the therapeutic approaches that utilize molecular monitoring.

The rapid decline of the BCR/ABL transcript has important prognostic significance. However, a three month follow-up may be too long in terms of disease progression and treatment management for patients, who experience blast crisis (BC) after EMR failure during the first months of treatment. Therefore, it is highly important to develop different biomarkers for early response prediction (13-15).

Additionally, the BCR/ABL transcript level at diagnosis helps prognostic scoring systems for predicting EMR response, also current research reveals the existence of different biomarkers for this prediction. (14-16). A recent study investigated the gene expression signature (GES) of chronic phase CML patients who failed the EMR and found that some candidate genes (IGFBP2, PRSS57, and CPXM1) were expressed higher in patients who failed to achieve EMR when compared to patients who achieved EMR (15). These genes may be candidate markers that can be used to predict response to therapy at diagnosis. This study aimed to investigate the relationship between the PRSS57 gene expression at diagnosis and EMR in our CML patients.

MATERIALS and METHODS

Patient samples

Peripheral blood or bone marrow samples of 20 CML patients at diagnosis and after treatment were used with informed consent (2023/409) from Istanbul University, Istanbul Faculty of Medicine, Pediatric Hematology/Oncology Department and at Bezmialem Vakif University Faculty of Medicine Department of Internal Medicine, Division of Hematology. Patients ranged in age between 1-85 years, including seven children (mean age 11) and 13 adults (mean age 51). The gender distribution of these 20 patients was 11 (55%) female and 9 (45%) male (Table 1).

Table 1: Demographic characteristics of the patients

		Child	Adult	Total
Gender	Male	1	8	9
	Female	6	5	11
Median age (range)		11 (1-17)	51 (22-76)	37 (1-76)
Total		7	13	20

Molecular responses of the patients to the treatment were detected by quantitative real-time polymerase chain reaction (real-time qPCR) at three, six, and 12 months if the patients' follow-up data was available. These patients received imatinib (400mg/day) treatment as a first-line treatment.

RNA Isolation and cDNA Synthesis

Total RNAs were isolated by Trizol protocol from the blood samples of patients. Total RNA was quantified by Nanodrop, and cDNA was synthesized with a Thermo High-Capacity cDNA Synthesis Kit (Thermo Scientific, Massachusetts, USA). The cDNA

was synthesized from 500ng total RNA. The cDNA reaction mix was collected by adding 10x Buffer, dNTP (100mM), Random Primer (10X), RNase Inhibitors (50mM), and Reverse Transcriptase (50U/µL) in 20µL reaction volume. The cDNA synthesis was performed as follows; for pre-incubation 10 minutes at 25°C, for main incubation 120 minutes at 37°C, and for final incubation 5 minutes at 85°C.

Quantitative Real-Time PCR

The cDNA was amplified by RT-PCR on the Light Cycler 480 (Roche Diagnostic, Mannheim, Germany) for 50 cycles. The Ct-values and concentrations were then analyzed on the LC480 software (Roche Diagnostic, Mannheim, Germany). A final reaction volume of 20µL was done, which contained the SYBR Master mix (Roche Diagnostic, Mannheim, Germany), primers (10pmol/µl) (Table 2), cDNA (500ng), and RNAse-free water by the manufacturer's instructions. The cDNA synthesis was validated by the housekeeping gene GAPDH amplification. The BCR/ABL fusion gene was analyzed using qRT-PCR as previously described (17).

Table 2: Primer sequences used for qRT-PCR

Gene symbol	Primer sequences	PCR product size (bp)
PRSS57	Forward: 5'-TCACCACACACCCGACTA-3' Reverse: 5'-CGGCAGCTCCTCAAAGTCAG-3'	194
GAPDH	Forward: 5'-AGAAGGCTGGGGCTCATTTG-3' Reverse: 5'-AGGGCCATCCAGAGTCTTC-3'	257

PRSS57: Serine protease 57, GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase, bp: Base pair

Statistical analysis

Statistical analyses were performed with SPSS software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) and GraphPad Prism 8.0 (GraphPad Software, Inc.). The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test normality. Independent sample T-test for parametric data. The Kruskal-Wallis and Mann-Whitney U tests were utilized for non-parametric data. Spearman's tests were used for the correlation analysis and $p<0.05$ was statistically significant for all statistical analyses.

Ethical approval

Ethics committee approval for this study was obtained from the Local Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (Date: 17.03.2023, No: 06).

RESULTS

In our study, the BCR/ABL IS ratio was determined from CML patients, who were followed for at least six months. The IS ratio of BCR/ABL did not fall below 10% in 6 patients in six months of follow-up, so we accepted an EMR failure. The remaining 14 patients were considered as EMR achieved (Table 3).

Table 3: Early molecular response status in patients

		Child	Adult	Total
Early molecular response: Failure	Male	1	1	2
	Female	2	2	4
Total		3	3	**6**
Early molecular response: Achieve	Male	-	7	7
	Female	4	3	7
Total		4	10	**14**

When the test of normality was performed, it found that the age and gender characteristics of patients showed normal distribution, while the PRSS57 gene concentration values and the BCR/ABL transcript values showed non-normal distribution.

The *PRSS57* gene expression analysis was normalized to the GAPDH housekeeping gene expression. Diagnosis samples for the EMR achieved and failure groups were examined in terms of *PRSS57* gene expression levels, it found that the EMR failure group had significantly higher gene expression levels (Figure 1). The *PRSS57* expression was approximately 4.47 times higher in the EMR failure group (p=0.002). The *PRSS57* gene expression was found to decrease in the post-treatment follow-up sample as compared to the diagnostic sample (p=0.000).

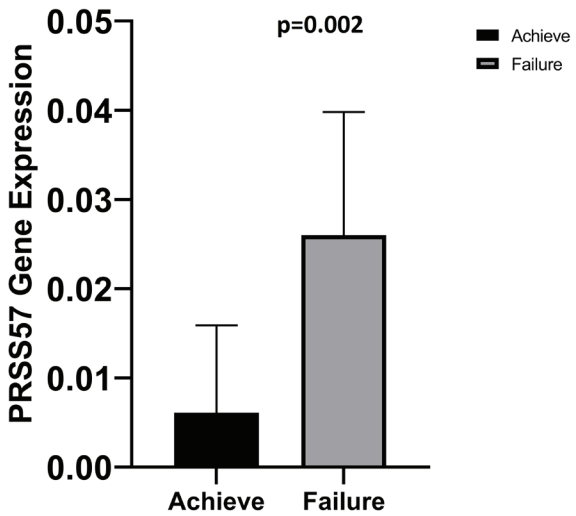


Figure 1: *PRSS57* gene expression levels in EMR achieve and failure patients at diagnosis (Achieve: EMR Achieved group, Failure: EMR Failure Group)
PRSS57: Serine protease 57

We analyzed the correlation between the *PRSS57* gene expression and the BCR/ABL IS value, and found that the *PRSS57* gene expression was moderately correlated with the BCR/ABL IS value (r=0.567, p=0.009) (Figure 2). We compared the *PRSS57* gene expression in terms of age and gender and we could not find any significant differences (p=0.125 and p=0.166 respectively).

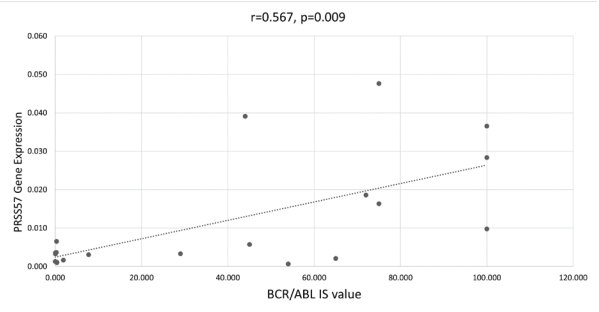


Figure 2: Correlation between *PRSS57* gene expression and BCR/ABL IS value
PRSS57: Serine protease 57

DISCUSSION

The guidelines determined many parameters (such as TKI treatment, post-treatment follow-up, and molecular response) that guide the clinical approach for patients with CML. Early prediction of disease prognosis is crucial for the treatment of the disease. In this context, showing the decrease in the BCR/ABL transcript level is an important parameter. Research has shown that when the BCR/ABL IS value is higher than 10% at three months, this is strong evidence for higher rates of disease progression (9). Observational studies report that the BCR/ABL ratio, which does not fall below 10% at three months post-treatment, carries a high risk of blast crisis progression and that the molecular response such as MMR-MR4.5 will occur at a low rate in 1-2 years. However, the prediction of treatment response at an early stage may change the prognosis of the disease with different treatment strategies (9). For these reasons, CML patients need different molecular markers to predict the prognosis of the disease at the time of diagnosis. This molecular marker may be a more accurate indicator than the BCR/ABL IS value, which can guide treatment at the time of diagnosis. In particular, the prognosis of the disease can be changed by the prediction of the patients at a high risk of EMR failure.

The therapeutic approaches used for leukemia may change as a result of the biological significance of the identified genes and their contribution to treatment resistance. Moreover, a diagnostic and prognostic biomarker found at the molecular level in research on hematological malignancies might allow for more precise evaluation. An early insight into treatment resistance will be provided by the evaluation of the achieve or failure status of the EMR. The study by Kok et al. analyzed the gene expression signature (GES) of chronic phase CML patients who failed EMR and determined that patients who failed EMR showed different gene signatures compared to those who achieved EMR (15). The results of this study showed that three genes (*IGFBP2, PRSS57*, and *CPXM1*) were expressed higher in patients who fail to achieve EMR when compared to patients who achieve EMR. Knowing the different gene signatures, whose prognostic value will be understood in the future, will improve the prediction of patients who fail EMR. While imatinib can be given to patients classified as low risk of EMR failure, patients with high risk of EMR failure can be identified at diagnosis and potentially recommended for stronger TKI therapy. Thus, the survival time in these patients could be extended.

The studies of Kok et al. and Harada et al. reported that one of the *PRSS57* genes show a different gene signature in patients who fail to achieve EMR when compared to those who achieve EMR. They found that the *PRSS57* gene expression levels were higher in patients who failed EMR (14, 15). This was determined by considering the IS ratio of the BCR/ABL for the prediction of EMR (15). Kok et al., and Harada et al. suggested that overexpression of the *PRSS57* gene is associated with poor prognosis (14, 15)

In this retrospective study, we analyzed the *PRSS57* gene expression both at the time of diagnosis and after treatment and then investigated its relationship with EMR. A significant positive correlation was found between EMR failure and the *PRSS57* gene expression at diagnosis. We found that the *PRSS57* gene expression was significantly higher in EMR failure patients than in EMR achieved patients. This study confirmed that the *PRSS57* gene is a biomarker that provides additional prognostic information. However, due to the small number of patients included in this study, it reveals the need for more comprehensive studies. Our results suggest that the *PRSS57* gene plays an important role in elucidating the pathogenesis of CML. In addition to providing positive results for clinical progress, this gene expression signature may also provide insight into the underlying resistance biology of CML.

Ethics Committee Approval: This study was approved by Istanbul University, Istanbul Faculty of Medicine (Date: 17.03.2023, No: 06).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.N.B., S.S.E.; Data Acquisition- E.N.B., S.S.E., G.Ç., S.K., A.Ü., Z.K., N.A.; Data Analysis/ Interpretation- E.N.B., S.S.E.; Drafting Manuscript- E.N.B., S.S.E.; Critical Revision of Manuscript- E.N.B., S.S.E., G.Ç., S.K., A.Ü., Z.K., N.A.; Final Approval and Accountability- E.N.B., S.S.E.; Material and Technical Support- E.N.B., S.S.E.; Supervision- S.S.E., E.N.B., G.Ç., S.K., A.Ü., Z.K., N.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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