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Research Article

Impact of MDR-1 Gene Polymorphism (rs1128503) on Response to Imatinib or Nilotinib in Iraqi Patients with Chronic Myeloid Leukemia: An Observational Study

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Abstract

Background: There is a significant molecular response to imatinib given at standard doses in individuals with chronic myeloid leukemia (CML) whose ABCB1 polymorphisms are present. ***Objective***: To investigate the impact of the polymorphism in the ABCB1 gene rs1128503 on the effectiveness of nilotinib or imatinib therapy. ***Methods***: From May 2022 until the end of January 2023, the current study was carried out in a single research institution, the National Center of Hematology, Baghdad Teaching Hospital at Medical City, Iraq. 76 people with chronic phase myeloid leukemia (CML-CP), who had previously received a diagnosis using the European Leukemia Net (ELN) criteria, enrolled in the trial. The PCR product was delivered to Macrogen Corporation, Korea, for Sanger sequencing on an automated DNA sequencer, the ABI3730XL. After receiving the results by email, Geneious Prime software was used for analysis. ***Results***: Patients receiving imatinib or nilotinib did not differ significantly in terms of age or gender. In contrast, BCR-ABL1 transcript levels were considerably greater at sampling in patients receiving nilotinib. Different types of the MDR-1 gene rs1128503 genotypes were not found in groups that were treated with either imatinib or nilotinib. ***Conclusions***: BCR-ABL1 transcript levels are lower in patients still receiving imatinib than in those receiving nilotinib.

Keywords: BCR-ABL fusion gene, Chronic myeloid leukemia, Imatinib, Nilotinib, MDR-1 gene.

تأثير تعدد أشكال الجين MDR-1 (rs1128503) على الاستجابة للعلاج بالإماتينيب أو النيلوتينيب في عينة من المرضى العراقيين في المرحلة المزمنة من سرطان الدم النخاعي: دراسة رصدية

الخلاصة

الخلفية: أظهر وجود تعدد أشكال ABCB1 لدى مرضى سرطان الدم النخاعي المزمن (CML) وجود علاقة ملحوظة مع الاستجابات الجزيئية الكبيرة للإيماتينيب الذي يتم إعطاؤه بجرعات تقليدية. **الهدف**: دراسة تأثير تعدد أشكال الجين ABCB1 rs1128503 على فعالية العلاج بالإيماتينيب أو النيلوتينيب. **الطريقة**: أجريت هذه الدراسة في المركز الوطني لأمراض الدم، مستشفى بغداد التعليمي في مدينة الطب، بغداد، العراق. خلال فترة ممتدة من مايو 2022 حتى نهاية يناير 2023. تم تسجيل ما مجموعه 76 مريضًا من مرضى سرطان الدم النخاعي المزمن (CML-CP) الذين تم تشخيصهم بالفعل من قبل أطباء أمراض الدم بناءً على معايير شبكة سرطان الدم الأوروبية (ELN) في الدراسة. تم إرسال منتج PCR لتسلسل Sanger باستخدام ABI3730XL، وهو جهاز تسلسل الحمض النووي الآلي، من قبل شركة ماكروجين الكورية. تم استلام النتائج عبر البريد الإلكتروني ثم تحليلها باستخدام برنامج Geneious Prime. **النتائج**: لم تسجل فروق ذات دلالة إحصائية في العمر أو الجنس بين المرضى الذين يتلقون إيماتينيب أو النيلوتينيب. من ناحية أخرى، كان لدى المرضى الذين يتلقون نيلوتينيب مستويات نسخة BCR-ABL1 أعلى بكثير عند أخذ العينات. لم يكن هناك وجود كبير للأنماط الجينية لجين MDR-1 rs1128503 في المجموعات المتلقية للإيماتينيب أو النيلوتينيب. **الاستنتاج**: كشفت هذه الدراسة أن المرضى الذين مازالوا يستخدمون إيماتينيب لديهم مستوى نسخة BCR-ABL1 أقل من المرضى الذين يستخدمون نيلوتينيب.

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INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative condition characterized by the presence of the Philadelphia chromosome, which results from a unique chromosomal rearrangement involving chromosomes 9 and 22 [1]. The resultant rearrangement creates the BCR-ABL1 fusion gene, which encodes a protein with continuous tyrosine kinase activity known as BCR-ABL1 kinase [2]. The BCR-ABL1 protein that develops as a result of this process disrupts myeloid cells' normal development and programmed cell death, resulting in unregulated cell division and conversion into less differentiated hemopoietic stem cells [3,4]. The BCR-ABL1 gene has been demonstrated to impact other genes in CML patients [5,6]. CML typically progresses in three stages: the chronic phase (CP), the accelerated phase (AP), and the blast crisis phase (BP). Chronic myeloid leukemia in the chronic phase (CML-CP) has the potential to go undiagnosed due to the limited presentation of symptoms in affected patients [7]. CML-CP is frequently detected incidentally via routine blood tests. As CML progresses to the accelerated phase (CML-AP) and blast phase (CML-BP), a large number of immature blast cells concentrate in the bone marrow and then spread throughout the bloodstream [8]. The malfunctioning of these blast cells causes a decrease in the production of functional blood cells, increasing the risk of infections, bleeding, and a variety of symptoms such as fatigue, weight loss, gastrointestinal discomfort, anemia, splenomegaly, and bone pain [8-10]. Biological drugs have recently received attention as a prospective treatment option for a wide range of illnesses, particularly those that are severe and potentially fatal [11]. Treatment resistance in cancer patients can develop through a variety of methods [12]. The advent of tyrosine kinase inhibitors (TKIs) that selectively target the BCR-ABL1 kinase has resulted in significant advances in the treatment of chronic CML. Tyrosine kinase inhibitors (TKIs) include imatinib, nilotinib, dasatinib, bosutinib, and ponatinib. The use of these drugs has resulted in a significant rise in the rates of long-term remission in CML patients. However, some people may develop resistance to TKIs after a prolonged term of use. Multiple theories have been proposed to explain the phenomena of TKI resistance in CML patients [13,14]. A number of these processes have been associated with point mutations in the BCR-ABL1 gene. The aforementioned changes produce a modified BCR-ABL1 kinase that is resistant to the inhibitory effects of tyrosine kinase inhibitors (TKIs) [15-17]. Furthermore, drug efflux pumps have been seen in cancer cells, which aggressively remove TKIs, resulting in a decrease in their intracellular concentration and thereby reducing their pharmacological activity [18-20]. Although target inhibition has been demonstrated to be successful, it is vital to recognize that further resistance mechanisms to TKIs may emerge. These methods include the reactivation of downstream signaling pathways or the activation of alternate parallel pathways, which have the potential to enhance carcinogenic activity [21-23]. The ABCB1 (or MDR1) gene encodes the protein ATP-binding cassette subfamily B member 1 (ABCB1), also known as P-glycoprotein or multidrug resistance protein-1. The protein in question plays an important function in aiding the movement of medicinal chemicals and harmful molecules out of cellular settings, potentially influencing the efficacy of specific therapeutic interventions. The existence of genetic polymorphisms in the ABCB1 gene has been linked to the development of resistance to chemotherapeutic drugs such TKIs [24-26]. Previous research has found a link between MDR-1 gene polymorphisms and imatinib resistance in CML patients [27,28]. Several earlier investigations have found evidence that MDR-1 rs1128503 genotypes affect the efficacy of imatinib or nilotinib [29,30]. The major goal of this study is to investigate the effect of the ABCB1 gene rs1128503 polymorphism on the efficacy of imatinib or nilotinib therapy in Iraqi patients with chronic myeloid leukemia in the chronic phase (CML-CP).

METHODS

Study design and setting

This is an observational cross-sectional study to find out how the rs1128503 polymorphism in the ABCB1 gene affects how well imatinib or nilotinib treatment works. The current study followed the STROBE standards for reporting cross-sectional observational research [31]. The current study was carried out at a single research facility, the National Center of Hematology, which is part of the Baghdad Teaching Hospital in Medical City, Baghdad. The institution serves a broad patient population from multiple governorates. Individuals who visited the facility on a regular basis for follow-up visits and medication administration were recruited to participate in the study. This process ran from May 2022 to the end of January 2023.

Sample size

The G*Power software version 3.1.9.7, with the Research Resource Identifier (RRID) SCR_013726, was used in the study to calculate the required sample size. The study employed a two-tailed alpha level of 0.05, a confidence interval of 95%, power of 95%, and an effect size of 0.38. As a result, it was decided that a sample size of approximately 79 was required. After screening patients and excluding those who were deemed unfit for the trial, the final study group consisted of 76 participants.

Inclusion criteria

This study included adult patients diagnosed with chronic myeloid leukemia in the chronic phase (CML-CP). They were either taking 400 mg of imatinib daily in the form of oral tablets or had an unsatisfactory molecular response to imatinib and

were switched to 800 mg of nilotinib daily in the form of oral tablets. This treatment regimen was based on the European Leukemia Network (ELN) recommendations from 2013 [32]. The study comprised 76 patients with CML-CP (Figure 1).

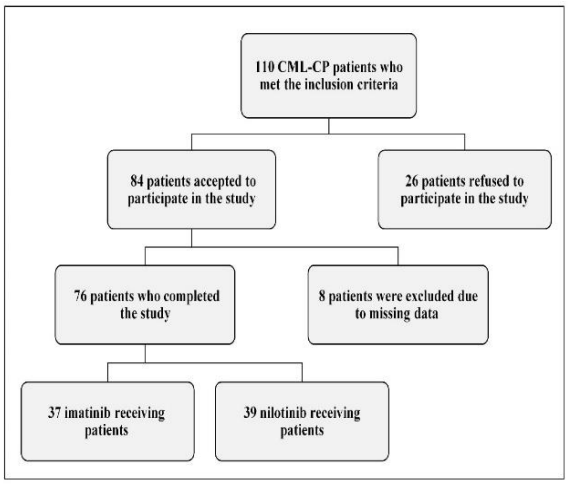

Figure 1: Flow chart of the participants' selection.

Patients were divided into four categories on an international basis. A major molecular response (MMR) was defined as a BCR-ABL qPCR transcript level of 0.1% or less (log 3), whilst MR4 (log 4) signified response levels of 0.01% or less BCR-ABL transcript. The MR4.5 (log4.5) represents a BCR-ABL1 transcript level equal to or less than 0.0032%. Suboptimal response is defined as a BCABL-1 score greater than 0.1, indicating a loss of minimum residual disease (MMR). Finally, failure occurs when the BCABL-1 value exceeds 1% [32,33].

Exclusion criteria

Patients who were taking interferon-alpha or hydroxyurea, had a history of hematopoietic stem-cell transplantation, had cardiovascular diseases or diabetes mellitus that were not under control, had chronic infections, had other types of cancer, were pregnant, or had congenital bleeding disorders or acquired bleeding disorders that were known before imatinib was given were not eligible.

Collection and processing of samples

Two milliliters of venous blood were obtained from all participants and then transferred into tubes containing ethylene diamine tetra-acetic acid (EDTA). The samples were stored at -20 degrees Celsius until DNA extraction.

DNA extraction

The genomic DNA was obtained from peripheral leukocytes using the "Promega ReliaPrepTM Blood gDNA Miniprep System." The concentration of the extracted genomic DNA was measured using the Nanodrop instrument at 260 nm. DNA purity was determined using the absorbance ratio at 260 and 280 nm. A DNA sample with a ratio of 1.8 or above was thought to contain "pure" DNA. Following genomic DNA extraction, the extracted DNA was tested for integrity using agarose gel electrophoresis. To make 1% agarose gel, add 1 g of agarose powder to the buffer and 2 l of safe green stain to the agarose solution. The UV transiluminator was employed to reveal the bromophenol blue dye-stained bands in the gel.

PCR amplification

This approach, known as polymerase chain reaction (PCR), was used to identify genotypes resulting from alterations in the ABCB1 gene. The DNA sequences for the ABCB1 gene were obtained from the NCBI GenBank database. Primer Premier 3 (RRID:SCR_003139) was used to design the PCR primers. Alpha and Company provided the lyophilized primers. The frozen primers were combined with nuclease-free water to a final concentration of 100 picomoles per microliter. This produced a stock solution. To create a workable primer solution with a concentration of 10 picomoles per microliter (10 pmol/µl), mix 90 microliters of nuclease-free water with 10 microliters of primer stock solution stored at -20°C. Table 1 contains information about the melting temperatures and sizes of PCR amplicons. The PCR procedure employed in this work contained a single 5-minute denaturation phase at 94°C. Following this, there were 35 cycles of denaturation, annealing, and extension. The denaturation stage was done at 94°C for 30 seconds, followed by an annealing step at 58°C for RS 1128503. The last elongation phase was carried out at 72°C for 5 minutes [25]. The amplicons were separated using the 1.5% agarose gel electrophoresis method. The gel was stained with ethidium bromide and viewed with a gel imaging system.

Table 1: The primers' characteristics

Primer name	Sequences	Annealing temperature (°C)	Amplicon size (bp)
MDR-1 (rs1128503-F)	5`-TGGGGCTTTTAGTGTTGGAC-3`	58	630
MDR-1(rs1128503-R)	5`-CATCTCACCATCCCCTCTGT-3`		

Figure 2 shows the generated images. The PCR products were transferred to Macrogen Corporation in Korea for Sanger sequencing on an automated DNA sequencer, the ABI3730XL. The data was analyzed with the Geneious Prime program (RRID: SCR_010519) [34].

Amplicons sequencing

PCR products were sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email then analyzed using Geneious Prime software.

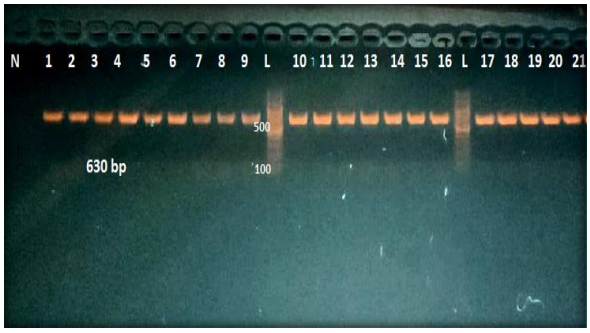

Figure 2: Human ABCB1-gene amplification was separated on 1.5% agarose gel electrophoresis stained with Ethidium Bromide. M:100-1500 bp ladder marker. Lanes1-21 resembles 630 bp PCR products.

Ethical consideration

According to the designated research reference number RECACPUB-3102020B, the Ethics Committee of the College of Pharmacy at Baghdad University approved the study protocol on November 24, 2021. Each participant signed informed consent before enrollment. No incentives were offered, and the participation was completely voluntary.

Statistical analysis

Descriptive statistics were used to examine the data in the present research. The data are presented in the form of a mean ± standard deviation. The data was statistically analyzed using the student's t-test. Binary logistic regression models are used to ascertain the association between a collection of independent factors and a binary dependent variable. Hardy-Weinberg Equilibrium is a valuable measure to assess if the studied genotypes were consistent with equilibrium or deviated from equilibrium. The statistical analysis was conducted employing the IBM SPSS Statistics (RRID: SCR 016479) version 27 software designed for the Microsoft Windows operating system.

RESULTS

The study included 76 patients diagnosed with chronic myeloid leukemia in the chronic phase (CML-CP), comprising 39 females and 37 males. The participants' ages ranged from 21 to 75 years, as shown in Table 2. Age and gender did not make a statistically significant difference between imatinib-responding and non-responding patients (p>0.05). However, non-responding patients exhibited significantly higher BCR-ABL1 at sampling levels (p=0.02) than responder patients (Table 2).

Table 2: Demographic and clinical characteristics

Variable		Imatinib group (n=37)	Nilotinib group (n=39)	*p*
Age (year)		44.19±13.05	47.10±10.31	0.074
Gender n(%)	Male	17(45.95)	20 (51.28)	0.6
	Female	20(54.05)	19 (48.72)	
BCR-ABL1 at sampling		0.093±0.06	0.43±0.396	0.02

Table 3 shows significant differences in deep molecular response (MR4 and MR4.5) and major molecular response, with a larger proportion of imatinib-treated patients reaching deep molecular response than nilotinib-treated patients. In contrast, the number of people who achieved MR3 in the nilotinib group was much higher than in the imatinib cohort.

Table 3: Frequency of molecular response difference between the study groups

Molecular response	Nilotinib group	Imatinib group	Total	*p*
MR 4	7	13	20	0.005
MR 4.5	7	11	18	
MR3	15	2	17	
S MR	10	11	21	

MR 3: BCR_ABL1 of <0.1%, MR 4: BCR-ABL1< 0.01%, MR 4.5≤ 0.0032 %, SMR: suboptimal molecular response more than 0.1 (loss of MMR).

Figure 3 depicts the approach used to investigate the rs1128503 single nucleotide polymorphism (SNP) inside the MDR-1 gene using Sanger sequencing. The single nucleotide polymorphism (SNP) rs1128503 is under consideration. A solitary "A" peak indicates the presence of a homozygous A allele. A solitary "G" peak indicates the presence of a homozygous G allele.

Figure 3: Analysis of of MDR-1 rs1128503 A/G SNP.

The presence of the "A" and "G" peaks in the data indicates the A^G heterozygous allele. Figure 4 depicts the high incidence of AA genotypes in the rs1128503 variant among Iraqi patients with CML-CP who participated in the current investigation.

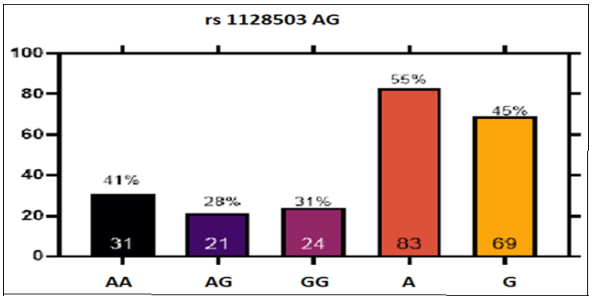

Figure 4: Genotypes frequencies of SNP in MDR-1 promoter region in all studied CML-CP patients (n=76).

The frequency of AA genotypes was roughly 41%, but the prevalence of the A allele was significantly higher, at around 55%. Furthermore, the findings of this study revealed no statistically significant occurrence of MDR-1 gene genotypes among patients receiving imatinib or nilotinib, as indicated in Table 4.

Table 4: Differences in genotypes and alleles frequency of MDR-1 gene SNPs in study groups

SNP	Genotypes	Imatinib group (n=37)	Nilotinib group (n=39)	*p*
Rs1128503	AA+	17(45.95)	14(35.9)	0.354
	AG	8(21.62)	13(33.33)	
	GG	12(32.43)	12(30.77)	
	A+	42(56.76)	41(52.56)	0.433
	G	32(43.24)	37(47.44)	

Values are expressed as frequency and percentage. Rs: reference SNP; wild genotypes are denoted +.

The results show no statistically significant variations in allele frequencies between the research groups. Table 5 shows the link between various MDR-1 genotypes and the risk of becoming a non-responder, as determined by binary logistic regression. Hardy-Weinberg equilibrium (HWE) analysis in individuals receiving imatinib and nilotinib revealed that the genotypes were consistent with the equilibrium.

Table 5: Relationship between the presence of multiple genotypes and tendency of being non-responder

Genotypes	Imatinib group (n=37)	Nilotinib group (n=39)	Odd ratio (95% CI)	*p*-value
Rs1128503	Imatinib R (n= 37)	Imatinib N-R (n= 39)		
Co-dominant				
AA	17(45.95)	14(35.9)	1.00 (Reference)	---
AG	8(21.62)	13(33.33)	1.81(0.64 to 5.25)	0.2
GG	12(32.43)	12(30.77)	0.93(0.35 to 2.48)	0.9
Dominant				
AA	17(45.95)	14(35.9)	1.00 (Reference)	---
AG+GG	20(54.05)	25(64.1)	1.52(0.60 to 3.86)	0.4
Recessive				
AA+AG	25(67.57)	27(69.23)	1.00 (Reference)	---
GG	12(32.43)	12(30.77)	0.93(0.35 to 2.48)	0.9
Allele				
A	42(56.76)	41(52.56)	1.00 (Reference)	---
G	32(43.24)	37(47.44)	1.18(0.62 to 2.26)	0.5

Values are expressed as frequency and percentage.

The observed and anticipated genotype frequencies of Rs1128503 in individuals taking nilotinib differed significantly (p<0.003), indicating a divergence from HWE Table 6.

Table 6: Numbers and percentage frequencies (Observed and Expected) of rs1128503 genotypes and their Hardy-Weinberg equilibrium (HWE) in the study groups

Groups Rs1128503		AA	AG	GG	*p*
Imatinib group	Observed	14	13	12	0.1
	Expected	10.7756	19.4487	8.7756	
Nilotinib group	Observed	17	8	12	0.003
	Expected	11.9189	18.1622	6.9189	

DISCUSSION

Pharmacogenomics has arisen as a subject devoted to studying individual differences in drug use, response to medication therapy, and the underlying processes that explain variability in drug reactions. This improvement has had a considerable impact on the development of tailored management approaches. The finding of DNA polymorphisms that have a significant influence in inter-individual variability in pharmacological reactions may help to improve treatment efficacy. In the current investigation, there were no statistically significant variations in the mean ages or gender distribution of patients receiving imatinib against those receiving nilotinib (p= 0.074 and p= 0.6). This conclusion is consistent with earlier studies that have found no discernable differences in the efficacy of tyrosine kinase inhibitor (TKI) treatment or other outcomes based on gender or age [36,37]. Radhi et al. studied sixty individuals with CML, with an average age of 46 years for those who responded to treatment and 50 years for those who did not. Statistical analysis revealed no significant differences between the two patient groups [38]. The median age of the patients included in this study is lower than the general population. This conclusion is consistent with the observations obtained in the 2020 ELN review, which indicated a median age of diagnosis of roughly 50 years [5]. The present study found a significant difference (p<0.0001) between CML patients who responded positively and those who failed treatment. The failure responder CML group had a higher average BCR-ABL1 transcript level (7.463%), while the optimal response group had a substantially lower average level (0.014%). The current study's findings are consistent with previous research, which found that patients who took nilotinib had higher levels of BCR-ABL1 than those who received imatinib [38]. This study looked at the frequency of the MDR-1 SNP in all individuals with chronic myeloid leukemia (CML-CP). The results showed that the AA genotype was the most common among those with the rs1128503 A/G genotype, accounting for 41% of cases. Furthermore, a significant proportion of patients (55% of the population) carried the A allele. The current discovery is consistent with the findings of the Rinaldi et al. study, which revealed that the prevalence of the rs1128503 genetic variation of MDR-1, specifically the wild type, was 40%. Mohammadi *et al*. found that all the evaluated single nucleotide polymorphisms (SNPs) were consistent with the Hardy-Weinberg equilibrium principles. This observation was true for both CML patients and the control group, with a p-value lower than 0.05. The genotype frequencies of the MDR-1 rs1128503 (c. 1236T) polymorphism were not significantly different between individuals who reacted positively to imatinib treatment and those who did not respond. Furthermore, there were no significant differences in the allele frequencies of MDR-1 single nucleotide polymorphisms (SNPs) between responder groups, which is consistent with the findings of the current

study. Individuals with the c.1236 AA genotype had a more than threefold higher risk of developing resistance than those with the AG/GG genotype. This study found a link between the prevalence of imatinib resistance and the number of A alleles in the MDR-1 gene (1236) [39]. Previous research conducted in Iraq looked at the relationship between genetic variants in numerous genes and the occurrence and severity of chronic myeloid leukemia in the chronic phase (CML-CP) [40-43]. This is the first study in Iraq to investigate the relationship between single nucleotide polymorphisms (SNPs) in the MDR-1 gene (rs1128503) and treatment outcomes in Iraqi patients with chronic myeloid leukemia in the chronic phase (CML-CP). Another study found that all of the genotypes investigated followed the Hardy-Weinberg equilibrium rules, which contradicts the current findings. There were no significant changes in the BCR-ABL transcript (an indicator of treatment response) across the research groups in terms of genetic variations of MDR-1 gene single nucleotide polymorphisms (SNPs) [29]. Additional research has looked into the effect of MDR-1 genetic polymorphisms on the efficacy of imatinib treatment. Dulucq and colleagues found a link between the rs1128503 AA genotypes and a higher MMR rate in patients. Nonetheless, the impact of ABCB1 polymorphisms on imatinib response remains unknown, as these findings were not replicated in a larger sample of patients [27,45]. Vivona *et al*. discovered no link between MDR-1 polymorphisms and the risk of chronic myeloid leukemia (CML) in the study group. These findings are consistent with the conclusions of the current investigation. The current study sought to investigate the relationship between MDR-1 polymorphisms and indications of imatinib response in patients with chronic myeloid leukemia (CML). The research approach used in the current examination was similar to that of a previous study, with the response criteria derived from the ELN's proposals. The study looked at the polymorphisms of MDR-1 c.1236C>T (rs1128503), c.3435 C>T (rs1045642), and c.2677G>T/A (rs2032582) [46].

Study limitations

The current investigation was conducted utilizing a single-center approach, and the findings must be verified by a comprehensive multicenter study. The tiny sample size was a serious limitation of this study. However, more trials are expected to be conducted using a large patient population cohort for long-term response monitoring.

Conclusion

The current study found that individuals who had an insufficient response to imatinib therapy and then switched to nilotinib treatment had greater levels of BCR-ABL1 transcript than patients who only got imatinib. There was no significant presence of (MDR-1) genotypes in either the imatinib or nilotinib treatment groups.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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