

Association between C1236T Genetic Variant of ABCBI Gene and Molecular Response to Imatinib in Indonesian Chronic Myeloid Patients

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Abstract

Objective: Imatinib is the first-line drug used for the treatment of chronic myeloid leukemia (CML) patients due to high molecular response and overall survival rate. However, some patients develop resistance to imatinib even after attaining a response. Mutation in *ABCBI* efflux transporters is one of the known mechanisms of resistance to imatinib in chronic myeloid leukemia patients. This study was aimed to investigate the association of *ABCBI* C1236T polymorphism in Indonesian chronic myeloid patients with molecular response to imatinib treatment. **Methods:** We analyzed 120 samples from chronic myeloid leukemia patients in the chronic phase, who had been on imatinib treatment for at least 12 months. We analyzed the C1236T variant of the *ABCBI* gene using PCR, followed by direct sequencing, and associate them with the achievement of major molecular response (MMR). **Results:** The major molecular response was achieved in 28% of patients. The frequencies of the SNPs were CC (40%), CT (46%), and TT (14%). Our result showed that there was a lack of association between polymorphism of *ABCBI* C1236T and imatinib response in Indonesian patients, with OR = 0.646 (95% CI: 0.283, 1.471; p>0.05). **Conclusion:** There was no association between *ABCBI* C1236T variants with the major molecular response in Indonesian chronic myeloid leukemia patients receiving imatinib treatment.

Keywords: Imatinib- *ABCBI*- C1236T- chronic myeloid leukemia

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Introduction

Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells, characterized by the Philadelphia (Ph) chromosome and driven by its product, the *BCR-ABL1* tyrosine kinase (Hochhaus et al., 2017; Hochhaus et al., 2018). Imatinib is the first tyrosine kinase inhibitor available for the treatment of chronic myeloid leukemia. The drug acts by inhibiting the *BCR-ABL1* oncoprotein, which will result in the inhibition of phosphorylation of proteins involved in cell signal transduction (Cohen et al., 2005; Johnson et al., 2003).

Upon treatment with imatinib, most patients restore normal hematopoiesis (Hochhaus et al., 2018). The long-term efficacy and safety imatinib in CML patients in the chronic phase have been established in IRIS Study (Hehlmann et al., 2017; Hochhaus et al., 2017; Hochhaus et al., 2018). After almost 11 years of follow-up, overall survival at ten years in imatinib-treated patients was 83.3% with no notable toxic effects (Hochhaus et al., 2017). Nonetheless, about 20 – 30 % of CML patients failed

to obtain a complete hematological response and major molecular response (MMR) after treatment with imatinib (Milojkovic and Apperley, 2009). Several mechanisms of resistance to imatinib have been proposed, which include amplification of *BCR-ABL1*, mutations in the ABL-kinase domain, as well as mutation or overexpression in *ABCBI* efflux transporters (Milojkovic and Apperley, 2009; Zu et al., 2014).

ABCBI, also known as P-glycoprotein or Multi-Drug Resistance 1 (MDR1), is a 170 – 190 kDa transmembrane protein that transports multiple endogenous compounds and xenobiotics. *ABCBI* involved in the absorption and elimination of many drugs. The action of *ABCBI* protein is to reduce intracellular drug accumulation via the efflux mechanism (Zu et al., 2014). *ABCBI* has been detected in CML patients and the circulating leucocytes in CML patients (Marzolini et al., 2004). *ABCBI* is highly polymorphic. Several studies had reported the most common variants in *ABCBI* gene, that are associated with imatinib response, C1236T, C3435T and G2677T in different populations (Angelini et al., 2013; Ben

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Hassine et al., 2017; Wang et al., 2015; Zu et al., 2014). However, the results had been inconclusive. Pooled data in a meta-analysis showed that in Asian CML patients, there is a significant association of imatinib resistance with *ABCBI C1236T* variant, but not with *C3435T* and *G2677T* variant. Therefore, in the present study, we aimed to investigate whether the *C1236T* genetic variant is associated with major molecular response in Indonesian patients treated with imatinib.

Materials and Methods

This was a nested case-control study, which included 120 stored whole blood samples from adult chronic myeloid leukemia patients in chronic phase treated with imatinib for at least 12 months. We compared those who failed to achieve major molecular response (case) and those who achieved MMR (control). Blood samples were collected from our previous research (Rinaldi et al., 2017). While Rinaldi et al., (2017) recruited 205 chronic myeloid leukemia patients, we only included samples from CML patients that have been treated with imatinib for at least 12 months. Our study was approved by the Ethics Committee Faculty of Medicine Universitas Indonesia/Ciptomangunkusumo Hospital, approval number 18-10-1194. All the samples were handled anonymously. Patients' clinical data were collected from the medical records of the respective patients. Molecular response to imatinib is shown as the last available data of major molecular response (MMR) (*BCR-ABL/ABL* transcript ratio of less than 0.1%).

DNA Isolation

DNA was isolated from whole blood samples using the Geneaid Blood DNA Isolation kit (Geneaid Biotech Ltd, Taiwan) according to the manufacturer's instruction. Only DNA with high purity were confirmed using spectrophotometry were continued for PCR analysis.

C1236T ABCBI genotyping

Genotyping of the *C1236T* variant of *ABCBI* (*rs1128503*) was performed by PCR using MyTaq HS Red Mix (Bioline, UK), followed by direct sequencing using primers and methods described previously (Ben Hassine et al., 2017). Before sequencing, the PCR product length was on electrophoresis. PCR products were sequenced for forward directions using the same primer used for PCR amplification. BioEdit Sequence Alignment Editor version 7.2.1 was used for sequence analysis.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.0. A chi-square test was done to test the significance of the genotype association with molecular response to imatinib treatment. The level of significance was set at $p < 0.05$. Odds Ratio was calculated to determine the odds of having *ABCBI C1236T* variants in patients who failed in achieving MMR than those achieving MMR.

Results

From 120 adult patients with chronic myeloid leukemia (CML) in the chronic phase, the median duration of imatinib treatment was 19 months, and the median dose was 400 mg (ranged from 200 to 500 mg). Patients consist of 54 (45%) male and 66 (55%) female, with a mean age of 42.52 years old. The majority of patients (82.5%) were treated with hydroxyurea prior to treatment with imatinib. Out of the 120 CML patients, only 34 (28.3%) achieved MMR, while 86 (71.7%) patients failed to achieve MMR.

Assessment of *C1236T ABCBI* genotype was analyzed using PCR followed by direct sequencing. The sample of results of domain sequence for the *C1236T* variant of *ABCBI* patients was given in Figure 1.

The distribution of *C1236T* genetic variant of *ABCBI* consists of 40% wild type (CC); 45.8% have a heterozygous mutation (CT), and 14.2% have a homozygous mutation (TT).

We found no association between the *C1236T* variant of the *ABCBI* gene with major molecular response (Table 1).

Discussion

Despite the radically improved outcome of CML patients treated with imatinib, about 20 – 30% of patients presented with resistance to this drug. Long term study with imatinib showed a 10-year survival of over 80% (Hehlmann et al., 2017; Hochhaus et al., 2017). However, our previous result in Indonesian patients showed a much

Table 1. Association between *C1236T* Variant of *ABCBI* Gene with Major Molecular Response in CML Patients Treated with Imatinib

Genotype	Not achieving MMR	Achieving MMR	OR (95% CI)	p
CT + TT	49	37		
CC	37	49	0.646 (0.283-1.471)	0.282
TT	11	6		
CC + CT	75	28	0.684 (0.249-2.087)	0.563

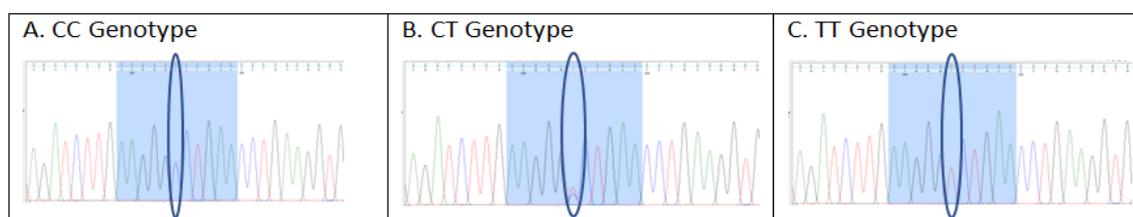


Figure 1. Domain Sequence for *C1236T* Variant of *ABCBI* in Patients with (A) Wild type CC Genotype (B) Mutant Heterozygote CT Genotype (C) Mutant Homozygote TT Genotype. Position of mutation is circled.

lower response rate and survival (Reksodiputro et al., 2010).

The role of *ABCBI* as efflux transporters of imatinib has been extensively studied, and thus far the results have been conflicting (Bedewy et al., 2019; Ben Hassine et al., 2017; Brambila-Tapia, 2013; Gromicho et al., 2013; Weiwei, 2012).

Polymorphism of *ABCBI* has been studied widely as one of the mechanisms of imatinib resistance in CML patients. *ABCBI* or widely known as MDR1 or P-glycoprotein, is an ATP-driven efflux transporter that contributes to imatinib resistance by decreasing drug concentrations in the cells (Maia et al., 2018). Among many SNPs identified for *ABCBI*, 3 variants had been extensively studied for their association with imatinib treatment, *C1236T*, *G2677T* and *C3435T*, with inconclusive results (Angelini et al., 2013; Ben Hassine et al., 2017; Maia et al., 2018; Wang et al., 2015; Zu et al., 2014). A recent meta-analysis concluded that *MDR1 C1236T* polymorphism is the risk factor for the non-optimal clinical response in Asian CML patients (Zu et al., 2014).

ABCBI variant *C1236T* (*rs118503*), located in exon 12, is a synonymous type of polymorphism, that do not produce altered coding sequences (Brambila-Tapia, 2013). A study showed that this kind of mutation provides a modified function of *MDR1*. The change in the gene did not alter *MDR1 mRNA* expression. However, it changed the substrate specificity of the protein (Kimchi-Sarfaty et al., 2007).

In contrast to other studies in CML patients treated with imatinib in different populations, our study showed low MMR achievement in CML patients after imatinib treatment (28.4%). The long duration of hydroxyurea treatment might explain the insufficient response to drug treatment in patients before imatinib treatment (Edesa and Abdel-malek, 2015; Rinaldi et al., 2017). We also found a high frequency of *ABCBI C1236T* polymorphism. In our study, we found 40% were CC, while the CT variant was 46%, and the TT variant was 14%. Our population showed a lower frequency of mutations compared to Thai, China or Korean populations (Ni et al., 2011; Pongstaporn et al., 2015; Seong et al., 2012; Yin et al., 2016). However, regarding the OR of the mutation in imatinib response, our result is quite similar to most Asian populations (Ni et al., 2011; Seong et al., 2012; Weiwei, 2012). We suspected that the higher frequency of homozygous mutation in other Asian populations, rather than heterozygous mutation type in our population, might be one the contributing factor.

In the present study, we could not explain patients' failure to achieve MMR with *C1236T* variant *ABCBI*. However, the presence of other drug transporters polymorphism has not been evaluated in our populations. Polymorphisms might not be things to be considered when we assess drug transporters. Studies had also shown that alterations in drug transporters' expressions. Studies had shown that changes in gene expression did not only occurred with *MDR1*, but also *hOCT1*, *ABCG2*, *OCTN1*, and *OATPIA2* might explain the lack of response to imatinib treatment (Angelini et al., 2013; Chhikara et al., 2017; Rinaldi et al., 2017).

Some studies demonstrated *ABCBI* haplotype, rather than individual SNPs, is a better predictor of imatinib response (Ali and Elsalakawy, 2014; Ben Hassine et al., 2017; Talaat et al., 2018; Vivona et al., 2014). While there were also reports that combinations of genetic variants in *ABCBI* with other drug transporters, mainly *OCT1* and *ABCG2* occurred in high frequency and lead to the reduced response of CML patients to imatinib (Galeotti et al., 2017; Yin et al., 2016).

There are still possibilities of other mechanism of imatinib resistance in our population that are needed to be investigated further, such as the presence of *MDR1* haplotypes or the mutations in BCR-ABL kinase domain (Ben Hassine et al., 2017; Kaleemet et al., 2015; Kimchi-Sarfaty et al., 2007; Milojkovic and Apperley, 2009).

In conclusion, our result suggested that the *C1236T* variant of *ABCBI* might not have an association with molecular response to imatinib in chronic myeloid leukemia patients.

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Conflict of interest statement

All of the authors declare no conflict of interest.

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