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The Association of Glutathione S-Transferase P1 Polymorphism to Imatinib Mesylate Resistance in Chronic Myelogenous Leukemia Patients According to Haematology Response at General Hospital Haji Adam Malik and Branch Hospital in Kota Medan

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ABSTRACT

Objective: Variations in the genes coding for drug metabolism enzymes may explain the variability response to treatment. Previous studies reported that GSTP1 polymorphism was associated with the incidence of Imatinib Mesylate resistance as first-line therapy in CML patients. This study aims to determine the association between GSTP1 polymorphism and Imatinib Mesylate resistance in CML patients according to haematology response.
Methods: Total of 46 people (consisting of 23 CML patients with Imatinib Mesilat resistance and 23 CML patients without Imatinib Mesilat resistance) at General Hospital H. Adam Malik and branch hospital in Medan City, Indonesia was analyzed in this study. Blood samples were taken for examination of GSTP1 polymorphism through Polymerase Chain Reaction (PCR). Resistance status was taken from medical records based on ELN criteria. Data were analyzed using Fisher's Exact test; *p value* <0,05 was applied to each statistical test as significant.
Result: The distribution of research subjects characteristic was divided into imatinib resistance and non-imatinib resistance groups. In both cases, the highest frequency was found in male (65,2% and 69,6%) in 35-44 years old group (34,8% and 39,1%). In the imatinib resistance group, the highest frequency was found in Javanese (39,1%) and GSTP1 polymorphism Ile/Ile (65,2%). In the non-imatinib-resistant group, the highest frequency was found in Batak tribe (43,5%) and GSTP1 polymorphism Ile/Val (52,2%). This study found 11 cases of leukocytosis and 7 cases of thrombocytosis in the imatinib resistance group. According to statistical measurement, p value was 0,142 (p>0,05).
Conclusion: The association of GSTP1 polymorphism to imatinib mesylate resistance in chronic myelogenous leukemia patients according to haematology response was not significant.

Keywords: *Chronic Myeloid Leukemia*, GSTP1 Polymorphism, Imatinib Mesylate Resistance

INTRODUCTION

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder of primitive haemopoietic stem cell which characterized by overproduction of myeloid cells. CML is identified by Philadelphia chromosome; ABL (Abelson) with BCR (break cluster region) gene at long arms of chromosome 22(22q11).[1]

CML is more common in older population (more than 50 years old). The incidence rate of this case is about 1-1,5 per 100.000 population each year.[2] The incidence of leukemia in Indonesia is estimated at 5,3 out of 100.000 population and the mortality rate is about 4,4 out of 100.000 population.[3]

Xenobiotic metabolizing enzyme plays an important role in the metabolism process of chemotherapy agents. Polymorphisms in genes encoding drug metabolizing enzymes were vary in enzymatic activity and pharmacokinetic variability. Therefore, this condition potentially modifying response and resistance to treatment or drug toxicity.[4]

Glutathione S-transferase P1 (GSTP1) is a Pi gene, located on chromosome 11q13.[4] GSTP1 polymorphism (Ile105Val) produced three types of GSTP1 genotype, namely: homozygous type Ile/Ile, heterozygous type Ile/Val, and homozygous variant Val/Val.[5]

Imatinib mesylate is a phenylaminopirimidine molecule that plays a role as BCR-ABL tyrosine kinase enzyme inhibitors. This enzyme is expressed on the leukemic cells of most patients with CML.[6] Based on previous studies, imatinib mesylate is a monoclonal antibody (STI 571) to inhibit the tyrosine kinase activity of the BCR-ABL gene fusion, but treatment resistance arises. Resistance mechanisms are generally divided into two: dependent and independent BCR-ABL.[7]

Due to the increasing of point mutations, new inhibitors have been developed with a rational drug design approach. The second-generation of inhibitor drugs were found to be able to resolve cases of resistance.[7]

To assess the response to therapy, monitoring must be carried out by assessing the hematological response, which consists of complete blood count, white blood cell differentiation and spleen measurement. The ideal response to treatment using TKI was detected during long-term treatment monitoring. The goal of TKI treatment is to achieve an ideal response haematologically, cytogenically, and molecularly.[8]

The association of GSTP1 polymorphism to imatinib mesylate resistance is still being debated. One study in Malaysia showed that patient with GSTP1 Ile105Val genotype are at risk of developing imatinib mesylate resistance. The heterozygote and homozygote variant had a significant value; 49,3% and 11% in imatinib resistance group, while in non-resistance group, it was 37,1% and 4,6%; p=0,013.[9] Similarly, in India it was found that the GSTP1 Ile105Val polymorphism had a significant relationship with the development of CML (p=0.0084); and Iran with p= 0.04.[10,11]

However, there was controversy in Romania where patients with the GSTP1 Ile105Val genotype had no associated risk of developing resistance to imatinib mesylate in CML patients, which was 22.9% in patients with CML and 17.4% in controls (p=0.07). Similarly, the study in Sudan found that no association between the GSTP1 polymorphism and the risk of CML (p=0.417); and Turkey with p= 0.199.[12,13]

METHODS

This study is an analytical study with case-control retrospective design which aims to determine the association between GSTP1 polymorphism and Imatinib Mesylate resistance in CML patients according to haematology response.

The target populations in this study were CML patients with or without imatinib mesylate resistance, while the accessible populations were CML patients with or without imatinib mesylate resistance who are outpatients at Haemato-Oncology Division, General Hospital Haji Adam Malik and branch hospital in Medan. The sampling technique used in this study was consecutive sampling, in which all subjects were involved in the study as long as they met the inclusion and exclusion criteria.

Inclusion Criteria:

a. CML patients who seek treatment at General Hospital Haji Adam Malik and branch hospital in Medan.
b. CML patients taking imatinib as the first line therapy.

Exclusion Criteria:

a. Pregnant

b. CML patients who are not willing to participate in this study.

The 46 samples (consisting of 23 CML patients with Imatinib Mesilat resistance and 23 CML patients without Imatinib Mesilat resistance) in this study was determined by total sampling method.

Procedures

All of CML patients with or without imatinib mesylate resistance who seek treatment at Haemato-Oncology Division, General Hospital Haji Adam Malik and branch hospital in Medan was selected according to inclusion and exclusion criteria. Samples in this study were asked to assign a written consent regarding subject willingness to participate in this study (informed consent).

Data of subjects (age, sex, religion, tribe, marital status, haematology or BCR-ABL response) were collected from medical records to determine the resistance status to imatinib mesylate. Subjects were carried out with blood sampling for PCR to detect GTSP1 polymorphism.

Measurements

***Chronic Myeloid Leukemia* (CML)**

Resistance Criteria

The criteria for resistance to treatment failure using imatinib were assessed relative to the duration of therapy with an assessment of hematological response (Leucocyte >10.000; Platelet >450.000) or BCR-ABL (>10%). The criteria used are in accordance with *European Leukemia Net* (ELN) criteria.

GSTP1 Polymorphism

GSTP1 polymorphism detected by PCR was divided into 3 groups (Ile/Ile, Ile/Val, and Val/Val).

Statistical Analysis

Univariate and bivariate analyzes were performed on the data in this study. Univariate analysis was performed to obtain the distribution of sample characteristics.

Bivariate analysis was performed to determine the relationship between the independent and dependent variables. This study used Fisher test as the statistical assessment; because the data was categorical and Shapiro Wilk normality test showed p = 0,0001. Statistical analysis was performed using the SPSS 20 (Statistical Package for Social Sciences) computer program where the p value <0.05 was significant.

Ethical Clearance

Ethical clearance is a written statement provided by the research ethics commission for research involving living things as well as humans, animals and plants, where it is stated that a research proposal is feasible after meeting certain requirements.

This research will be conducted after obtaining permission to carry out research from the research supervisor, approval from the USU Medical Faculty Research Ethics Commission, and after obtaining permission from the head of the hemodialysis unit at RSUP HAM. As ethical considerations, researchers believe that respondents' rights is secured by paying attention to aspects such as Self Determination, Informed Consent, Privacy, Anonymity and Confidentiality, and Protection from Discomfort

RESULTS

Sample characteristics

Table 1 shows that based on age, imatinib resistance and non-resistance were most commonly found at the age of 35-44 years, respectively 8 patients (34.8%) and 9 patients (39.1%). As for gender, cases of imatinib resistance and non-resistance were most commonly found in male, respectively 15 patients (65.2%) and 16 patients (69.6%). There was no difference in frequency based on religion between patients with resistance and non-imatinib resistance. Based on marital status, the highest number of imatinib resistance and non-imatinib resistance was found in married patients, respectively 22 patients (95.7%) and 20 patients (87.0%). Based on

ethnic, it was found that the highest imatinib resistance rate occurred in Javanese (39.1%), while the highest non-imatinib resistance rate occurred in Bataknese (43.5%).

Table 1. Sample Characteristics

Variable	Imatinib Resistance (n=23)	Non Imatinib Resistance (n=23)
Age Group		
25-34	4 (17,4%)	7 (30,4%)
35-44	8 (34,8%)	9 (39,1%)
45-54	7 (30,4%)	5 (21,7%)
55-64	2 (8,7%)	2 (8,7%)
65-74	2 (8,7%)	0 (0,0%)
Gender		
Male	15 (65,2%)	16 (69,6%)
Female	8 (34,8%)	7 (30,4%)
Religion		
Muslim	16 (69,6%)	16 (69,6%)
Christian	7 (30,4%)	7 (30,4%)
Ethnic		
Batak	6 (26,1%)	10 (43,5%)
Karo	1 (4,3%)	2 (8,7%)
Nias	1 (4,3%)	0 (0,0%)
Mandailing	3 (13,0%)	1 (4,3%)
Jawa	9 (39,1%)	6 (26,1%)
Melayu	3 (13,0%)	4 (17,4%)
Marital Status		
Married	22 (95,7%)	20 (87,0%)
Unmarried	1 (4,3%)	(13,0%)

GSTP1 Polymorphism and Haematology Response Distribution

According to PCR-RFLP, the PCR results in this study were 433 basepair (bp). Wild-type (AA/ Ile105Ile) was found at 433 bp, heterozygous variant (AG/Ile105Val) was found at 433 bp and 327 bp, while homozygous variant (GG/Val105Val) was found at 327 and 106 bp. GSTP1 polymorphism pattern were served in figure 1.

Table 2 shows that the GSTP1 Ile/Ile genotype was found to be the most common in imatinib resistance cases (65.2%) and Ile/Val genotype in non-resistance cases (52.2%).

The hematological response of the resistance group was: (1) mean value of Hb: 10.74 ± SD 1.99; (2) Leukocytes: 34724 ± SD 57568; (3) Platelets: 497869 ± SD 476381. Leukocytosis and thrombocytosis were found in imatinib resistance group, respectively 11 patients and 7 patients. Meanwhile, the haematological response of non-resistance group was: (1) mean value of Hb: 11.68 ± SD 1.65; (2) Leukocytes: 7393 ± SD 1847; (3) Platelets: 244565 ± SD 80385.

Table 2. GSTP1 Polymorphism and Haematology Response

Variable	Imatinib Resistance (n=23)	Non Imatinib Resistance (n=23)
GSTP1 Polymorphism		
Ile/Ile	15 (65,2%)	8 (34,8%)
Ile/Val	7 (30,4%)	12 (52,2%)
Val/Val	1 (4,3%)	3 (13,0%)
Haematology Response		
Hemoglobin (Hb)	Mean 10,74 ± SD 1,99	Mean 11,68 ± SD 1,65
Leukocyte (Leu)	Mean 34724 ± SD 57568	Mean 7393 ± SD 1847
Platelet (Plt)	Mean 497869 ± SD 476381	Mean 244565 ± SD 80385
Interpretation of Haematology Response		
Leucocytosis	11	0
Non Leucocytosis	12	23
Thrombocytosis	7	0
Non Thrombocytosis	16	23

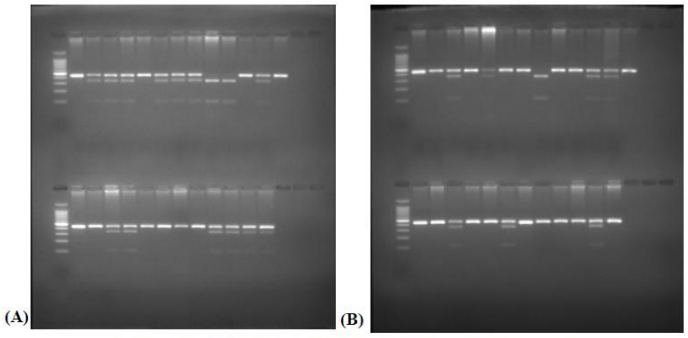

Figure 1. GSTP1 polimorphism pattern (a) Control (non-resistance); (b) Imatinib mesylate resistance

1.1 Association of GSTP1 Polymorphism to Imatinib Mesylate Resistance

Data of this study were not normally distributed (with Shapiro Wilk, p=0,001), therefore the statistical test carried out with fisher exact test.

Table 3 shows that in this study the GSTP1 Ile/Ile genotype (the supposed homozygous type) was 15 in imatinib resistance group and 8 in non-resistance group. Ile/Val polymorphism was 7 in resistance group and 12 in controls. In addition, the homozygous Val/Val polymorphism was found in 1 patient of resistance group and 3 patients of non-resistance group. The association between variables in this study is not statistically significant (p = 0,142).

Table 3. Association of GSTP1 Polymorphism to Imatinib Mesylate Resistance

GSTP1 Polymorphism	Status		p = 0,142*
	Resistance	Non Resistance	
Ile/Ile	15	8	
Ile/Val	7	12	
Val/Val	1	3	

DISCUSSION

Variations in the genes coding for drug metabolism enzymes may explain the variability response to treatment. This can be used as a determinant of the assessment of the response therapy. Polymorphism can lead to a lack of enzyme activity and a reduced detoxification role in GST.[4]

In this study, the most GSTP polymorphisms were Ile/Ile, followed by Ile/Val, and Val/Val with the least number found in resistance cases. A study in Romania by Banescu et al. (2014) found that the spread of the GSTP 1 polymorphism in CML patients was Ile/Ile (61.9%), Ile/Val (30.4%), and Val/Val (7.7%). In concordance with the study in Sudan by Idris et al., Ile/Ile was found as much as 65%, Ile/Val 31.5%, and Val/Val 3.5%. A study in Turkey by Karkucak et al showed the percentage of Ile/Ile was 64.2%, Ile/Val 29.6%, and Val/Val 7.04%.[11,12,13]

In addition to the GSTP1 polymorphism, this study also assessed the description of the hematological response of the study subjects and found the average leukocyte count in resistance group was 34724 ± SD 57568, with an average hemoglobin level of 10.74 ± SD 1.99, and an average platelet count. 497869 ± SD 476381. Karkucak et al showed the average level of leukocyte was 129,590 ± SD 130,947. Meanwhile, Elhoseiny et al showed an average value of leukocyte count was 88,800 ± 83,600; hemoglobin: 9.5 ± 2; and platelet count: 371,600 ± 286,000. The results of each study were almost similar, where the hematological response of leukocytes must reach <10 x 10^9/L. However, due to imatinib mesylate resistance, the target was not achieved. There are quite varied data regarding hematological parameters.[13,14,15]

Although there have been a number of studies conducted in different populations to evaluate the role of GST polymorphisms in the incidence of CML and response to therapy, the significance of this association remains controversial. This inconsistent nature may be related to geographical and ethnic differences in study samples. However, involvement of certain demographics in incidence of imatinib mesylate resistance is still a matter of debate. The distributions of samples in this study found that ethnic group with the highest risk for imatinib resistance were Javanese (39.1%), followed by Bataknese (26.1%) and Mandailing (13.0%). In Indonesia, there is still no study similar to the research conducted by researchers, so it is very limited to show other data.[10]

Elhoseiny et al. (2014) showed that there was an association between the GSTP1 polymorphism and poorer haematological response. Patients who did not achieve complete remission had polymorphisms (50% homozygous and 50% heterozygous) with p value = 0.05 based on haematological response and p = <0.001 based on cytologic response. Makhtar's (2017) in Malaysia found that the frequency of GSTP1 heterozygous variant polymorphisms in the resistant group was significantly higher compared to the group

that had a good response to therapy (p = 0.041). An assessment of the association of the GSTP1 Ile105Val polymorphism with response to imatinib mesylate was conducted and it was found that heterozygous and homozygous variants were associated with resistance to imatinib mesylate (OR= 1.951; 95% CI= 1.186 – 3.209; P= 0.009 – OR= 3.450; 95% CI= 1.305 – 9.606; P= 0.013.[4,14]

In this study, the association of GSTP1 polymorphism to imatinib mesylate resistance in CML patients was found to be insignificant (p = 0.142). This is in line with research by Sailaja et al. (2010), where there was no association between hematological response and GSTP1 polymorphism. Meanwhile, the frequency of Ile/Val and Val/Val genotypes was found to be increased in the poor (41.6%) and minor (53.57%) cytogenetic response groups compared to the major (38.51%) response groups. This was also stated by Karkucak et al where there was no association between CML resistance and GSTP1 polymorphism (P = 0.822; P = 0.138).[9,13]

Although there were no significant results on the association between imatinib mesylate resistance and GSTP polymorphism in this study, the number of cases used was still smaller than the population of other polymorphism studies. Due to these limitations, further studies require a larger sample with a wider geographic and ethnic spectrum. Apart from the sample size, this study did not have sufficient information regarding other environmental factors that influence treatment response.

Several studies with insignificant results were also carried out using a smaller sample size and using a wider confidence interval. So, this research needs to be confirmed by further research using a larger population with a larger number of controls. Other variables that influence the incidence of imatinib mesylate resistance in CML patients are also recommended to be included in further research.

CONCLUSION

Based on the results of the research described above, the conclusions that can be drawn are: (1) imatinib resistance and non-imatinib resistance are most commonly found at the age of 35-44 years, male gender, Muslim religion, and married patients. Javanese are the most common in the resistance group and Bataknese in the non-resistance group; (2) The most common GSTP1 polymorphism pattern found in the imatinib resistance group was Ile/Ile. Meanwhile, Ile/Val was the most common pattern in the non-imatinib resistance group. Leukocytosis (11 cases) and thrombocytosis (7 cases) were found in imatinib resistance group; (3) the association of GSTP1 polymorphism to imatinib mesylate resistance in chronic myelogenous leukemia patients according to haematology response was not significant.

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