The Association of G2677T Polymorphism in MDR1 Gene with Neutropenia Incidence in Breast Cancer Patients Treated by Doxorubicin based Chemotherapy

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Abstract: MDR1 gene is a gene that encoded P-glycoprotein (P-gp), an active efflux pump for a variety of carcinogens and cytostatics. It has been suggested that MDR1 polymorphisms G2677T contribute to the variability of therapeutic outcome and side effects. The present study was conducted to investigate the relation of G2677T polymorphisms in MDR1 gene with neutropenia incidence in breast cancer patients treated with doxorubicin based chemotherapy. As many as 147 Indonesian women' isolated DNA samples were amplified using the PCR method. The analysis process of G2677T polymorphism were done by using PCR-RFLP method. The frequencies of MDR1 G2677T genotype for homozygous GG, heterozygous GT and variant TT was 50 (34.01%), 78 (53.1%), and 19(12.9%) respectively. No association were found between MDR1 G2677T polymorphisms with degree of neutropenia ($p > 0.05$). However, there was no significant deviation of allele and genotype frequency from Hardy-Weinberg Equilibrium

1 INTRODUCTION

Breast cancer is the second most frequent cancer experienced by women in both well developed and developing countries with the number of newly diagnosed cases being 1.7 million women in the world.(World Cancer Research, 2015). The administration of chemotherapy as one of the important things in the management of breast cancer patients increased life expectancy but also various side effects (Vulsteke et al, 2013). One of the most dangerous side effects of chemotherapy is bone marrow suppression that can be measured through absolute neutrophil count (ANC). The condition of neutropenia may cause patients at risk for infection and result in delayed chemotherapy (Fung et al, 2014).

Current studies have showed, the development of medical technologies such as gene sequencing, DNA analysis and popularization of the idea of "personalized medicine" have contributed significant advances on how genetic patterns can be used to predict the efficacy and safety of chemotherapy in breast cancer (Milojkovic et al, 2011). The presence of genetic polymorphisms in the MDR1 G2677T (rs2032582) gene in exon 21 which encoded P-glycoprotein (P-gp) associated with the increased of neutropenia incidence due to chemotherapy. P-gp is a transporter protein that acts as an active effluent pump for various toxins including carcinogens and medicines such as antineoplastic drugs like doxorubicin and taxan. Interestingly, P-gp is mainly expressed in bone marrow and peripheral leukocytes, the presence of P-gp in bone marrow and peripheral leukocytes certainly has a protective effect of cells against drug accumulation into cells (Taheri et al, 2010). Several studies showed the relation of MDR1 polymorphism with hematological toxicities, but the results was inconsistent and there was no many data about MDR1 polymorphism in Indonesia (Cascorbi et al, 2011).

Therefore, we evaluated the relationship of G2677 polymorphism with degree of neutropenia both individually breast cancer patients who treated by doxorubicin based chemotherapy. The results of this study are expected to provide information related to

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the role of pharmacogenomics in response to treatment.

2 MATERIAL AND METHODS

147 Indonesian women who met the inclusion criteria were included in the study. The isolated DNA samples were then amplified using the PCR method [Syarifah et al, 2016]. DNA Amplification of Gen MDR1 G2677T using *GoTaq® Green Master Mix (Promega)* of 12.5 µl, F5'- TGC AGG CTA TAG GTT CCA GG – 3' and R5'- TTT AGT TTG ACT CAC CTT CCC G – 3' to amplify 224bp fragment. respectively 1 µl, nuclease free water as much as 7.5 µl and 3 µl DNA with final volume is 25 µl. The amplification process consisted of an initiation denaturation stage of 5 min at 94°C, followed by a 30-second denaturation step at 58°C at 35 cycles, annealing stage for 30 seconds at 72^0, extension stage for 45 seconds at 72°C and elongation at 72°C for 10 minutes. [Sailaja et al, 2010].

The analysis process of G2677 polymorphism is done by using PCR-RFLP method. [16] For SNP MDR1 G2677T analysis, 5 µl of PCR product will be rested with restriction enzyme Ban I (Promega) of 1 unit, incubated at 37°C for 1 hour. The distilled fragment was then separated by electrophoresis on 4% agarose gel for 60 min at 90 mV and analyzed after staining with Ethidium Bromide under UV light. The electrophoresis pattern shows one band (272 bp) for TT variant genotype, 2 bands (198 and 26 bp) for homozygous GG genotype and two band (224 and 198 bp) for heterozygous GT genotype. Data on neutropenia will be classified according to *Common Terminology and Criteria of Adverse Events* (CTCAE) v.4.0

Data analysis will use IBM SPSS ver.23.0, Comparison of degree of neutropenia with MDR1 G2677T polymorphism will be calculated by using Kruskal Wallis test. $P < 0.05$ was statistically significant. The frequency distribution of alleles and genotypes will use Hardy-Weinberg Equilibrium.

3 RESULT AND DISCUSSIONS

3.1 Characteristic of Subjects

Characteristics of subjects can be seen in Table 1. Frequencies of GG, GT and TT genotypes were 50 (34.01%), 78 (53.1%), and 19(12.9%) respectively. The electrophoresis pattern of MDR1 G2677T polymorphism can be seen in Figure 1. We found five ethnics include Bataknese, Malay, Javanese,Acehnese and others (Tionghoa and India). Distribution of MDR1 G2677T was varied among these ethnics. Bataknese had the highest frequency for three type of G2677T polymorphism which are homozygous GG genotype (wildtype) with 25 (17%), heterozygous variant (GT) with 44 (29.9%) and homozygous variant (TT) with 7(4.7%)

Table 1. Characteristic of Subjects

Variables	N(%)
Group of age	
<40	13(8.5)
40-50	63(41.2)
51-60	53(34.6)
>60	18(11.8)
Ethnic	
Bataknese	76(49.7)
Javanese	39(25.5)
Acehnese	16(10.5)
Malay	12(7.8)
Others	4(2.6)
BMI Classification	
Underweight	2(1.3)
Normal	60(39.2)
Overweight	63(41.2)
Obese	22(14.4)
Stages of breast cancer	
II A	15(9.8)
II B	26(17.0)
III A	51(33.3)
III B	41(26.8)
IV	14(9.2)
Histopathology of breast cancer	
Infiltrative ductal carsinoma	14(9.2)
Invasive ductal carsinoma	133 (90.8)
Histopatology grading	
Unknown	14 (9.5)
Grade I	41(27.9)
Grade II	70(47.6)
Grade III	22(15.0)

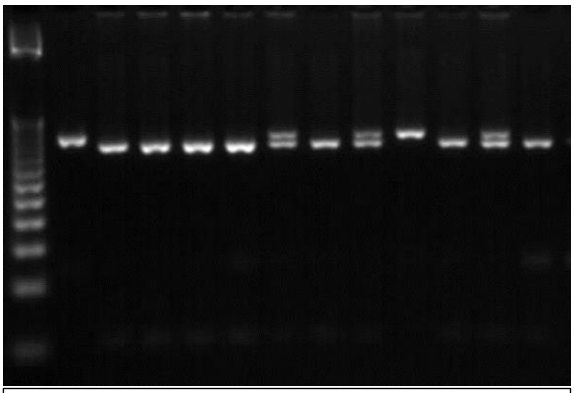

Figure 1: Electrophoresis pattern of MDR1 G2677T by PCR-RFLP: GG genotype (B-E), TT genotype (I), GT genotype (F,H,K), 25 bp DNA ladder marker (1), Undigested PCR product (A).

3.2 Frequency of Allele and Genotype of MDR1 G2677T Polymorphism

Frequency of allele and genotype can be seen in Table 2 below.

Table 2: Frequency of allele and genotype of MDR1 G2677T polymorphism.

Polymorphism	Genotype	n (%)	Allele	(%)	Hardy-Weinberg p
G2677T	GG	50 (34.01)			
	GT	78 (53.1)	G	62	0,56
	TT	19 (12.9)	T	38	

The G allele frequencies tend to be higher than T allele in G2677T. Based on the distribution of polymorphism, the frequency of alleles and genotype in this study more closely related to Asian populations than Caucasians. In this study, p> 0,05 shows that there is no significant genotype and allele frequency deviation based on Hardy-Weinberg Equilibrium.

3.3 The Association of MDR1 G2677T Polymorphism with Neutropenia

Polymorphism	Degree of Neutropenia						Total		P
	Normal		Degree 1-2		Degree 3-4				
	n	%	n	%	n	%	n	%	
GG	24	16.3	18	12.2	8	5.4	50	34	0.093
GT	45	30.6	27	18.4	6	4.1	78	53.1	
TT	**6**	**4.1**	**10**	**6.8**	**3**	**2**	**19**	**12.9**	
Total	75	51	55	37.4	17	11.5	147	100	

Kruskal-wallis Test

In this study, as shown in Table 3, 55 people (37.4%) had mild neutropenia (1-2 degrees) and 17 people (11.5) had severe neutropenia (grade 3-4). G2677T polymorphisms had no significant association with neutropenia (p> 0.05). The results of this study are in line with a study by Cizmarikova et al (2009) which shows no significant association between MDR1 polymorphism and bone marrow suppression events. Studies conducted by Chang et al (2008) in 121 cancer patients who received paclitaxel chemotherapy also showed that there was no association between MDR1 polymorphism with the incidence of neutropenia of grade 3 and 4. The results of this study contradict the studies conducted by Tran et al (2010) and Sissung et al (2006) which indicate that the homozygous variant of TT has a relationship to the incidence of severe neutropenia.

The presence of a TT variant is known to cause lower P-gp expression. The absence of any association between MDR1 G2677T with the occurrence of neutropenia may be due to other influencing factors such as the presence of other gene polymorphisms and the influence of MDR1 C3435T, C1236T polymorphism, it is known that the common haplotype in the MDR1 gene were C3435T, C1236T and G2677T (Syarifah, 2016)

4 CONCLUSIONS

In this study, all forms of GG, GT and TT polymorphisms in G2677T were found. There was no significant association between MDR1 G2677T polymorphisms with neutropenia grading. Advanced research is needed with larger sample quantities to

confirm and compare the results obtained with respect to gene polymorphisms related to treatment response.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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