Single Nucleotide Polymorphism of MDR1 C1236T Gene and Its Association with Neutropenia Event in Breast Cancer Patients Treated by Chemoterapy

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Abstract: Neutropenia event was one of the side effect which could be occurred in breast cancer patients receiving chemotherapy. 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 C1236T contribute to the variability of therapeutic outcome and side effects. The present study was conducted to investigate the association of C1236T polymorphisms in MDR1 gene with neutropenia incidence in breast cancer patients treated with antracycline based chemotherapy. As many as 144 Indonesian women' isolated DNA samples were amplified using the PCR method. The analysis process of MDR1 C1236T polymorphism were done by using PCR-RFLP method. The frequencies of MDR1 C1236T genotype for homozygous CC, heterozygous CT and variant TT was 13 (9,03%), 93 (64,58%), and 38(26,39%) respectively. There was no association between MDR1 C1236T polymorphisms with neutropenia event ($p > 0.05$). However, 69 patients (47.9%) .suffered for neutropenia event. Limitation: the data of patients were collected only after 3 cycles of chemotherapy.

1 INTRODUCTION

The incidence of breast cancer increased every year, especially in developing countries due to the increased of life expectancy, lifestyle, urbanization, and the majority of cases detected when it is already achieved in advanced stage (World Cancer Research, 2015). Chemotherapy as one of the important things in the management of breast cancer patients increased life expectancy but also various side effects (Vulsteke, 2013). Neutropenia event is one of the side effects which can be harmful to the patients due to increased of infection's risk and delayed chemotherapy (Fung, 2009).

The pharmacogenomic studies have contributed significant advances on how genetic patterns can be used to predict the efficacy and safety of chemotherapy in breast cancer (Franke, 2010). The presence of genetic polymorphisms in the MDR1 C1236T gene in exon 12 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 (Tazzite, 2016). 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 (Milojkovic, 2016).

Therefore, we evaluated the relationship of C1236T 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 the role of pharmacogenomics in response to treatment.

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2 MATERIALS AND METHODS

We conducted a cohort study recruited 144 breast cancer patients receiving anthracycline base chemotherapy in Adam Malik Hospital.

2.1 Study Area

This study was conducted at Adam Malik Hospital and Facullty of Medicine, Universitas Sumatera Utara.

2.2 Samplings

Protocol of this study has been approved by Medical Ethics Committee Universitas Sumatera Utara (No. 386/TGL/FK/KEPK FK USU-RSUP HAM/2018).

2.2.1 Recruiting Methods

Subjects of this study were purposively selected according to the inclusion criteria. Most of the subjects who had been diagnosed and treated by anthracycline base chemotherapy at Adam Malik Hospital were recruited for this study.

2.2.2 Subjects Characteristics

This study has recruited 144 breast cancer patients. The characteristic of the patients fulfil the inclusion criteria: had histologically confirmed breast cancer, had been planned to receive anthracycline base regimen of chemotherapy, ages 16– 68 years old, had normal liver function and kidney function, had a normal complete blood count (CBC).

2.2.3 Data Collection

The data of subjects's characteristic and neutropenia were collected from medical records for three cycles of chemotherapy. The data of MDR1 C1236T polymorphism were collected using PCR-RFLP method. DNA Amplification of MDR1 C1236T using *GoTaq® Green Master Mix (Promega)* of 12.5 µl, primer of exon forward 12 MDR1 5-'GCCACAGTCTGCCCACTC-3 'and reverse exon 12 MDR1 5-'CCCATCGAAAAGAAATTAAG-3', 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, followed by a 30-second denaturation step, annealing stage, extension stage and elongation stage for 10 minutes (Syarifah, 2016)

3 RESULTS

The results consist of characteristic of subjects, frequency of allele and genotype of MDR1 C1236T polymorphism, the association of MDR1 C1236T polymorphism with neutropenia.

3.1 Characteristic of Subjects

This study include 144 subjects. We found five ethnics include Bataknese, Malay, Javanese,Acehnese and others (Tionghoa and India). The majority of the subjects were Bataknese (52,8%), the group of age were 40-50 years old (39,6%), had job as housewives (63,9%). Most of the subjects were detected at late stage (61,8%) and 69 patients suffered for neutropenia after 3 cycles of chemotherapy (47,9%)[Table 1].

Table 1: Characteristic of subjects

Variables	N (%)
Group of age	
<40	14(8.5)
40-50	57(39.6)
51-60	54(37.5)
>60	18(11.8)
Ethnic	
Bataknese	76(52.8)
Javanese	40(27.8)
Acehnese	14(9.7)
Malay	11(7.6)
Others	3(8.4)
Job	
Housewives	92(63.9)
Public Employee	35(24.3)
Private Employee	10(6.9)
Farmer	7 (4.9)
Stages of breast cancer	
II	41(28.5)
III	89(61.8)
IV	14(9.7)
Degree of neutropenia	
Normal	75(52.1)
Degree 1-2	52(36.1)
Degree 3-4	17(11.8)

3.2 Frequency of Allele and Genotype of MDR1 C1236T Polymorphism

Frequency of allele and genotype can be seen below [Table 2]

Table 2: Frequency of allele and genotype of MDR1 C1236T polymorphism

Polymorphism	Genotype	n (%)	Allele	(%)	Hardy-Weinberg p
C1236T	CC	13 (9.02)			
	CT	93 (64.58)	C	62	0,61
	TT	38 (26.38)	T	38	

3.3 The Association of MDR1 C1236T Polymorphism with Neutropenia

The association of MDR1 C1236T polymorphism with neutropenia can be seen below [Table 3]

C1236T	**Degree of Neutropenia**						**Total**	
	Normal		**Degree 1-2**		**Degree 3-4**			
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
CC	7	4.8	5	3.4	1	0.6	13	34
CT	47	32.6	36	25	10	6.9	93	53.1
TT	21	14.6	11	7.6	6	4.2	38	12.9
Total	75	52	52	36	17	12	144	100
P: 0.096 (Kruskal- wallis Test)								

4 DISCUSSION

The C allele frequencies tend to be higher than T allele in C1236T. 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.In this study, as shown in Table 3, 52 subjects (36%) had mild neutropenia (1-2 degrees) and 17 people (12%) had severe neutropenia (grade 3-4). C1236T polymorphisms had no significant association with neutropenia (p> 0.05). The results of this study are in line with a study which shows no significant association between MDR1 polymorphism and bone marrow suppression events (Cizmarikova, 2010). Studies conducted) 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 (Chang, 2010). The results of this study contradict the studies which indicate that the homozygous variant of TT has a relationship to the incidence of severe neutropenia (Taheri, 2010) (Sissung, 2006). The presence of a TT variant is known to cause lower P-gp expression. The absence of any association between MDR1 C1236T 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)

5 CONCLUSION

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.

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