Association of Multi-Drug Resistance-1 (MDR1) Gene Polymorphism with Leukocytopenia in Breast Cancer Patients treated with Chemotherapy

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Abstract— **Breast cancer incidence rates tend to increase in Indonesia and worldwide. Chemotherapy is an important breast cancer treatment which improve survival rate but also has many side effects. Leukocytopenia is one of the most common side effects that can be life-threatening due to opportunistic infection. Genetic polymorphism has been linked to inter-individual variations in terms of toxicity response of anticancer drugs. C3435T polymorphism in exon 26 of Multi-Drug Resistance 1 (MDR-1) gene which encodes P-glycoprotein (P-gp) is considered to be associated with increase of leukocytopenia incidence during chemotherapy. This study aim to investigate the association between MDR1 C3435T polymorphism with the grading of leukocytopenia in breast cancer patients treated with chemotherapy. 72 Indonesian female breast cancer patients from Haji Adam Malik Hospital who received chemotherapy containing doxorubicin-paclitaxel were selected for this cohort study. DNA was extracted from peripheral leukocytes and MDR1 C3435T polymorphism was analyzed with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Patient data were collected for 3 cycles of chemotherapy. Association between MDR1 C3435T polymorphism with the grading of leukocytopenia was assessed using Kruskal-Wallis test. Decline of absolute leucocyte count during 3 cycles of chemotherapy was assessed using Wilcoxon test. Genotype deviation and allele frequencies were also determined by Hardy-Weinberg Equilibrium. Patients were divided into 4 ethnics: Batakanese, Minangkabau, Javanese and Acehnese. Distribution of MDR1 C3435T polymorphism was varied among these ethnics. The frequencies of MDR1 C3435T genotype for wildtype (CC) was 22 (30,6%), heterozygous (CT) was 38 (52,8%) and homozygous mutant (TT) was 12 (16,7%). There was no association between MDR1 C3435T polymorphism and the grading of leukocytopenia (p>0,05). The average of absolute leukocyte count was differ after the second chemotherapy and after the third chemotherapy (p<0,05). The allele and genotype frequency from Hardy-Weinberg Equilibrium showed no significant deviation. MDR1 C3435T polymorphism had no association with leukocytopenia in breast cancer patients treated with doxorubicin-taxan regimen, meanwhile there was a trend of absolute leukocyte count declining post chemotherapy cycle 2.**

Keywords— **MDR1 C3435T polymorphism, leukocytopenia, doxorubicin-paclitaxel, breast cancer**

I.INTRODUCTION

Breast cancer is the second most common cancer overall in women worldwide with nearly 1,7 million new cases in 2012 [1]. In Indonesia, breast cancer is the most common cancer among women with incidence rates are 26 per 100.000 women and breast cancer patients have the highest number of hospitalization with percentage is 16,86%. The total number of hospitalized breast cancer patients in Adam Malik Hospital, Medan, Indonesia, were 532 women in 2012 and majority of cases were diagnosed in late stages [2].

Chemotherapy is the important breast cancer treatment which improve survival rates but also has many side effects. One of the most common adverse event is leukocytopenia which can be life threatening definitely by opportunistic infection [3]. These resulting in reduction of anticancer drug's doses and delays chemotheraphy which compromise treatment outcomes [4], [5]. Human Multidrug Resistance Gene (MDR1) encodes P-glycoprotein (P-gp), a 170-kDa multidrug transporter/ efflux pump. P-gp responsible for efflux of wide variety of liphophilic compounds, including multiple chemotherapeutics agents such as antracycline (doxorubicin), taxanes (docetaxel, paclitaxel), alcaloid vinca, epidopolitoxin and tamoxifen. P-gp is expressed primarily in gastrointestinal tract, liver, kidney, testis, placenta,

blood-tissue barrier,breast, bone marrow and peripheral leukocytes [6],[7],[8].

Nowadays, more than 100 variants of MDR1 gene have been reported. One of these, a silent single nucleotide polymorphism which located in nucleotide position 3435 that change cytosine base (C) to Thymin base (T) on exon 26 (C3435T) has been found to be associated with altered P-gp function [9],[10]. Several studies showed SNP C345T, TT homozygotes had significantly decreased duodenal P-gp expression, in CD56+ natural killer cells and in cell lines derived from ovary, mammary and kidney [11], [12]. In hematological toxicities, homozygous variant (TT) have 1,5 times of absolute neutrophil count declining than group which have wildtype genotype (CC) [13].

Based on differential expression, those patients with low-expressing MDR1 genetics variant (TT homozygous) could be more likely to doxorubicin-paclitaxel induced leukocytopenia. Therefore, we investigated the association of MDR1 gene C3435T polymorphism with the grading of leukocytopenia in breast cancer patients.

II.METHOD

72 Indonesian breast cancer patients from Haji Adam Malik Hospital who had been diagnosed and treated with doxorubicin-paclitaxel regimen were selected for this cohort study from February to August 2014. Eligible patients had histological confirmed breast cancer, had planned to get paclitaxel-doxorubicin regimen, range of age 16-68 years old, had a normal of liver function and kidney function, had a normal complete blood count (CBC), available to sign informed consent. Patient who smoking, had a history of cardiac disease, hematologic disease and had previous radiotherapy 3 month before were excluded from this study.This study was approved by medical faculty Ethic Committe.

Blood samples were taken from anticoagulated (EDTA) peripheral venous blood. Genomic DNA were extracted using genomic DNA Purification Kit (Promega Corporation, USA). SNP in MDR1 (C3435T in exon 26) was analyzed by PCR-RFLP assay using the primer sequences 5'-TGTTTTCAGCTGCTTGATGG-3' and 5'-AAGGCATGTATGTTGGCCTC-3'. 5 μl of PCR product was digested by 1 unit restriction endonuclease enzyme Sau3AI (Promega) at 370C for 2 hours. Digested products were separated by 2% agarose gel electrophoresis and observed directly under UV light after staining with Ethidium Bromide. Electrophoresis patterns showed one band (158 bp) for homozygous wildtype (CC), two bands (197 and 158 bp) for heterozygous variant (CT) and one band (197 bp) for homozygous variant (TT). Leukocytopenia data and characteristic of subjects was collected from medical records for 3 cycles of chemotherapy. Leukocytopenia will be classified into grade 1-4 based on Common Terminology and Criteria of Adverse events (CTCAE).

SPSS software for Windows (SPSS version 19.0) was used for statistical analyses. The association of polymorphism with grading of leukocytopenia will be analyse by using Kruskal Wallis test. To analyse trend of absolute neutrophil count declining, we use wilcoxon test. $p < 0,05$ was considered statistically significant. Distribution of allele frquency and genotype will calculate by using Hardy-Weinberg Equilibrium.

III. RESULT

Patients characteristic are shown in Table 1. The CC genotype was found in 22 (30,6%) patients, CT genotype in 38 (52,8%) patients and TT genotype was found in 12 (16,7%) patients. We found 4 ethnics that were Bataknese, Minangkabau, Javanese and Acehnese. Distribution of MDR1 C3435T was varied among these ethnics. Bataknese had the highest frequency of homozygous CC genotype (wildtype) with 12 (54,50%), Javanese had the highest frequency of heterozygous varian (CT) with 21 (55,30%) and we found homozygous variant (TT) as the lowest among all ethnics. There was no association between MDR1 C3435T polymorphism with the grading of leukocytopenia (p>0,05) for post chemotherapy cycle 1, cycle 2 and cycle 3. The results were summarized in table 2, table 3 and table 4, respectively.

TABLE I
PATIENTS CHARACTERISTIC

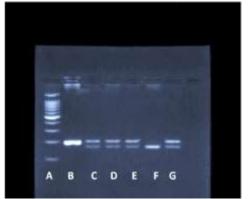| Variables	N (%)
Group of age	
<35	2 (2,8%)
36-55	51 (19,4%)
56-65	17 (40,3%)
>65	2 (27,8%)
Education History	
Elementary school	11 (15,3%)
Junior High school	15 (20,8%)
Senior High school	36 (50%)
Academician degree	10 (13,8%)
Ethnic	
Javanese	30 (41,7%)
Bataknese	29 (40,3%)
Acehnese	10 (13,9%)
Minangkabau	3 (4,2%)
Family History	
Negative	70 (97,2%)
Positive	2 (2,8%)
Contraceptive oral history	
Negative	54 (75%)
Positive	18(25%)
Stadium	
II	14 (19,4%)
III	40 (55,6%)
IV	18 (25%)

Fig 1. Electrophoretic pattern of MDR1 C3435T evaluated by PCR-RFLP assay: CC genotype (F column), TT genotype (B column), CT genotype (C,D,E,G column), A (100 bp DNA ladder marker)

TABLE II
ASSOCIATION OF MDR1 C3435T WITH LEUKOCYTOPENIA
POST CHEMOTHERAPY CYCLE 1

Polymorphism	Post chemotherapy cycle I						Total		P
	Normal		Degree I		Degree III				
	n	%	n	%	n	%	n	%	
CC	20	90,9	2	9,1	0	0	22	100	0,390
CT	34	89,4	2	5,3	2	5,3	38	100	
TT	11	91,7	1	8,3	0	0	12	100	
Total	65	90,3	5	6,9	2	2,8	72	100,0	

Kruskal-Wallis Test

In this study, no association between polymorphism of MDR1 gene C3435T and the grading of leukocytopenia was observed (p > 0,05). In this study, we can find trend of absolute leukocyte count declining (table 5). Distribution of frequency of alelle and genotype was using Hardy-Weinberg Equilibrium (Table 6). Based on table 6, p > 0,05 which showed that there was no any significant deviation of alelle and genotype frequency from Hardy-Weinberg Equilibrium.

TABLE III
ASSOCIATION OF MDR1 C3435T WITH LEUKOCYTOPENIA
GRADING POST CHEMOTHERAPY CYCLE II

Polymorphism	Post chemotherapy cycle II								P
	Normal		degree I		degree II		Degree III		
	n	%	N	%	n	%	n	%	
CC	18	81,8	4	18,2	0	0	0	0	0,790
CT	31	81,6	5	13,2	1	2,6	1	2,6	
TT	10	83,4	1	8,3	1	8,3	0	0	
Total	59	81,9	10	13,8	2	2,8	1	1,5	

Kruskal-Wallis Test

TABLE IV
ASSOCIATION OF MDR1 C3435T WITH LEUKOCYTOPENIA
GRADING POST CHEMOTHERAPY CYCLE 3

Polymorphism	Post chemotherapy cycle III								P
	Normal		degree I		degree II		Degree III		
	n	%	N	%	n	%	n	%	
CC	17	77,4	3	13,6	1	4,5	1	4,5	0,476
CT	28	73,6	7	18,5	3	7,9	0	0	
TT	7	58,3	5	41,7	0	0	0	0	
Total	52	72,2	15	20,8	4	5,6	1	1,4	

Kruskal-Wallis Test

TABLE V
TREND OF ABSOLUTE LEUKOCYTE DECLINING

Absolute leukocyte count	Range	Mean	Median	SD	p
Pre chemotherapy cycle I	3660-13840	8421,67	8435,00	2125,664	-
Post cemotherapy cycle I	1090-25680	8160	7315	3967,495	0,156
Post chemotherapy cycle II	1058-14670	6941,92	6460	2884,774	0,015

Post chemotherapy cycle III	1010-14790	6322,99	5960	3417,35	0,037

Wilcoxon Test

Based on table 5, we can find the trend of absolute leukocyte declining with more chemotherapy cycles.

TABLE VI
DISTRIBUTION OF ALELLE AND GENOTYPE FREQUENCY MDR1 C3435T

Genotype	CC	CT	TT	
Observed	30,55	52,77	16,66	P = 0,4094
Expected H-W Freq	32	48	18	
	32,65%	48,98%	18,37%	
Allele Frequencies		C= 57,14%	T=42,96%	

IV. DISCUSSION

The breast cancer incidence varied among 4 ethnics. Several studies had confirmed female breast cancer incidence vary considerably across racial and ethnic group. Difference between incidence rates and survival rates from different ethnic resulted from culture factor and environment such as exposure to carcinogenic agents, socioeconomic factor and genetic. Unpreventable risk factor including age when get the first menarche, age when get menopause and age when born first child. These was associated with the duration of estrogen exposure. Reproductive hormones are thought to influence breast cancer risk by increasing cell proliferation [14], [15]. Majority of patients didn't have family history of breast cancer, this finding was contradictive with studies showed that women with a family history of breast cancer are at increased risk of developing breast cancer [16]. Majority of the patients had no history of contraceptive oral. The risk of breast cancer increases with age, most breast cancer are diagnosed after age 50. The contradictive finding showed that breast cancer risk factors are multifactorial [15]. From this study, frequency of polymorphism MDR1 C3435T varied among the ethnics (Table.7)

TABLE VII
GENOTYPE FREQUENCIES OF MDR1 C3435T AMONG ETHNICS

Population	N	C3435T (%)			Reference
		CC	CT	TT	
Serbia	158	19	54	27	[17]
Germany	461	21	50	29	[18]
Jordania	100	17	50	33	[19]
Japan	154	36	47	17	[20]
	114	35	53	12	[21]
Chinese	200	30	53	17	[22]
	98	24	44	32	[23]
Malays	99	25	46	28	[23]
Indians	93	18	39	43	[23]
Philipines	60	38	42	20	[24]
Indonesia	19	-	26	74	[25]
Indonesia	72	30,6	52,8	16,7	This study

The frequency of wild type and variant alleles in position 3435 detected in this study did not differ significantly from those reported for Japanese and Chinese population. Different result with Indonesia population due to different ethnic and the number of subject were fewer than subject from this study.[25] This result showed that drug response differentiation based on interindividual and interethnics difference become important for certain population.

From this study, polymorphism of MDR1 C3435T had no association with leukocytopenia. This result was relevant with several studies that showed there was no association between polymorphism of MDR1 C3435T with leukocytopenia event [26]. This result was contradictive with other study in 58 cancer patient who get docetaxel monotherapy that there was an association between homozygous variant (TT) of MDR1 C3435T with grade III leukocytopenia. [27],[28] Another study showed polymorphism of MDR1 C3435T had association with grade III and IV leukocytopenia in acute lymphoblastic leukemia patients. [29]

This contradictory result may indicate that SNP of MDR1 gene should be analyzed according to complete haplotypes. Collection of several SNP that had link were known as haplotype. The most common haplotype was the polymorphism of C3435T combined with G27T and C1236T [30]. Haploptype would be considerable for differentiation of P-gp function than just one SNP.

V. CONCLUSION

There was no association of MDR1 C3435T polymorphism with leukocytopenia meanwhile there was a trend of absolute leukocyte declining with more chemotherapy cycles. A study with a larger sample size must be carried out to make definite conclusions. It is hoped therefore that pharmacologic results present here will encourage investigators to analyze haplotype of MDR1.

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