The Relation of Haplotype ATP-Binding Cassette B1 (ABCB1) and Gluthation S-Transferase P1 (GSTP1) A313G Gene with Haematological Toxicity in Indonesian Breast Cancer Patients Receiving Chemotherapy

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ABSTRACT

Objectives: Hematological toxicity induced by chemotherapy is known to be caused by multiple factors, including genetic factors, such as polymorphisms. The polymorphisms may occur in drug efflux transporter proteins and enzymes involved in drug metabolism. In our study, we investigate the incidence of hematological toxicities and its relation to the haplotype ATP-Binding Cassette B1 (ABCB1) which were polymorphism of C1236T, C3435T, G2677T and Glutathione S-Transferase P1 (GSTP1) A313G gene in Indonesian breast-cancer patients who receives anthracycline during chemotherapy.

Methods: One hundred and thirty-eight breast-cancer patients in H. Adam Malik Hospital, Medan, Indonesia who were in the inclusion criteria were recruited in this retrospective cohort study. The DNA of patients was extracted from the peripheral leukocytes. Single nucleotide polymorphism (SNP) ABCB1 and GSTP1 were examined by the PCR-RFLP method. Data on patient characteristics and the incidence of hematological toxicity were obtained from patient medical records after three cycles of chemotherapy. Trend of absolute neutrophil count (ANC) and anemia were analyzed using the Friedmann test and the Wilcoxon signed-rank test. The Kruskal-Wallis test was performed to understand the association of ABCB1 and GSTP1 polymorphisms with the incidence of anemia and neutropenia. The frequency distribution of genotypes and alleles were

determined using the Hardy-Weinberg Equilibrium (HWE).
Results: A decrease of ANC was found after post chemotherapy on cycles 3 **(**Mean ± SD: 5644.48± 2962.545/mm^3 vs 3034.89±2049.635/mm^3), and the anemia (12.1478±1.50057 gr/dl vs 11.2746± 1.31221 gr/dl) after the patients underwent three chemotherapy cycles ($p < 0.05$). There was no relation between ABCB1 polymorphism, either in each SNP or in the form of haplotype (the combination of more than one SNP) on the incidence of anemia and neutropenia ($p > 0.05$). In GSTP1 polymorphisms, no correlation between polymorphisms and anemia and neutropenia incidence ($p > 0.05$) was found. In our study, the ABCB1 and GSTP1 genotypes and alleles frequency distribution showed no deviation from HWE ($p > 0.05$).
Conclusions: Patients who have performed the three cycles of chemotherapy demonstrated a susceptibility to the side effects of hematological toxicity (such as anemia and neutropenia); however, there was no relationship between ABCB1 and GSTP1 polymorphisms to hematological toxicity.

Keywords: *ABCB1, GSTP1, polymorphism, hematological toxicity, chemotherapy, breast cancer*

Introduction

Breast cancer is the most common cancer affecting women globally, both in developed and developing countries.[1] The incidence of breast cancer is increasing every year[1], and there were 2,261,419 new cases and 684,996 deaths from the breast cancer worldwide.[2] In Indonesia, breast cancer cases are increasing, in which 65,868 women were diagnosed with a mortality rate of 44 people per 100,000 population.[2] Even though there are several risk factors that can be prevented via certain attempts such as reduction of hormonal intake, nutrition (alcohol), body weight (obesity) and inactive lifestyles, most of breast cancer cases have been detected at an advanced stage[1]; for instance, the data from Adam Malik Hospital in Medan City showed that 532 women were diagnosed with late-stage breast cancer in 2012.[3,4]
During the chemotherapy, the management of breast-cancer increases the recovery rate as well as produces adverse reactions, such as hematological toxicity. This toxicity includes anemia, neutropenia, and thrombocytopenia due to bone marrow suppression which was caused by the myelosuppression induction of chemotherapy agents, such as anthracycline, taxane, cyclophosphamide, 5-FU, and vinblastine.[5] The occurrence of this hematological toxicity influences the physician to delay the chemotherapy; furthermore, the delay increases the morbidity and mortality of the treatment.[6]

Anthracycline is one of the most widely used drugs for chemotherapy medications both its combination with cyclophosphamide and taxane which acts as adjuvant or neoadjuvant therapy; consequently, these may cause many side effects such as cardiotoxicity, gastrointestinal, and hematological toxicities.[7-8] However, there is a large difference in the clinical response of breast cancer patients who receive chemotherapy, especially to the reaction of hematological toxicities. It is suspected that there is an involvement of genetic factors. Additionally, polymorphisms of genes encoding drug transporters, enzymes that metabolize drugs, have been considered to play a role in causing differences in therapeutic responses.[9]

Several studies have suggested that there are associations between therapeutic response and SNP in a particular gene, whereas the response and therapeutic efficacy involved multiple pathways and genes. Therefore, it is more reasonable for a study to analyze several genes and several SNPS that is in one gene (haplotype). [9] The ABCB1 is known as a gene-encoded P-glycoprotein (P-GP) and an efflux transporter for various toxins, including chemotherapy agents.[4] Whilst, the GSTP1 gene encodes for GSTP1 is an essential enzyme for certain drugs' metabolism, including anthracycline and cyclophosphamide.[10-11]

The polymorphism of the ABCB1 gene in C1236T (rs1128503) in exon 12, C3435T (rs1045642) in exon 26, and G2677T (rs2032582) has been suggested to have relations to hematological toxicities, induced by chemotherapy.[12] Although previous studies have shown that there was no association of *ABCB1* C3435T with neutropenia event in breast cancer patients,[4] other studies showed there was a relation of ABCB1 polymorphism with hematological toxicities. [13-14]

Moreover, a study that investigated the GSTP1 A313G (rs1695) relationship to chemotherapy has also shown contradictory results, which suggested the relation of GSTP1 polymorphism with hematological toxicities,[10-11] but the other showed no association with hematological toxicities.[11] The results above imply to the unclear investigation of gene polymorphism to the hematological toxicities. Therefore, this study focuses in analyzing the relationship of GSTP1 and ABCB1 polymorphisms both by single SNP and in haplotype form with the incidence of hematological toxicity in breast cancer patients receiving anthracycline-based chemotherapy.

Methods

Our study was a cohort retrospective study, which was conducted from September to December 2020. The patient data were obtained from the medical records of breast cancer patients at H. Adam Malik Hospital, Medan, Indonesia. Hematological toxicity data were collected from the records of the patients that have performed three cycles of chemotherapy. The study was conducted after receiving the ethical approval from the Medical Ethics Committee of Universitas Sumatera Utara N0.879/KEP/USU/2020.

Subjects

The subjects of our study consist of 138 Indonesian women who met the inclusion criteria. They had normal liver and renal function, and typical blood test result was collected before chemotherapy administration. The patients age were in between 18-68 years old, and received chemotherapy regimen that was contained anthracycline combination (willing to be the subjects by signing the informed consent). The other participants that are excluded from our study are those who had radiotherapy history three weeks before chemotherapy administration, hematologic disorder, cardiac disease and smoking history.

DNA amplification by PCR-Method

The DNA was isolated from leukocytes via a genomic DNA kit commercial based on a manual standard procedure (Promega, USA). The PCR method was also applied in order to amplify the isolation process with GoTaq® Green Master Mix (Promega) was used to amplify ABCB1 C3435T, C1236T and G2677T. The amplifying process for ABCB1 C3435T was referred in a previous study[4], the amplifying process for ABCB1 C1236T followed from a previous study[15], and the amplifying process for ABCB1 G2677T was done using forward primer F5’- TGC AGG

CTA TAG GTT CCAGG– 3' and reverse primer R5'- TTT AGT TTG ACT CAC CTT CCC G – 3' DNA. The annealing stage was run for 30 seconds at 72º extension stage, and then it was followed for 45 seconds at 72°C, and finally was performed for elongation for 10 minutes at 72°C in 35 cycles.[16] The amplifying process for GSTP1 A313G was performed by following a previous study.[10]

SNP ABCB1 C1236T, C3435T and G2677T analysis were carried out by using the PCR-RFLP method. For the SNP *ABCB1* C3435T analysis, the restriction enzyme of Sau3AI were used[4], for the SNP *ABCB1* C1236T analysis, 5 µl of amplified PCR product was digested with 1 unit of restriction enzyme HaeIII (Promega), and it was incubated at 37°C for 1 hour.[15] Restriction enzyme Ban I (Promega) was used for SNP ABCB1 G2677 analysis, while the PCR-RFLP products were electrophoresed in agarose gel 4%.[16]

The electrophoresis pattern for ABCB1 C1236T consists of three forms, one band (272 bp) for homozygous CC genotype, two bands (272 bp and 250 bp) for heterozygous CT genotype and one band (250 bp) for variant homozygous TT genotype. The electrophoresis pattern for *ABCB1* G2677T has two bands (198 dan 26 bp) for homozygous GG, two bands (224 dan 198 bp) for heterozygous GT and one band (224 bp) for variant homozygous TT. The electrophoresis pattern for *ABCB1* C3435T consists of three forms, CC, CT and TT.[4,15] The electrophoresis pattern for GSTP1 A313G has two bands (292 and 132 bp) for homozygous AA, four fragments for heterozygous AG (292,222,132, and 70 bp) and three fragments for variant homozygous GG (222,132 and 70 bp).[10]

The assessment of hematological toxicities

In our study, the neutropenia and anemia were selected to be analysed for the hematological toxicities since these two were the most frequent hematological toxicity events in breast-cancer patients that has been treated with chemotherapy at H. Adam Malik Hospital. The occurrence of neutropenia and anemia causes a delay in the schedule of the next cycle of chemotherapy.

Neutropenia and anemia were classified into normal, grade 1 to 5 according to Common Terminology and Criteria of Adverse Events v.5.0 (CTCAE v.5.0), and these data were collected from patients' medical record for 3 cycles of chemotherapy. Degree of neutropenia (grade 1= <2,500- 1,500/mm3, grade 2= <1,500-1,000/mm3, grade 3= <1,000-500/mm3, grade 4= life-threatening, grade 5= death). Degree of anemia (grade 1= Hb LLN-10 gr/dl, grade 2= <10-8 gr/dl, grade 3=<8 gr/dl, grade 4=life threatening, grade 5= death).[17] Because the data only shows grade 1 to 3, the results are shown as normal, and grade 1 to 3 categorize as neutropenia and anemia.

Statistical Analysis

Data were analyzed statistically via IBM SPSS software. After three cycles of chemotherapy, the trends of neutropenia and anemia were analysed by using the Friedmann test and the Wilcoxon Sign Rank Test. The association of ABCB1 *(*each SNP and haplotype) and GSTP1 with neutropenia and anemia grading were assessed by using the Kruskal-Wallis test, and $p < 0,05$ was considered as statistically significant. The evaluation of deviation between allele and genotype frequency were performed via using Hardy-Weinberg Equilibrium (HWE), and $p > 0,05$ was considered as no deviation

Results

In 138 female breast-cancer patients, the highest number of age-range was 90 people (62.5%) within the range of 44-59 years of age with the majority of the people were Bataknese with 68 people (49.3%), and most of these patients were housewife, which accounted 87 people (63%). In term of the cancer, most of the subjects were categorized to suffer advanced stage cancer (stage IIIB) for 78 people (56.5%), and the highest percentage was found to have invasive ductal carcinoma for 109 people (79%). 67 patients (48.6%) experienced neutropenia (with grade 1 to 3), while 98 patients (71%) experienced anemia (with grade 1 to 3). The characteristics of the subjects are displayed in the following Table 1.

Table 1. Characteristic of subjects

Variables	n (%)
Age group	
28-35	4(2.9)
36-43	23(16.7)
44-51	45(32.6)
52-59	45(32.6)
60-68	21(15.2)
Ethnicity	
Bataknese	68(49.3)
Javanese	45(32.6)
Acehnese	16(11.6)
Tionghoa	1(0.7)
Malay	5(3.6)
Minangkabau	3(2.2)
Occupation	
Housewives	87(63)
Farmer	6(4.3)
Enterpreneur	9(6.5)
Teacher	1(0.7)
Pastor	1(0.7)
Government employees	34(24.6)
Body Mass Index (BMI)	
underweight	1(0.7)
normal	58(42)
overweight	58(42)
obese	21(15.2)
Staging	
Staging IIA	13(9.4)
Staging IIB	25(18.1)
Staging IIIA	8(5.8)
Staging IIIB	78(56.5)
Staging IV	14(10.1)

Histopathology	
infiltrating ductal carcinoma	13(9.4)
invasive ductal carcinoma	109(79)
invasive lobular carcinoma	15(10.9)
carcinoma mucinous mammae	1(0.7)
Chemotherapy	
Anthracycline (doxorubicin)-paclitaxel	71(51.4)
Cyclophospamide-anthracycline-5 Fluorouracil (CAF)	67(48.6)
Neutropenia grading	
Normal	71(51.4)
Grade 1	35(25.4)
Grade 2	15(10.9)
Grade 3	17(12.3)
Anemia grading	
Normal	40(29.0)
Grade 1	80(58.0)
Grade 2	16(11.6)
Grade 3	2(1.4)

In our study, the evaluation of possibility of neutrophils decreases after being treated for three cycles of chemotherapy was carried to see the trend. Based on Table 2, it is shown that there was a decrease of absolute neutrophils count (ANC) after the patients were treated by chemotherapy. The analysis of neutrophils decreases was carried out in the first cycle, post-second cycle, and the post-third cycle of chemotherapies, which would display the trends. Based on the data, after the three cycles, the ANC within the patient blood experienced a significant decrease, in which the neutrophil trends accounted for $p < 0.05$ (Friedmann test), and for every cycle of chemotherapy, the subjects tended to experience a decrease in neutrophils with $p < 0.05$ (Wilcoxon signed-rank test).

Tabel 2. Trend of Absolute Neutrophil Count Decrease for 3 cycles of chemotherapy

Absolute Neutrophil Count (ANC) /mm^3	N	Mean	SD	Minimum	Maximum	p value*	p value**
pre-chemotherapy	138	5644.48	2962.545	1741	23890	-	0,000
post chemotherapy cycle 1	138	4073.96	2813.130	580	19710	0,000	
post chemotherapy cycle 2	138	3540.47	2430.071	210	19870	0,018	
post chemotherapy cycle 3	138	3034.89	2049.635	200	9520	0,025	

*Wilcoxon signed- rank test, **Friedmann test

In our study, the analysis of anemia was also carried out to determine the trend of the patients that were also part of the chemotherapy. From the Table 3, it can be seen that there was a decrease in number of Hb after the patients were treated for the three cycles of chemotherapy ($p < 0.05$). It can also be seen that the patients experienced decreases in the first cycle, post-second cycle, and post-

third cycle of chemotherapy ($p < 0.05$).

Table 3. Trend of Anemia (Hb level decreasing) for 3 cycles of chemotherapy

Anemia (gr/dl)	N	Mean	SD	Minimum	Maximum	p value*	p value**
pre-chemotherapy	138	12.1478	1.50057	8.00	15.90	-	0,000
post chemotherapy cycle 1	138	11.6203	1.40272	8.40	15.10	0,000	
post chemotherapy cycle 2	138	11.4189	1.23324	8.50	14.10	0,005	
post chemotherapy cycle 3	138	11.2746	1.31221	6.80	14.60	0,007	

*Wilcoxon Sign Rank Test, **Friedmann test

The ABCB1 patterns for each SNP and its haplotype, along with the GSTP1 gene, can be seen in Table 4. In the GSTP-1 gene, the most common form of polymorphism was the homozygous wildtype, which was found in 72 people (52.5%). The second and third most common form were for heterozygote wildtype with 55 people (39.9%) and homozygote variant (GG) with 11 people (8%). In ABCB1 C3435T, the form of homozygote wildtype was found in 45 people (32.6%), whereas the homozygote variant was in 26 people (18.8%). In ABCB1 C1236T, the most common polymorphism was in wildtype heterozygote with 89 people (64.5%), and the least common form was found in 12 people (8.7%), in which the form was wildtype homozygote (CC). The highest percentage of wildtype heterozygote which was 72 people (52.2%) was also found in ABCB1 G2677T polymorphism, and the 19 people (13.8%) were found to have homozygote variant. In the haplotype ABCB1 form, there was a homozygous variant in the three SNPs (TT-TT-TT) which was found in 10 people only (7.2%). The homozygote variant of ABCB1 (3SNPs) and GSTP-1 (1 SNP) genes (The form of TT-TT-TT-TT) only found collectively in 4 people (2.9%). Both of these polymorphisms had value of allele distribution frequencies for more than 0.05 ($p > 0.05$) which implied to the no deviation of allele frequencies, based on Hardy-Weinberg Equilibrium (HWE).

Table 4. Polymorphism of GSTP1, ABCB1 (C3435T, C1236T, G2677T) And ABCB1 Haplotype

Polymorphism	n (%)	Allele	%	p(HWE)*
GSTP-1				
AG	55(39.9)	A	72,1	0,122
AA	72(52.2)	G	27,9	
GG	11(8.0)			
ABCB1 C3435T				
CT	67(48.6)	C	56,8	0,144
CC	45(32.6)	T	43,12	
TT	26(18.8)			
ABCB1 C1236T				
CC	12(8.7)	C	40,9	1,53
CT	89(64.5)	T	59,06	
TT	37(26.8)			
ABCB1 G2677T				
GG	47(34.1)	G	60,14	1,07
GT	72(52.2)	T	39,86	

TT	19(13.8)
Haplotype ABCB1	
Non-TT-TT-TT	128(92.8)
TT-TT-TT	10(7.2)
GSTP1 and ABCB1	
Non-variant- non variant	134(97.1)
Variant (AG/GG)-Variant (TT-TT-TT)	4(2.9)

*Chi-squared(chi^2)

In our study, the relationships between polymorphisms and the degree of neutropenia were evaluated. From the total of 138 patients, 71 people had no experience in neutropenia, and the others experienced grade 1, grade 2 and grade 3 which were respectively 35 people (25.4%), 15 people (10.9%), and 17 people (12.3%). The relations of GSTP1 and ABCB1 gene polymorphisms with their SNPs and haplotypes are displayed in the following Table 5. Based on Table 5, there was no significant relationship between GSTP-1, ABCB1 C3445T polymorphisms as well as the relationship of ABCB1 C1236T and ABCB1 G2677T with its haplotype shapes to the incidences of neutropenia ($p > 0.05$).

Table 5. Association of polymorphism GSTP-1 and ABCB1 with neutropenia

Gene	Polymorphism	Neutropenia								Total		p*
		Normal		Grade 1		Grade 2		Grade 3				
		n	%	n	%	n	%	n	%	n	%	
GSTP-1	AA	31	43,6	23	65,7	10	66,7	8	47,1	72	52,1	0,277
	AG	33	46,5	10	28,6	4	26,7	8	47,1	55	39,9	
	GG	7	9,9	2	5,7	1	6,7	1	5,8	11	8,0	
Total		71	100,0	35	100,0	15	100,0	17	100,0	138	100,0	
ABCB1	C3435T											
	CC	22	31,0	13	37,1	5	33,3	5	29,4	45	32,6	0.366
	CT	39	54,9	14	40,0	6	40,0	8	47,1	67	48,6	
	TT	10	14,1	8	22,9	4	26,7	4	23,5	26	18,8	
Total		71	100,0	35	100,0	15	100,0	17	100,0	138	100,0	
	C1236T											
	CC	6	8,5	2	5,7	3	20,0	1	5,9	12	8,7	0,574
	CT	44	62,0	27	77,1	8	53,3	10	58,8	89	64,5	
	TT	21	29,5	6	17,2	4	26,7	6	35,3	37	26,8	
Total		71	100,0	35	100,0	15	100,0	17	100,0	138	100,0	
	G2677T											
	GG	23	32,4	10	28,6	6	40,0	8	47,1	47	34,1	0,739
	GT	42	59,2	19	54,3	5	33,3	6	35,3	72	52,2	
	TT	6	8,4	6	17,1	4	26,7	3	17,6	19	13,7	
Total		71	100,0	35	100,0	15	100,0	17	100,0	138	100,0	

ABCB1	Haplotype											
	Non-TT-TT-TT	67	94,4	33	94,3	14	93,3	14	82,4	128	92,8	0,374
	TT-TT-TT	4	5,6	2	5,7	1	6,7	3	17,6	10	7,2	
Total		71	100,0	35	100,0	15	100,0	17	100,0	138	100,0	
GSTP1 + ABCB1	Non variant-variant	69	97,2	35	100,0	15	100,0	15	88,2	134	97,1	
	Variant (AG/GG)-Variant (TT-TT-TT)	2	2,8	0	0	0	0	2	11,8	4	2,9	0.456
Total		71	100,0	35	100,0	15	100,0	17	100,0	138	100,0	

*Kruskall- wallis test

The relations between GSTP1 and ABCB1 genes polymorphisms to the incidence of anemia is shown in Table 6. Based on Table 6, 40 people (29%) have experienced no anemia, in which the highest percentage was found in anemia grade 1 with 80 people (58%); the other of the patients experienced grade 2 for 16 people (11.6%). The grade 3 was experienced by 2 people (1.4%), which indicated no significant relationships of ABCB1 and GSTP1 polymorphisms in individual SNP and haplotype ($p > 0.05$).

Table 6. Association of polymorphism GSTP-1 and ABCB1 with anemia

Gene	Polymorphism	Anemia								Total		p*
		Normal		Grade 1		Grade 2		Grade 3				
		n	%	n	%	n	%	n	%	n	%	
GSTP-1	AA	22	55,0	39	48,8	11	68,8	0	0	72	52,2	0,859
	AG	15	37,5	33	41,2	5	31,3	2	100,0	55	39,9	
	GG	3	27,3	8	10,0	0	0,0	0	0	11	8,0	
Total		40	100,0	80	100,0	16	100,0	2	100,0	138	100,0	
ABCB1	C3435T											
	CC	15	37,5	26	32,5	4	25,0	0	0	45	32,6	0,418
	CT	16	40,0	41	51,3	9	56,3	1	50,0	67	48,6	
	TT	9	22,5	13	16,3	3	18,7	1	50,0	26	18,8	
Total		40	100,0	80	100,0	16	100,0	2	100,0	138	100,0	
	C1236T											
	CC	3	7,5	8	10	1	6,3	0	0	12	8,7	0,711
	CT	26	65,0	54	67,5	9	56,3	0	0	89	64,5	
	TT	11	27,5	18	22,5	6	37,5	2	100	37	26,8	
Total		40	100,0	80	100,0	16	100,0	2	100,0	138	100,0	
	G2677T											
	GG	16	40,0	29	36,3	2	12,5	0	0	47	34,1	0,184
	GT	18	45,0	41	51,3	12	75,0	1	50,0	72	52,2	
	TT	6	15,0	10	12,5	2	12,5	1	50,0	19	13,8	
Total		40	100,0	80	100,0	16	100,0	2	100,0	138	100,0	

ABCB1	Haplotype											
	Non-TT-TT-TT	39	97,5	73	91,3	15	93,8	1	50,0	128	92,8	0,149
	TT-TT-TT	1	2,5	7	8,8	1	6,3	1	50,0	10	7,2	
Total		40	100,0	80	100,0	16	100,0	2	100,0	138	100,0	
GSTP1 + ABCB1	Non variant-variant	40	100,0	78	97,5	15	93,8	1	50,0	134	97,1	
	Variant (GG)-Variant (TT-TT-TT)	0	0	2	2,5	1	6,2	1	50,0	4	2,9	0,627
Total		40	100,0	80	100,0	16	100,0	2	100,0	138	100,0	

*Kruskall-wallis test

Discussion

Based on our study results, the most dominant ethnicity of the breast-cancer patients is Bataknese which were mainly detected at an advanced stage. Our results are also in line with previous studies which showed that the majority of patients were diagnosed as advanced stage. [18]
Most of the patients developed neutropenia and anemia after performing three cycles of chemotherapy. This finding is in accordance to a previous study, in which 50% of the patients experienced neutropenia in varying degrees and 20% experienced febrile neutropenia after three cycles of chemotherapy.[19] Our results contradict a previous study that showed that the risk of developing neutropenia was highest in the first cycle of chemotherapy.[20] The tendency of neutropenia after three cycles of chemotherapy could be due to other factors such as poor nutrition and older age (we did not analyze in our study). Previous studies have also shown that hematological toxicity is a common side effect of giving chemotherapy drugs such as anthracyclines, cyclophosphamide and taxane.[8,21]. The chemotherapy drug 5-Fluorouracil (5-FU) can also cause side effects such as anemia, leukopenia and thrombocytopenia[22] as anemia, neutropenia and thrombocytopenia are other forms of myeloid toxicity. Since myeloid cells have a rapid division rate, this makes them vulnerable targets of cytotoxic drugs.[14,23-24]

The distribution of allele and genotype of ABCB1 (C1236T, G2677T and C3435T) in our study is in accordance with previous studies, which showed a similar proportion to the proportion of C1236T, G2677T and C3435T polymorphisms in Japanese and Chinese.[25-26]Meanwhile, there was a differentiation of polymorphism distribution with populations in Russia, Serbia and Germany.[27-28]The distribution of alleles and genotype found in our study had more similarity in Asian populations than that in Caucasians. The distribution of GSTP1 in our study is also in accordance with previous studies, in which most patients had the homozygous wildtype (AA) form compared to heterozygous and homozygous mutants.[10] Regarding the relationship between ethnicity and ABCB1 and GSTP1 polymorphisms, there was no significant relationship between ethnicity and polymorphisms.

In our study, no relations were found in between the ABCB1 and GSTP1 polymorphisms, and anemia and neutropenia. These findings are in accordance to previous studies that explained there was no relation between ABCB1 and GSTP1 with neutropenia.[11,13,28-29]. Our study also showed the same result as previous study conducted on 882 patients involved in the SWOG trial S0221

which showed none of the SNPs of ABCB1 were associated with haematological toxicity.[30]However, our results were contradictory with the previous research, which showed ABCB1 C1236T, T allele was associated to the increase of hematological toxicity and ABCB1 G2677T, in which the presence of the T allele was associated with an increased risk of leukopenia and neutropenia.[13] By contrast, a study has reported that the A TT variant in the ABCB1 gene which is commonly known as a factor could lower P-GP expression, and the presence of the variant in ABCB1 (C3435T, C1236T, G2677T) causes more significant changes of P -GP function compared to only one SNP.[31] The contradictive results which explained the involvement of ABCB1 C3435T, C1236T, G2677T polymorphism can cause less functional P-GP.[29,32] In this study, most subjects had a non-homozygous variant of haplotype ABCB1 form (92.8%) than homozygous variant (TT-TT-TT), which was only 7.2%. The minority of TT variant in SNP and haplotype of ABCB1 and GSTP1 found in our subjects would affect the result in this study.

Lack of significant relationship between ABCB1 and GSTP1 polymorphisms either individually (one SNP) or in the form of haplotypes with the incidence of hematological toxicity can be caused by several factors, such as the small number of subjects, the involvement of other genes in the pathway of the use of taxane and cyclophosphamide as the medicines and administration of filgrastim, a granulocyte-colony stimulating factor in patients with neutropenia.[24] The previous study has shown that the inconsistency of the association between ABCB1 polymorphisms and breast cancer treatment outcomes may be due to small sample-sized studies, interethnic variations and the involvement of other gene polymorphisms involved in metabolic enzyme pathways.[33]It is known that there are 16 genes involved in the drug pathway alone, such as the ABCC1, ABCC2, CYP3A5, MAPT, TP53, XRCC1 genes.[9] In the anthracycline pathway (doxorubicin and epirubicin), several other genes were also involved; for instance, not only ABCB1 as an efflux drug transporter and GSTP1, which plays a role in drug detoxification but even genes CBR1, CBR3, AKR1A1 and AKR1C3, which play a role in phase 1 of drug biotransformation.[24]

Regarding genetic factors, apart from considering the polymorphisms, the epigenetic factors which are the changes in gene expression without altering the nucleotide sequences but rather the DNA methylation, miRNA and modified histones, affect not only cancer initiation and development but also the cancer chemotherapy response.[34] Other factors that must be considered are non-genetic factors that can help to determine the clinical response to treatment, such as diet, chemotherapy, drug interactions with other drugs, and the lifestyle that can affect treatment response.[24]Healthy diets such as the microbiota regulating diet, very low ketogenic diet, Mediterranean and Japanese diet have a role in suppressing specific reactive oxygen (ROS) and nitrogen species which affect epigenetic modification. However, their effects on direct hematological toxicity have not been proven yet.[35] Obesity, unhealthy diet, smoking and inadequate physical activities contribute a significant role in reducing cancer patient survival and clinical response to chemotherapy.[36]

All forms of homozygous, heterozygous and variant in ABCB1 and GSTP1 gene were found, although there was no relationship between ABCB1 and GSTP1 polymorphisms on the incidence of anemia and neutropenia. The existence of a trend of hematological toxicity after chemotherapy administration certainly requires further study with a larger sample size involving the other genetic and non-genetic factors to determine the important factors that contribute mainly to the incidence of hematological toxicity after chemotherapy treatments. There are limitations in our study that need to be considered in interpreting our result. First, we only selected two genes that play a role

in the pharmacokinetic pathway of anthracycline and cyclophosphamide. However, it is important to consider the multiple SNPs and genes involved and examine the effects on patient outcomes. Second, we did not analyze other non-genetic factors such as diet, physical activity, smoking that could affect clinical response to chemotherapy.

Conclusion

The incidence of neutropenia and anemia in breast cancer patients receiving anthracycline-based chemotherapy was found. However, there was no association between ABCB1 and GSTP1 polymorphisms on hematological toxicity.

Conflict of Interest

The authors declare that they have no conflict of interest

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Author Contributions

SS, TW and DH were responsible for research design. SS, DH, TH were responsible for collecting data. R, MIS was responsible for data analysis and result writing. All authors have read and agree to the final manuscript.

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References

1. World Health Organization [Internet].Cancer country profile. [updated 2020]. Available from: http://www.who.int/cancer/country-profiles/en/

2. World Health Organization [Internet]. Breast,source GLOBOCAN 2020. cancer: prevention and control [updated 2021, Marc 05 cited 2021 Marc 10]. Available from: https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf.

3. Al-Lawati JA, Al-Zakwani I, Fadhil I, Al-Bahrani BJ. Cancer Incidence in Oman (1996-2015). Oman Medical journal, 2019;34(4): 271-273.

4. Syarifah S, Siregar KB, Siregar Y. Association of ATP-binding cassette sub-family B member 1 gene C3435T polymorphism with neutropenia in breast cancer patients treated with chemotherapy. Med J Indones, 2016; 25(3):156-162.

5. Shahrasbi A, Armin A, Ardebili A, Rafie KS, Ansari M, Rabani M. Hematologic Adverse Effects following Systemic Chemotherapy. J Oncol Med &Pract 2017,2:1.

6. Remesha.Toxicities of anticancer drugs and itsmanagement.Int J Basic Clin Pharmacol. 2012;1(1):2-12.

7. Leong SL, Chaiyakunapruk N, Lee SWH. Candidate Gene Association Studies of Anthracycline-induced Cardiotoxicity: A Systematic Review and Meta-analysis. 2017;7 (1), 39. doi: 10.1038/s41598-017-00075-1.

8. Schneider BP, Shen F, Gardner L, Radovich M, Li L, Miller KD et al. (2017). Genome-Wide Association Study for Anthracycline-Induced Congestive Heart Failure. Clin. Cancer Res. 2017; 23 (1), 43–51. doi: 10.1158/1078-0432.CCR-16-0908.

9. González-Neira A. (2012). Pharmacogenetics of chemotherapy efficacy in breast cancer. Pharmacogenomics. 13 (6), 677–690. doi: 10.2217/pgs.12.44.

10. Hasni D, Siregar KB, Lim H. The influence of glutathion S-transferase P-1 polymorphism A313G rs1695 on the susceptibility to cyclophosphamide hematologic toxicity in Indonesian patients. Med J Indones. 2016; 25(2):118-26.

11. Tulsyan S, Chaturvedi P, Agarwal G, Lal P, Agrawal S, Mittal RD. Pharmacogenetic influence of GST polymorphisms on anthracycline-based chemotherapy responses and toxicity in breast cancer patients: a multianalytical approach. Mol. Diagn. Ther. 2013;17, 371–379. doi:10.1007/s40291-013-0045-4

12. Hodges LM, Markovac SM, Chinnc LW, Gowc JM, Kroetzc DL, Kleina TE, et al. Very important pharmacogene summary: ABCB1 (MDR1, Pglycoprotein). Pharmacogenet Genomics. 2011; 21(3):152–161

13. Chaturvedi P, Tulsyan S, Agarwal G, Lal P, Agarwal S, Mittal RD, et al. Influence of ABCB1 genetic variants in breast cancer treatment outcomes. Cancer Epidemiol. 2013; 37 (5), 754–761. doi: 10.1016/j.canep.2013.04.012

14. Angelini S, Botticelli A, Onesti CE, Giusti R, Sini V, Durante V, et al. Pharmacogenetic Approach to Toxicity in Breast Cancer Patients Treated with Taxanes. Anticancer Res. 2017; 37 (5), 2633–2639. doi: 10.21873/anticanres.11610

15. Syarifah S, Hamdi T, Widyawati T,Sari MI,Anggraini DR. Relation of polymorphism C1236T and C3435T in ABCB1 gene with bone marrow suppression in chemotherapy-treated breast cancer patients. IOP Conference Series: Earth and Environmental Science. 2018; 125(1): 012126

16. Sailaja K, Surekha D, Rao DN, Raghunadharao D, Vishnupriya S. Association of MDR1 gene polymorphism (G2677T) with . chronic myeloid leukemia. Biology and Medicine. 2010;2(4): 17-21.

17. ctep.cancer.gov [Internet]. Cancer therapy evaluation program: common terminology criteria for adverse events version 5.0 (CTCAE v.5.0). [update 2017; cited 20 Feb 2021]. Available from: http://ctep.cancer.gov/.

18. Zahid KF, Kumar S, Al-Bimani K, Ahmed T,Al-Ajmi A, Burney IA et al. Outcome of Omani Women with Breast Cancer-associated Brain Metastases Experiences from A University Hospital. Oman Medical Journal, 2019;34(5):412-19

19. Bidadi B, Liu D, Kalari KR, Rubner M, Hein A, Beckmann MW, et al. Pathway-Based Analysis of Genome-Wide Association Data Identified SNPs in HMMR as Biomarker for Chemotherapy- Induced Neutropenia in Breast Cancer Patients. Front. Pharmacol.2018; 9, 158. doi: 10.3389/fphar.2018.00158.

20. Ozer H. The timing of chemotherapy-induced neutropenia and its clinical and economic impact. Oncology (Williston Park). 2006 Apr;20(5 Suppl 4):11-5.

21. Frederiks CN, Lam SW, Guchelaar HJ, Boven E. Genetic polymorphisms and paclitaxel- or docetaxel-induced toxicities: A systematic review. Cancer Treat Rev. 2015; 41(10), 935–950. doi: 10.1016/j.ctrv.2015.10.010

22. Shajahan J, Pillai PS, Jayakumar KNL. A Prospective Comparative Study of the Toxicity Profile of 5-Flurouracil, Adriamycin, Cyclophosphamide Regime vs Adriamycin, Paclitaxel Regime in Patients with Locally Advanced Breast Carcinoma. J. Clin. Diagn. Res. 2015;9 (12), FC01–FC06. doi: 10.7860/JCDR/2015/15939.6864

23. Kurtin, S. Myeloid Toxicity of Cancer Treatment. J. Adv. Pract. Oncol. 2012; 3(4), 209–224. doi: 10.1111/bjh.15077

24. Al-Mahayri1 NZ, Patrinos PG, Ali BR. Toxicity and Pharmacogenomic Biomarkers in Breast Cancer Chemotherapy. Frontiers in pharmacology.2020;11(445);1-16

25. Taheri M, Mahjoubi F, Omranipour R. Effect of MDR1 polymorphism on multidrug resistance expression in breast cancer patients. Genet Mol Res.2010;9(1):34–40.

26. Milojkovic et al. Frequency of the C1236T, G2677T/A and C3435T MDR1 gene polymorphisms in the Serbian population. Pharmacological Reports.2011

27. Erdélyi DJ, Kámory E, Zalka A, Semsei AF, Csókay B, Andrikovics H, et al. The role of ABC-transporter gene polymorphisms in chemotherapy induced immunosuppression, a retrospective study in childhood acute lymphoblastic leukaemia. Cell Immunol.2006. 244(2);121-4.

28. Cizmarikova M, Wagnerova M, Schonova L, Habalova V, Kohut A, Linkova A, etal. MDR1 (C3435T) polymorphism: relation to the risk of breast cancer and therapeutic outcome. Pharmacogenomics J.2010; 10: 62-9.

29. Faraji A, Dehghan Manshadi HR, Mobaraki M, Zare M, Houshmand M. Association of ABCB1 and SLC22A16 gene polymorphisms with incidence of doxorubicin-induced febrile neutropenia: a survey of iranian breast cancer patients. Plos One 2016;11(12)

30. Yao S, Sucheston LE, Zhao H, et al. Germline genetic variants in ABCB1, ABCC1 and ALDH1A1, and risk of hematological and gastrointestinal toxicities in a SWOG Phase III trial S0221 for breast cancer. Pharmacogenomics J. 2014 Jun;14(3):241-7.

31. Reed K, Parissenti AM. The effect of ABCB1 genetic variants on chemotherapy response in HIV and cancer treatment. Pharmacogenomics. 2011 Oct;1 2(10): 1465-83.

32. Farhat K, Waheed A, Hussain A, Pasha A K, Ismail M, Mansoor Q. Polymorphism of the ABCB1 gene in a pakistani population in comparison to the published data on asians and europeans Ann. Pak. Inst. Med. Sci. 2014;10(1)3–6

33. Tulsyan S, Mittal RD, Mittal B. The effects of ABCB1 polymorphisms on the outcome of breast cancer treatment. Pharmacogenomics and Personalized Medicine.2016;9:47-58

34. Jin-Feng Lv, Hu L, Zhuo W, Zhang CW, Zhou HH, Fan L. Epigenetic alternations and cancer chemotherapy response. Cancer chemotherapy and pharmacology.2016;77;673-84.

35. Soldati L, Di Renzo L, Jirillo E, Ascierto PA, Marincola FM, De Lorenzo A. The influence of diet on anti-cancer immune responsiveness. J Transl Med. 2018 Mar 2016(1):75. doi: 10.1186/s12967-018-1448-0. PMID: 29558948; PMCID: PMC5859494.

36. Vijayvergia N, Denlinger CS. Lifestyle Factors in Cancer Survivorship: Where We Are and Where We Are Headed. J Pers Med. 2015;5(3):243-263. doi:10.3390/jpm5030243