ORIGINAL ARTICLE

Oman Medical Journal [2022], Vol. 37, No. 2: e357

The Relation of Haplotype ATP-binding Cassette B1 and Glutathione S-transferase P1 *A313G* Genes with Hematological Toxicity in Indonesian Breast Cancer Patients Receiving Chemotherapy

Siti Syarifah[1]*, Tri Widyawati[1], Dita Hasni[2], Mutiara Indah Sari[3], Rusdiana Rusdiana[3] and Tasrif Hamdi[4]

[1]*Department of Pharmacology and Therapeutic, Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia*
[2]*Department of Pharmacology, Medical Faculty, Universitas Baiturrahmah, Padang, Indonesia*
[3]*Department of Biochemistry, Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia*
[4]*Department of Anesthesiology and Incentive Therapy, Faculty of Medicine, Universitas Sumatra Utara, Medan, Indonesia*

ARTICLE INFO

Article history:
Received: 19 March 2021
Accepted: 28 June 2021

Online:
DOI 10.5001/omj.2022.36

Keywords:
ABCB1 Protein; GSTP1 Protein, Human; Polymorphism, Gene; Toxicity; Chemotherapy; Breast Cancer.

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. We investigate the incidence of hematological toxicities and their relation to the haplotype ATP-binding cassette B1 (ABCB1) which were polymorphisms of *C1236T*, *C3435T*, *G2677T,* and glutathione S-transferase P1 (*GSTP1*) *A313G* genes in Indonesian breast cancer patients who received anthracycline during chemotherapy. ***Methods***: This retrospective cohort study was conducted on 138 breast cancer patients who underwent three cycles of chemotherapy in H. Adam Malik Hospital, Medan, Indonesia, who satisfied the inclusion criteria. The DNA of these patients was extracted from the peripheral leukocytes. Single nucleotide polymorphism (SNP) ABCB1 and GSTP1 were examined by the polymerase chain reaction-restriction fragment length polymorphism method. Data on patient characteristics and the incidence of hematological toxicity after each of the three cycles of chemotherapy were obtained from the medical records. The variations in absolute neutrophil count (ANC) and anemia were analyzed using the Friedmann test and the Wilcoxon signed-rank test. The Kruskal-Wallis test was used to investigate the association of ABCB1 and GSTP1 polymorphisms with anemia and neutropenia. The frequency distributions of genotypes and alleles were determined using the Hardy-Weinberg Equilibrium (HWE). ***Results:*** Post the chemotherapy cycles, there was decrease in ANC (Mean±SD: 5 644.4±2 962.5 mm^3 vs. 3 034.8±2 049.6 mm^3) and increase in anemia (12.1±1.5 g/dL vs. 11.2± 1.3 g/dL) ($p < 0.050$ for each). No relation was observed between ABCB1 polymorphism, either in each SNP or in the form of haplotype (the combination of more than one SNP), and the incidence of anemia and neutropenia ($p > 0.050$). There was also no correlation between GSTP1 polymorphisms, anemia and neutropenia incidence ($p > 0.050$). The ABCB1 and GSTP1 genotypes and alleles frequency distribution showed no deviation from HWE ($p > 0.050$). ***Conclusions:*** Indonesia breast cancer patients who underwent three cycles of chemotherapy demonstrated susceptibility to hematological toxicity by developing side effects such as anemia and neutropenia. However, no relationship was found between hematological toxicity and ABCB1 and GSTP1 polymorphisms.

The prevalence of breast cancer, the most common cancer affecting women globally, is increasing every year.[1] The year 2020 saw nearly 2.3 million new cases (65 868 in Indonesia alone) and 0.7 million deaths from breast cancer worldwide.[2] Mortality rates in breast cancer tend to be high, partly because most patients present at advanced stages.[1,3,4]

While chemotherapy has improved recovery rates, it also produces adverse reactions such as hematological toxicity. Chemotherapeutic agents such as anthracycline, taxane, cyclophosphamide,

*Corresponding author: ✉siti.syarifah@usu.ac.id

5-Fluorouracil (5-FU), and vinblastine may cause myelosuppression leading to bone marrow suppression, triggering anemia, neutropenia, and thrombocytopenia.[5] When hematological toxicity occurs, the physician may decide to delay the chemotherapy. Such breaks in treatment can cause resurgence of the cancer, increasing the risk of morbidity and mortality.[6] Anthracycline is one of the most widely used drugs for chemotherapy, often in combination with cyclophosphamide and taxane as adjuvant or neoadjuvant therapy. The combination carries the risk of serious side effects such as cardio, gastrointestinal, and hematological toxicities.[7,8]

There are large differences between the clinical responses of individual breast cancer patients in chemotherapy induced hematological toxicities. Involvement of genetic factors is suspected, especially because polymorphisms in genes which encode the drug transporters and enzymes that metabolize drugs are known to affect an individual's responses to chemotherapy.[9]

Several studies have suggested associations between therapeutic response and single nucleotide polymorphism (SNP) in a particular gene, whereas the response and therapeutic efficacy involve multiple pathways and genes. Therefore, it is more reasonable to analyze the genes and SNPs haplotype-wise.[9] The gene ATP-binding cassette B1 (*ABCB1*) is suggested as promoting drug resistance by encoding P-glycoprotein (P-GP), an efflux transporter for toxins including chemotherapeutic agents.[4] The *GSTP1* gene, by contrast, encodes an enzyme that metabolizes certain drugs, including anthracycline and cyclophosphamide.[10,11]

Polymorphisms of the *ABCB1* gene in *C1236T* (rs1128503) located in exon 12, *C3435T* (rs1045642) in exon 26, and *G2677T* (rs2032582) are suggested to be involved in hematological toxicities induced by chemotherapy.[12] However, various studies have yielded mixed results. Some studies in breast cancer patients found no association of ABCB1 C3435T with neutropenia,[4] while others linked ABCB1 polymorphism with hematological toxicities.[13,14] Another study that investigated the relationship of GSTP1 A313G (rs1695) to chemotherapy also linked GSTP1 polymorphism with hematological toxicities,[10] but a similar study showed no association.[11] These results imply insufficient understanding of the role of gene polymorphism in hematological toxicities in breast cancer patients subjected to chemotherapy, which points to the need for further study. Therefore, this study focuses on 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 who received anthracycline-based chemotherapy.

METHODS

This was a cohort retrospective study, conducted from September to December 2020 at H. Adam Malik Hospital, Medan, Indonesia. The data pertaining to patients who underwent three cycles of chemotherapy during the study period was collected from their medical records. The study began after receiving the ethical approval from the Medical Ethics Committee of Universitas Sumatera Utara (No.879/KEP/USU/2020).

Our subjects comprised N=138 Indonesian women who met the following inclusion criteria: aged 18–68 years, normal liver and renal function, typical blood test result (blood collected before chemotherapy), and completed three cycles of chemotherapy regimen that contained anthracycline combination. Prospective subjects who fulfilled the criteria were invited to the study. Written informed consent was received from the 138 women who were willing to participate. Excluded were patients who underwent radiotherapy within three weeks before the start of chemotherapy, and those who had pre-existing hematologic disorders, cardiac disease, or history of smoking.

The DNA was isolated from leukocytes via a standard procedure using a commercial genomic DNA kit (Promega, USA). The polymerase chain reaction(PCR) method was applied 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 as per Syarifah et al,[4] and that for ABCB1 C1236T followed Syarifah et al.[15] The amplifying process for ABCB1 G2677T was conducted 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 72 °C 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 as per Hasni et al.[10]

SNP ABCB1 C1236T, C3435T, and G2677T analyses were carried out using the PCR-RFLP method. For the SNP *ABCB1* C3435T analysis, the restriction enzyme of Sau3AI was 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 then 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 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 pattern for *ABCB1* C3435T consists of three forms, CC, CT and TT.[4,15] The 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]

Neutropenia and anemia were selected to study the effect of hematological toxicities since these two were the most frequently observed conditions in breast cancer patients treated with chemotherapy at the hospital. In addition, neutropenia and anemia disrupt the treatment schedule by delaying 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), the data pertaining to which was collected from patients' medical records for three cycles of chemotherapy. Degree of neutropenia (grade 1: < 2500–1500 mm^3; grade 2: < 1500–1000 mm^3; grade 3: < 1000–500 mm^3; grade 4: life-threatening; grade 5: death). Degree of anemia (grade 1: hemoglobin (Hb) 10 g/dL; grade 2: < 10–8 g/dL; grade 3: < 8 g/dL; grade 4: life threatening; grade 5: death).[17]

The data was analyzed statistically using IBM SPSS. After three cycles of chemotherapy, the trends of neutropenia and anemia were analyzed by using

Table 1: Characteristics 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	
Homemaker	87 (63.0)
Farmer	6 (4.3)
Entrepreneur	9 (6.5)
Teacher	1 (0.7)
Pastor	1 (0.7)
Government employee	34 (24.6)
Body Mass Index	
Underweight	1 (0.7)
Normal	58 (42.0)
Overweight	58 (42.0)
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 (79.0)
invasive lobular carcinoma	15 (10.9)
carcinoma mucinous mammae	1 (0.7)
Chemotherapy	
Anthracycline (doxorubicin)-paclitaxel	71 (51.4)
Cyclophospamide-anthracycline-5 Fluorouracil	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)

Table 2: Trend of absolute neutrophil count decrease for three cycles of chemotherapy.

Absolute neutrophil count mm^3	N	Mean	SD	Minimum	Maximum	*p-value**	*p*-value**
pre chemotherapy	138	5 644.4	2 962.5	1 741	23 890	-	< 0.001
post chemotherapy cycle 1	138	4 073.9	2 813.1	580	19 710	< 0.001	
post chemotherapy cycle 2	138	3 540.4	2 430.0	210	19 870	0.018	
post chemotherapy cycle 3	138	3 034.8	2 049.6	200	9 520	0.025	

*SD: standard deviation. *Wilcoxon signed- rank test; **Friedmann test.*

Table 3: Trend of anemia (Hemoglobin level decreasing) for three cycles of chemotherapy.

Anemia (g/dL)	N	Mean	SD	Minimum	Maximum	*p-value* *	*p-value* **
pre-chemotherapy	138	12.1	1.5	8.00	15.90	-	< 0.001
post chemotherapy cycle 1	138	11.6	1.4	8.40	15.10	< 0.001	
post chemotherapy cycle 2	138	11.4	1.2	8.50	14.10	0.005	
post chemotherapy cycle 3	138	11.2	1.3	6.80	14.60	0.007	

*SD: standard deviation. *Wilcoxon sign rank test; **Friedmann test.*

the Friedmann test and the Wilcoxon sign rank test. The association of ABCB1 (each SNP and haplotype) and GSTP1 with graded neutropenia and anemia was assessed by using the Kruskal-Wallis test, and $p < 0.050$ was considered statistically significant. The evaluation of deviation between allele and genotype frequency was performed using Hardy-Weinberg Equilibrium (HWE), and $p > 0.050$ was considered as no deviation.

RESULTS

Among the N = 138 breast cancer patients who participated in the study, 90 (65.2%) were in the age range = 44–59 years. Nearly half (n = 68, 49.3%) were of Batak ethnicity. Most patients (n = 87, 63.0%) were homemakers. Most (n = 78, 56.5%) had advanced cancer (stage IIIB). The vast majority (n = 109, 79.0%) had been diagnosed with invasive ductal carcinoma, and 67 (48.6%) patients had neutropenia (grades 1 to 3), while 98 patients (71.0%) had anemia (grades 1 to 3). More details are in Table 1.

There was a progressive decrease in absolute neutrophils count (ANC) after each cycle of chemotherapy [Table 2]. 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 $p < 0.050$ (Friedmann test), and for every cycle of chemotherapy the subjects tended to experience a decrease in neutrophils with $p < 0.050$ (Wilcoxon signed-rank test).

The changes in anemia in the subjects were also monitored. Table 3 shows progressive mean Hb decline after each cycle of chemotherapy ($p < 0.050$), falling to the lowest level post-third cycle ($p < 0.050$).

The ABCB1 patterns for each SNP and its haplotype, along with the *GSTP-1* 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 (52.2%) patients. The second and third most common forms were for heterozygote wildtype in 55 (39.9%) patients and homozygote variant (GG) in 11 (8.0%) patients. In ABCB1 C3435T, the form of homozygote wildtype was found in 45 (32.6%) patients, whereas the homozygote variant was present in 26 (18.8%) patients. In ABCB1 C1236T, the most common polymorphism was wildtype heterozygote with 89 (64.5%) patients, and the least common form, found in 12 (8.7%) patients, was the wildtype homozygote (CC). Wildtype heterozygote was present in 72 (52.2%) patients who also had ABCB1 G2677T polymorphism. The homozygote variant was present in 19 (13.8%) patients. In the haplotype ABCB1 form, there was a homozygous variant in the three SNPs (TT-TT-TT), found in 10 (7.2%) patients. The homozygote variant of ABCB1 (3SNPs) and GSTP-1 (1 SNP) genes (TT-TT-TT-TT) was found in only 4 (2.9%)patients . Both these polymorphisms had value of allele distribution frequencies of > 0.050 ($p > 0.050$) which implied no

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

Polymorphism	n (%)	Allele	%	*p*-value (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	43.1	
TT	26 (18.8)			
ABCB1 C1236T				
CC	12 (8.7)	C	40.9	1.,530
CT	89(64.5)	T	59.1	
TT	37 (26.8)			
ABCB1 G2677T				
GG	47 (34.1)	G	60.1	1.070
GT	72 (52.2)	T	39.9	
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 (χ^2).*

deviation of the allele frequencies, based on Hardy-Weinberg Equilibrium (HWE).

The relationships between polymorphisms and the degree of neutropenia were evaluated. Among N = 138 subjects, 71 did not have neutropenia, while the others variously had grade 1 (25.4%), grade 2 (10.9%), and grade 3 (12.3%) neutropenia. 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 ($p > 0.050$).

The relations between GSTP1 and ABCB1 genes polymorphisms to the incidence of anemia is shown in Table 6, which shows 40 (29.%) patients experienced no anemia. Grade 1 anemia was present in 80 (58.0%) patients, grade 2 in 16 (11.6%) patients, while grade 3 was present only in two (1.4%) patient, which indicated no significant relationships of ABCB1 and GSTP1 polymorphisms in individual SNP and haplotype ($p > 0.050$).

DISCUSSION

Based on our study results, the most dominant ethnicity of the breast cancer patients is Batak. This is to be expected as the hospital is in North Sumatra, which has Batak majority. Most breast cancer cases were detected at advanced stages, similar to the trend reported from the Middle Eastern countries such as Oman.[18]

Most patients developed neutropenia and anemia after three cycles of chemotherapy. This finding is in accordance with a previous study, which reported that 50% patients experienced neutropenia in varying degrees and 20% had febrile neutropenia after three cycles of chemotherapy.[19] Our results appear to partly contradict some previous studies that showed that the risk of developing neutropenia was highest in the first cycle of chemotherapy.[20] The trend of neutropenia over three cycles of chemotherapy could also be due to other factors such as poor nutrition and older age (the present study did not analyze this). Hematological toxicity is a common side effect of chemotherapy drugs such as anthracyclines, cyclophosphamide and taxane.[8,21] The chemotherapy drug 5-FU can also cause side effects such as anemia,

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

Gene	Polymorphism	Neutropenia								Total		*p*-value*
		Normal (n = 71)		Grade 1 (n = 35)		Grade 2 (n = 15)		Grade 3 (n = 17)		(n = 138)		
		n	%	n	%	n	%	n	%	n	%	
GSTP-1	AA	31	43.7	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	
ABCB1	C3435T											
	CC	22	31.4	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	
	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	17.1	4	26.7	6	35.3	37	26.8	
	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	8.5	6	17.1	4	26.7	3	17.6	19	13.8	
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	
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	0.0	2	11.8	4	2.9	0.456

**Kruskall- wallis test.*

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

Gene	Polymorphism	Anemia								Total		*p*-value*
		Normal (n = 40)		Grade 1 (n = 80)		Grade 2 (n = 16)		Grade 3 (n = 2)		(n = 138)		
		n	%	n	%	n	%	n	%	n	%	n
GSTP-1	AA	22	55.0	39	48.8	11	68.8	0	0.0	72	52.2	
	AG	15	37.5	33	41.3	5	31.3	2	100	55	39.9	
	GG	3	27.3	8	10.0	0	0.0	0	0.0	11	8.0	
ABCB1	C3435T											0.418
	CC	15	37.5	26	32.5	4	25.0	0	0.0	45	32.6	
	CT	16	40.0	41	51.3	9	56.3	1	50.0	67	48.6	
	TT	9	22.5	13	16.3	3	18.8	1	50.0	26	18.8	
	C1236T											0.711
	CC	3	7.5	8	10.0	1	6.3	0	0.0	12	8.7	
	CT	26	65.0	54	67.5	9	56.3	0	0.0	89	64.5	
	TT	11	27.5	18	22.5	6	37.5	2	100	37	26.8	
	G2677T											0.184
	GG	16	40.0	29	36.3	2	12.5	0	0.0	47	34.1	
	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	
ABCB1	Haplotype											0.149
	Non-TT-TT-TT	39	97.5	73	91.3	15	93.8	1	50.0	128	92.8	
	TT-TT-TT	1	2.5	7	8.8	1	6.3	1	50.0	10	7.2	
GSTP1 + ABCB1	Non variant-variant	40	100.0	78	97.5	15	93.8	1	50.0	134	97.1	0.627
	Variant (GG)-variant (TT-TT-TT)	0	0.0	2	2.5	1	6.2	1	50.0	4	2.9	

**Kruskall-wallis test.*

leukopenia, and thrombocytopenia,[22] these being various expressions of myeloid toxicity. The rapid division rates of myeloid cells render them vulnerable targets of cytotoxic drugs.[14,23,24]

The distribution of allele and genotype of ABCB1 (C1236T, G2677T, and C3435T) among our subjects was in accordance with previous studies and in proportions similar to that of C1236T, G2677T, and C3435T polymorphisms among the Japanese and Chinese populations,[25,26] but differed from the polymorphism distribution found in populations in Russia, Serbia, and Germany.[27,28] Nevertheless, the polymorphism distribution of ABCB1 between Asian and Caucasian populations did not differ significantly.[29] 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] We found no significant relationship between ethnicity and ABCB1 and GSTP1 polymorphisms.

In our study, no relation was found between the ABCB1 and GSTP1 polymorphisms, and anemia and neutropenia. Several previous studies also found no relation between ABCB1 and GSTP1 and neutropenia.[11,13,28,30] Our results were also similar to those of a previous study conducted on 882 patients involved in the SWOG trial S0221 which showed that none of the SNPs of ABCB1 was associated with hematological toxicity.[31] Some previous studies have yielded results at variance with ours, suggesting a lack of association between ABCB1 polymorphism and the incidence of neutropenia and leukopenia (SWOG Trial 2021). Meanwhile Chaturvedi et al,[13] (2013) showed a link between ABCB1 C1236T polymorphism and neutropenia. By contrast, a study has reported that the TT variant in the *ABCB1* gene, which is known to be a factor that 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.[32] The contradictory explanations for the involvement of ABCB1 C3435T, C1236T, and G2677T polymorphism can cause less functional P-GP.[29,33] In this study, most subjects had a non-homozygous variant of haplotype ABCB1 form (92.8%) while the homozygous variant (TT-TT-TT) 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 hematological toxicity might be due to several factors, such as the small number of subjects, the involvement of other genes in the metabolism of taxane and cyclophosphamide, or administration of filgrastim to treat neutropenia.[24] It has also been 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.[34] It is known that there are 16 genes involved in the drug pathway alone, including *ABCC1*, *ABCC2*, *CYP3A5*, *MAPT*, *TP53*, and *XRCC1*.[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 also genes *CBR1*, *CBR3*, *AKR1A1,* and *AKR1C3*, which are involved in phase 1 of drug biotransformation.[24]

Apart from the polymorphisms, the epigenetic factors—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 chemotherapy response.[35] Also to be considered are non-genetic factors relevant to the clinical response to treatment, such as diet and lifestyle, chemotherapy, and inter-drug interactions.[24] Healthy food habits, such as microbiota-friendly diet, very low ketogenic diet, and Mediterranean and Japanese diets, may help suppressing the specific reactive oxygen and nitrogen species which affect epigenetic modification. However, their effects on direct hematological toxicity have not been proven yet.[36] Obesity, unhealthy diet, smoking, and inadequate physical activity contribute significantly reducing cancer patient survival and clinical response to chemotherapy.[37]

The present study found all forms of homozygous, heterozygous variants in *ABCB1* and *GSTP1* genes, but no relationship between ABCB1 and GSTP1 polymorphisms and 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 factors linked to hematological toxicity post chemotherapy.

There are limitations in our study that need to be considered in interpreting our results. First, we selected only two genes that affect 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 non-genetic factors such as diet, physical activity, and 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 by this study. However, no association was found between ABCB1 and GSTP1 polymorphisms on hematological toxicity.

Disclosure

The authors declared no conflicts of interest. This research is funded by the Research Institute of Universitas Sumatera Utara.

Acknowledgements

We thank the Universitas Sumatera Utara Research institute committee for their tremendous support. We also acknowledge the support received from Dr. Kamal Basri Siregar (surgical oncologist) and Ms. Mardiah Nasution (laboratory technician).

REFERENCES

1. World Health Organization. Cancer country profile. [cited 2021 Mar 10]. Available from: http://www.who.int/cancer/country-profiles/en/.
2. World Health Organization. Source: Globocan 2020. [cited 2021 Mar 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 Med J 2019 Jul;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. Hematologic adverse effects following systemic chemotherapy. J Oncol Med &Pract 2017;2(1):110.
6. Remesh A. Toxicities of anticancer drugs and its management. Int J Basic Clin Pharmacol 2012;1(1):2-12 .
7. Leong SL, Chaiyakunapruk N, Lee SW. Candidate gene association studies of anthracycline-induced cardiotoxicity: a systematic review and meta-analysis. Sci Rep 2017 Feb;7(1):39.
8. Schneider BP, Shen F, Gardner L, Radovich M, Li L, Miller KD, et al. Genome-wide association study for anthracycline-induced congestive heart failure. Clin Cancer Res 2017 Jan;23(1):43-51.
9. González-Neira A. Pharmacogenetics of chemotherapy efficacy in breast cancer. Pharmacogenomics 2012 Apr;13(6):677-690.
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):118-126 .
11. Tulsyan S, Chaturvedi P, Agarwal G, Lal P, Agrawal S, Mittal RD, et al. Pharmacogenetic influence of GST polymorphisms on anthracycline-based chemotherapy responses and toxicity in breast cancer patients: a multi-analytical approach. Mol Diagn Ther 2013 Dec;17(6):371-379.
12. Hodges LM, Markova SM, Chinn LW, Gow JM, Kroetz DL, Klein TE, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). Pharmacogenet Genomics 2011 Mar;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 Oct;37(5):754-761.
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 May;37(5):2633-2639.
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 Conf Ser Earth Environ Sci 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. Biol Med (Aligarh) 2010;2(4):17-21.
17. Cancer therapy evaluation program: common terminology criteria for adverse events (CTCAE) v5.0. [cited 2021 Feb 20]. Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf.
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 experience from a university hospital. Oman Med J 2019 Sep;34(5):412-419.
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 Mar;9:158.
20. Ozer H. The timing of chemotherapy-induced neutropenia and its clinical and economic impact. Oncology (Williston Park) 2006 Apr;20(5)(Suppl 4):11-15.
21. Frederiks CN, Lam SW, Guchelaar HJ, Boven E. Genetic polymorphisms and paclitaxel- or docetaxel-induced toxicities: A systematic review. Cancer Treat Rev 2015 Dec;41(10):935-950.
22. Shajahan J, Pillai PS, Jayakumar KN. 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 Dec;9(12):FC01-FC06.
23. Kurtin S. Myeloid toxicity of cancer treatment. J Adv Pract Oncol 2012 Jul;3(4):209-224.
24. Al-Mahayri ZN, Patrinos GP, Ali BR. Toxicity and Pharmacogenomic Biomarkers in Breast Cancer Chemotherapy. Front Pharmacol 2020 Apr;11:445. .
25. Taheri M, Mahjoubi F, Omranipour R. Effect of MDR1 polymorphism on multidrug resistance expression in breast cancer patients. Genet Mol Res 2010 Jan;9(1):34-40.
26. Milojkovic M, Stojnev S, Jovanovic I, Ljubisavljevic

S, Stefanovic V, Sunder-Plassman R. Frequency of the C1236T, G2677T/A and C3435T MDR1 gene polymorphisms in the Serbian population. Pharmacol Rep 2011;63(3):808-814.

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 Dec;244(2):121-124.
28. Cizmarikova M, Wagnerova M, Schonova L, Habalova V, Kohut A, Linkova A, et al. MDR1 (C3435T) polymorphism: relation to the risk of breast cancer and therapeutic outcome. Pharmacogenomics J 2010 Feb;10(1):62-69.
29. Balram C, Sharma A, Sivathasan C, Lee EJ. Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic-genotypic correlates. Br J Clin Pharmacol 2003 Jul;56(1):78-83. .
30. 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 Dec;11(12):e0168519.
31. Yao S, Sucheston LE, Zhao H, Barlow WE, Zirpoli G, Liu S, 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):241-247.
32. Reed K, Parissenti AM. The effect of ABCB1 genetic variants on chemotherapy response in HIV and cancer treatment. Pharmacogenomics 2011 Oct;12(10):1465-1483.
33. Farhat K, Waheed A, Azhar H, Pasha AK, 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.
34. Tulsyan S, Mittal RD, Mittal B. The effect of ABCB1 polymorphisms on the outcome of breast cancer treatment. Pharmgenomics Pers Med 2016 Apr;9:47-58.
35. Lv JF, Hu L, Zhuo W, Zhang CM, Zhou HH, Fan L. Epigenetic alternations and cancer chemotherapy response. Cancer Chemother Pharmacol 2016 Apr;77(4):673-684.
36. 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;16(1):75.
37. Vijayvergia N, Denlinger CS. Lifestyle factors in cancer survivorship: where we are and where we are headed. J Pers Med 2015 Jul;5(3):243-263.