JETROMI
Journal of Endocrinology, Tropical Medicine, and Infectiouse Disease
Journal homepage: https://jetromi.usu.ac.id

THE RELATIONSHIP BETWEEN SLC22A16 GENE POLYMORPHISM AND HEMATOLOGICAL TOXICITY IN BREAST CANCER PATIENTS RECEIVING DOXORUBICIN-BASED CHEMOTHERAPY

Sri Melinda Kaban [*1], Siti Syarifah [1], Yunita Sari Pane [1], Tri Widyawati [1]

[1]*Departement of Pharmacology and Therapeutic, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*

[*]Corresponding Author: sri.melinda@usu.ac.id

ARTICLE INFO

Article history:
Article history:
Received 14 October 2024
Revised 08 January 2025
Accepted 08 January 2025
Available online xxx

E-ISSN: 2686-0856
P-ISSN: 2686-0872

How to cite:
Sri Melinda Kaban (2025). The Relationship Between SLC22A16 Gene Polymorphism And Hematological Toxicity In Breast Cancer Patients Receiving Doxorubicin-Based Chemotherapy. Journal of Endocrinology, Tropical Medicine, and Infectious Disease (JETROMI) Vol.07, No.01 (2025)
27-33. (make in IEEE style)

10.32734/jetromi.v7i1.18520

ABSTRACT

Background: Drug transporter polymorphisms are widely associated with the risk of toxicity in many chemotherapy drugs for cancer. This paper aims to assess the relationship of polymorphisms of this transporter, namely SLC22A16, which are associated with side effects of Doxorubicin-based chemotherapy drugs, namely hematological toxicity (anemia, neutropenia, leukopenia, and thrombocytopenia) in breast cancer patients.
Methods. This cross-sectional study will be conducted by testing polymorphism using the ARMS PCR method, which will assess the distribution of AA, AG, and GG genotypes.
Results. Most patient ages are under 50 years (55%), with overweight BMI (41,7%) and Batak ethnicity (43,3%). There is no relationship between SLC22A16 Gene Polymorphism and Hematological Toxicity in Breast Cancer Patients Receiving Doxorubicin-Based Chemotherapy.

Keyword: SLC22A16, Polymorphism, Breast Cancer, Doxorubicin, Hematology Toxocity

ABSTRAK

Latar belakang *Polimorfisme transporter obat banyak dikaitkan dengan risiko toksisitas pada banyak obat kemoterapi pada kanker. Tulisan ini bertujuan untuk menilai hubungan polimorfisme transporter yaitu SLC22A16 yang dikaitkan dengan efek samping obat kemoterapi berbasis Doksorubisin yaitu toksisitas hematologi (anemia, neutropenia,leukopenia dan trombositopenia).*
Metode*. Penelitian potong lintang ini akan dilakukan dengan pengujian polimorfisme dengan menggunakan metode ARMS PCR dimana akan dinilai distribusi genotip AA,AG, dan GG..*
Hasil*. Usia pasien terbanyak adalah dibawah 50 tahun (55%), dengan BMI overweight (41,7%) dan suku Batak (43,3%). Tidak terdapat hubungan antara Polimorfisme Gen SLC22A16 dengan Toksisitas Hematologi pada Pasien Kanker Payudara yang Mendapat Kemoterapi Berbasis Doksorubisin.*
Kesimpulan. *Tidak terdapat hubungan antara polimorfisme SLC22A16 pada kejadian toksisitas hematologi pada pasien kanker payudara yang mendaptakan kemoterapi berbasis Doksorubisin pada populasi kota Medan.*

Keyword: *SLC22A16,Polimorfisme, Kanker Payudara, Doksorubisin, Toksisitas Hematologi*

1. Introduction

Breast cancer is the most commonly diagnosed cancer globally. Breast cancer accounts for 1 in 4 cancer cases among women and is the leading cause of cancer death in women. An estimated. Million new cases indicate that one in every 10 cancers diagnosed in 00 will be breast cancer [1]. Female breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated .3 million new cases (11.7%), followed by lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach (5.6%) cancers [2]. Even in Indonesia, a low-middle-income country, breast cancer is the most common cancer diagnosed in women [3].

Breast cancer treatment involves three main modalities: surgery, radiation, and chemotherapy. One of the most commonly used chemotherapy drugs for breast cancer treatment is the Anthracycline class, namely Doxorubicin. Anthracyclines, including doxorubicin, epirubicin, daunorubicin, and idarubicin, are considered the most potent chemotherapeutics used in many types of solid tumors and leukemia. In addition to their cardiac effects, hematological toxicity, gastrointestinal toxicity, and the incidence of febrile neutropenia are all dose-limiting side effects of anthracyclines [4].

Hematologic toxicity is the most common side effect of cytotoxic agents. In a study of 85 Chinese breast cancer patients receiving adjuvant chemotherapy with doxorubicin and cyclophosphamide, grade 3 and 4 neutropenia were found to be higher compared to Caucasians [5]. Doxorubicin is known to have side effects of myelosuppression such as anemia, leukopenia, thrombocytopenia, and even neutropenia are all forms of myeloid toxicity. Neutropenia can cause dangerous immune system suppression, especially in cases of infection, and become a hematological toxicity that is quite life-threatening, especially in cancer patients. These "cytopenias" are the most common toxicity encountered due to chemotherapy. This manifestation can be explained by the rapid mitotic rate of myeloid cells, which makes them vulnerable to cytotoxic agents. While lymphopenia is less common,

Previous studies have not found any association between ABCB1 and GSTP1 polymorphisms with the incidence of anemia and neutropenia in patients receiving chemotherapy [7]. However, an association was found between AA homozygotes compared to AG and GG in the GSTP1 gene polymorphism with febrile neutropenia in the Japanese population [8]. In another study, it was found that polymorphisms in the CYP2B6 and ERCC1 genes were studied concerning the occurrence of grade 4 neutropenia in breast cancer patients who received chemotherapy that was also doxorubicin-based [9]. However, there are many types of polymorphisms that are suspected of being associated with the incidence of anemia and neutropenia in patients receiving chemotherapy including Doxorubicin, one of which is the SLC22A16 gene which is a protein-coding gene.

There are 3 steps of anthracycline drug metabolism including doxorubicin (DOX-ol), daunorubicin (DNR-ol), and epirubicin (EPI-ol). First, promote the organic cation transporter SLC22A16 of anthracycline drugs into cells, then metabolized into secondary alcohols via cytoplasmic NADPH-dependent carbonyl (Carbonyl reductase, CBR) and aldehyde-ketone (Aldo-keto reductase, AKR) reductase. Polymorphisms of genes involved in anthracycline pharmacokinetics (including AKR1A1 rs2088102, CBR1 rs20572, ABCG2 rs2231142, SLC22A16 rs6907567) are associated with myelosuppression in breast cancer patients with anthracycline-based drug therapy including doxorubicin. Drug metabolizing enzymes with gene polymorphisms alter pharmacokinetics including drug absorption, detoxification, and excretion [10].

Polymorphisms in SLC22A16 gene are suspected of influencing the incidence of hematological toxicity in Doxorubicin administration where SLC22A16 is related to the transport of inorganic cations/anions and amino acids/oligopeptides as well as the pharmacokinetics of the Doxorubicin pathway. This study will examine whether there is a relationship between SLC22A16 (rs12210538A/G) polymorphism with the incidence of hematological toxicity in breast cancer patients receiving Doxorubicin chemotherapy.

2. Methods

This study was conducted on 60 breast cancer patients who received doxorubicin-based chemotherapy. This is a descriptive-analytical research in which samples were selected from the DNA of breast cancer patients stored in the Terpadu Laboratory of the Faculty of Medicine of Universitas Sumatera Utara. Sample selection was carried out using a non-probability method, the purposive sampling method which used DNA samples that met the inclusion and exclusion criteria. The selected DNA will be examined for SLCC22A16 polymorphism (rs12210538A) using the ARMS PCR method. The final concentration for PCR is as follows.

Reagen	Volume (uL)
ddH2O	17,3
PCR Buffer(10×)	2.5
$MgCL_2$ (50mM)	1
dNTPs (10 mM)	0.5
Wild Forward Primer (10 uM)	0.8
Mutant Forward Primer (10uM)	0.8
Common Reverse Primer (10 uM)	0.8
DNA (600 ng)	1
Taq DNA polymerase (5 U/uL)	0.3
Total volume	25

After the final concentration is made, the mixture will be inserted into the PCR device and will be processed using the following method. The PCR program was as follows: 1 cycle: first denaturation at 95˚C for 5 minutes; 35 cycles: denaturation at 95˚C for 1 minute, annealing at 59˚C for 50 seconds, and extension at 72˚C for 50 seconds; and 1 cycle: final extension at 72˚C for 5 minutes. The PCR products were then separated by electrophoresis on 1.5% agarose gel at 80–100 V for 40–50 minutes. The gel was stained with ethidium bromide and visualized. To assess the accuracy of the ARMS-PCR primers, 3 PCR products representing wild homozygote, heterozygote, and homozygote mutant of each polymorphism studied were sequenced. In electrophoresis, a band will be seen at 215 bp as a control, and 581bp if polymorphism is found.

3. Results

Based on Table 1, a total of 60 samples were examined in this research. Secondary data has been collected in previous research and distributed as in the table below. From secondary data in previous studies, the samples were distributed based on age, body mass index, and ethnicity. The selection of secondary data was carried out because this data was still complete. From Table 1 it can be seen that the majority of ages are under 50 years (55%), with overweight BMI (41,7%) and Batak ethnicity (43,3%).

Table 1 Sample Characteristic Distribution

Characteristic	**Frekuensi (%)**
Age (yr)	
< 50	27 (45)
> 50	33(55)
BMI (kg/m^2)	
Underweight (<18.5)	1 (1.7)
Normoweight (18.5-22.9)	23(38.3)
Overweight (23-29.9)	25(41.7)
Obese (30<)	11(18.3)
Ethnic group	
Batak	26 (43.3)
Aceh	7 (11.7)
Java	24 (40)
Padang	3 (5)

Based on Table 2, it was found that the most frequently found genotypes in the sample were allele A and genotype AA. The Hardy-Weinberg Equilibrium (HWE) test was performed on the samples using the χ^2 test to see the differences between the frequencies of the genotypes studied. From Table 2 it can be seen that the population in this study is in line with the HWE balance where the p-value> 0.05.

Table 2 Distribution and Hardy Weinberg Equilibrium

	Frequency	**%**	**HWE**
Allele			**1,0068**
A	97	80,8	**(>0,05)**
G	23	19,2	
Total	120	100	
Genotype			
AA	38	63,3	
AG	21	35	
GG	1	1,7	
Total	60	100	

Based on Table 3, it can be found that hematological toxicity occurred in breast cancer patients who received doxorubicin-based chemotherapy. Although hematological toxicity was found in breast cancer patients receiving doxorubicin-based chemotherapy, based on statistical analysis there was no relationship between hematological toxicity and SLC22A16 polymorphism in breast cancer patients in this population (p>0,05). Table 3 shows that the incidence of hematological toxicity such as anemia, neutropenia, leukopenia, and thrombocytopenia, is not associated with the SLC22A16 polymorphism (rs12210538 A/G (p>0,05).

Table 3 Distribution of Polymorphism SLC22A16 and their relationship to hematological toxicity

Parameter		AA	%	AG+GG	%	*P*
Hb	Normal	14	23	7	12	0.694[a]
	Low	24	40	15	25	
	Total	38	63	22	37	
Neutrophil	Normal	24	40	16	27	0.449[a]
	Low	14	23	6	10	
	Total	38	63	22	37	
Leukocite	Normal	30	50	13	22	0.100[a]
	Low	8	13	9	15	
	Total	38	63	22	37	
Trombocite	Normal	36	60	21	35	0.902[a]
	Low	2	3.3	1	1.7	
	Total	38	63.3	22	36.7	

[a]Chi square test

4. Discussions

Currently, more sciences are studying the relationship between polymorphisms and various diseases, especially infectious diseases and oncology. This relationship is related to drug susceptibility and response including drug side effects. The relationship with susceptibility, for example, is related to polymorphisms in the interleukin gene, glutathione peroxidase (GPX1), TNF alpha, vitamin D receptor (VDR), toll-like receptor, interferon-gamma, and others. Meanwhile, drug response is often associated with polymorphisms in the ATP Binding Cassette (ABC), Solute Carrier (SLC) transporter gene, ABCC1, ABCC2, CYP3A5, MAPT, TP53, XRCC1, and others. One of the most frequently performed polymorphism studies in oncology is polymorphisms related to the drug transporter Solute Carrier (SLC) transporter gene [8,12,13].

The SLC22 transporter plays a critical role in moving small molecule endogenous metabolites, drugs, and toxins (exogenous and endogenous) between interacting tissues and body fluids (e.g., renal proximal tubule, hepatocytes, choroid plexus). This transporter is one of the most studied SLC families concerning drugs. The SLC22 family can be divided into at least six subclades or subfamilies and SLC22A16 belongs to the

OCT/OCTN-related class [14]. The SLC22A family of transporters share a similar membrane topology: 12 α-helical transmembrane domains, a large extracellular loop between domains 1 and 2, and a large intracellular loop between domains 6 and 7 [10].

Among the SLC22A16 polymorphisms, rs12210538 is located in domain 8. In addition, SLC22A16 may have profound adverse effects on hematopoiesis through doxorubicin accumulation, as the expression of the gene is restricted to hematopoietic cells which may lead to hematological toxicity [15]. SLC22A16 is a gene that acts as a mediator of doxorubicin absorption in cancer cells, so if there is a polymorphism in this gene, it will affect the absorption of this drug and the risk of [16]. Drug metabolizing enzymes with gene polymorphisms alter pharmacokinetics including drug absorption, detoxification, and drug excretion [10].

Carriers of the SLC22A16 rs12210538 allele were observed to have an increased incidence of leukopenia but no difference in survival. Expression of the SLC22A16 gene in cancer cells is associated with increased cytotoxicity of doxorubicin. Patients with the variant genotype may have greater uptake of doxorubicin into normal and tumor cells, which may result in greater toxicity. 20–25% of Caucasians are known to carry the variant, while the frequency of the allele in Africans is estimated to be 38% [17]. In the study by Lal et al the relationship between this gene and the occurrence of hematological toxicity in breast cancer patients receiving doxorubicin was not analyzed, but 4 polymorphisms were found in the SLC22A16 gene [18]. In this study, identification was carried out in breast cancer patients who received doxorubicin and searched for SLC22A16 gene polymorphism (rs12210538 A/G). This polymorphism is a non-synonymous polymorphism that replaces methionine with threonine at position 409 [11].

Although SLC22A16 polymorphism was not statistically associated with the incidence of hematological toxicity in breast cancer patients receiving doxorubicin-based chemotherapy, it appears that the incidence of hematological toxicity is more common in patients with AA homozygous genotype compared to AG heterozygotes and GG homozygotes. This result is similar to the study in Iran which also found no association between this gene and the incidence of neutropenia in the Iranian population [11].

A study in Poland found that AA homozygotes in one of the SLC22A16 polymorphisms (rs6907567) were shown to increase the risk of neutropenia in breast cancer patients receiving chemotherapy -fluorouracil, doxorubicin, and cyclophosphamide by 3-fold (OR 3.15; 95% CI 1.00–9.92; p = 0.049), together with the A polymorphic allele of the CYP2C19 variant p.Pro227= (rs4244285), although the strongest factor for recurrent neutropenia was the rare AA homozygote of the ERCC1 c.1510C>A polymorphism (rs3212986) [19]. Patients with reduced CYP2D6 activity, as a result of either their genotype or induction by the coadministration of other drugs that inhibit CYP2D6 function, produce little endoxifen and hence derive limited therapeutic benefit from tamoxifen; the same can be said about the different classes of therapeutics in breast cancer. PG studies of breast cancer therapeutics should provide patients with breast cancer with optimal and personalized therapy [20].

5. Conclusions

Although statistically, there was no association between the SLC22A16 polymorphism and the incidence of myelosuppression in breast cancer patients who received doxorubicin chemotherapy, from this study it can be seen that administration of this drug has the side effect of myelosuppression, including anemia, neutropenia, leukopenia and thrombocytopenia. In addition, no identification of other gene polymorphisms was carried out, which may be related to the occurrence of myelosuppression in breast cancer patients who received doxycycline chemotherapy either alone or in combination. Further research is needed to determine the cause of this myelosuppressive effect, whether it is related to other drug transporter polymorphisms or other genes related to the pharmacology of doxorubicin.

Competing interests

The authors declare no conflict of interest in this research

Acknowledgments

The author would like to express gratitude to all patients, lecturers, and laboratory staff, Mrs. Winda who have helped in carrying out this research. In particular, thank you to the Faculty of Medicine Universitas Sumatera Utara for funding this research.

References

[1] Hyuna Sung, Jacques Ferlay, Rebecca L. Siegel, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal et al: Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries CA Cancer J Clin 2021;71:209-249. https://doi.org/10.3322/caac.21660

[2] Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. Global Cancer Statistics 2020: Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 2021;*71*(3), 209–249. https://doi.org/10.3322/caac.21660

[3] Solikhah, S., Promthet, S., & Hurst, C. Awareness level about breast cancer risk factors, barriers, attitude, and breast cancer screening among Indonesian woman. *Asian Pacific Journal of Cancer Prevention*, 2019;*20*(3: 877–884. https://doi.org/10.31557/APJCP.2019.20.3.877

[4] Al-Mahayri, Z. N., Patrinos, G. P., & Ali, B. R.. Toxicity and Pharmacogenomic Biomarkers in Breast Cancer Chemotherapy. In *Frontiers in Pharmacology* 2020; Vol. 11. Frontiers Media S.A. https://doi.org/10.3389/fphar.2020.00445

[5] Ma, B., Yeo, W., Hui, P., Ho, W. M., & Johnson, P. J. *Acute toxicity of adjuvant doxorubicin and cyclophosphamide for early breast cancer retrospective review of Chinese patients and comparison with a historic Western series*. 2002. www.elsevier.com/locate/radonline.

[6] Smith, R., Associates, C. O., Smith, R. E., & Carolina, S. Trends in Recommendations for Myelosuppressive Chemotherapy for the Treatment of Solid Tumors. In *Journal of the National Comprehensive Cancer Network* 2006; Vol. 4. www.cancer.gov/clinical_trials

[7] Kurtin, S. Myeloid Toxicity of Cancer Treatment. J. Adv. Pract. Oncol. 2012; 3(4), 209– 224. Doi: 10.1111/bjh.1507

[8] Syarifah, S., Widyawati, T., Hasni, D., Sari, M. I., Rusdiana, & Hamdi, T. 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. Oman Medical Journal, 2022;37(2). https://doi.org/10.5001/omj.2022.36

[9] Sugishita, M., Imai, T., Kikumori, T., Mitsuma, A., Shimokata, T., Shibata, T.et al. Pharmacogenetic association between GSTP1 genetic polymorphism and febrile neutropenia in Japanese patients with early breast cancer. *Breast Cancer*, 2016;*23*(2), 195–201. https://doi.org/10.1007/s12282-014-0547-x

[10] Cui, L., Huang, J., Zhan, Y., Qiu, N., Jin, H., Li, J., Huang, H., & Li, H. Association between the genetic polymorphisms of the pharmacokinetics of anthracycline drug and myelosuppression in a patient with breast cancer with anthracycline-based chemotherapy. Life Sciences, 2021;276. https://doi.org/10.1016/j.lfs.2021.119392

[11] Faraji, A., Manshadi, H. R. D., 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. In PLoS ONE 2016; Vol. 11(12). Public Library of Science. https://doi.org/10.1371/journal.pone.0168519

[12] Ikeda, M., Tsuji, D., Yamamoto, K., Kim, Y. il, Daimon, T., Iwabe, Y., et al.. Relationship between ABCB1 gene polymorphisms and severe neutropenia in patients with breast cancer treated with doxorubicin/cyclophosphamide chemotherapy. Drug Metabolism and Pharmacokinetics, 2015;30(2), 149–153. https://doi.org/10.1016/j.dmpk.2014.09.009

[13] Kaban, S.M., Sinaga, B.Y.M., & Nasution, T.A. Association of polymorphism +874 t/a interferon-gamma gene with susceptibility to pulmonary tuberculosis in Medan, Indonesia. Foundation SPIROSKI, Skopje, Republic of Macedonia Open Access Macedonian Journal of Medical Sciences. 2021 Aug 09; 9(A):515-519.https://doi.org/10.3889/oamjms.2021.6480

[14] Nigam, S. K. *The SLC22 Transporter Family: A Paradigm for the Impact of Drug Transporters on Metabolic Pathways, Signaling, and Disease*. https://doi.org/10.1146/annurev

[15] Okabe, M., Unno, M., Harigae, H., Kaku, M., Okitsu, Y., Sasaki, T., et al. Characterization of the organic cation transporter SLC22A16: A doxorubicin importer. *Biochemical and Biophysical Research Communications*, 2005;*333*(3), 754–762. https://doi.org/10.1016/j.bbrc.2005.05.174

[16] Takeuchi, A., Oguri, T., Fukuda, S., Sone, K., Kagawa, Y., Uemura, T., Takakuwa, O., Maeno, K., Fukumitsu, K., Kanemitsu, Y., Tajiri, T., Ohkubo, H., Takemura, M., Ito, Y., & Niimi, A. (2020). Variants of SLC22A16 predict the efficacy of platinum combination chemotherapy in

advanced non-small-cell lung cancer. *Anticancer Research*, *40*(8), 4245–4251. https://doi.org/10.21873/anticanres.14426

[17] Nthontho, K. C., Ndlovu, A. K., Sharma, K., Kasvosve, I., Hertz, D. L., & Paganotti, G. M. (2022). Pharmacogenetics of Breast Cancer Treatments: A Sub-Saharan Africa Perspective. In *Pharmacogenomics and Personalized Medicine* (Vol. 15, pp. 613–652). Dove Medical Press Ltd. https://doi.org/10.2147/PGPM.S308531

[18] Lal, S., Wong, Z. W., Jada, S. R., Xiang, X., Shu, X. C., Ang, P. C. S et al Novel SLC22A16 polymorphisms and influenceon doxorubicin pharmacokinetics in Asian breast cancer patients. *Pharmacogenomics, 8(6):* 567–575. https://doi.org/10.2217/14622416.8.6.567

[19] Tecza, K., Pamula-Pilat, J., Lanuszewska, J., Butkiewicz, D., & Grzybowska, E. Pharmacogenetics of toxicity of 5-fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer patients. In *Oncotarget* 2018;Vol.9(10). www.impactjournals.com/oncotarget.

[20] Sarah Rofaiel, Esther N Muo, Shaker A Mousa. Pharmacogenetics in breast cancer: steps toward personalized medicine in breast cancer management. Pharmacogenomics and Personalized Medicine 2010:3 129–143