Relation of polymorphism C1236T and C3435T in ABCB1 gene with bone marrow suppression in chemotherapy-treated breast cancer patients

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Abstract. ABCB1 is a gene that encoded P-glycoprotein (P-gp), a transmembrane active efflux pump for a variety of carcinogens and cytostatics. ABCB1 polymorphisms C1236T and C3435T contribute to the variability of therapeutic outcome and side effects. The present study was conducted to investigate the relation of C1236T and C3435T polymorphisms in ABCB1 gene with bone marrow suppression in breast cancer patients treated with chemotherapy. The frequencies of ABCB1 C1236T genotype for homozygous CC, heterozygous CT and variant TT was 11(15.28%), 42(58.33%), 19(26.39%), respectively. No association was between ABCB1 C1236T and C3435T polymorphisms in both individually and haplotypes with bone marrow suppression event (p > 0.05). There was no specific deviation of allele and genotype frequency from Hardy-Weinberg Equilibrium. There was a linkage between heterozygous CT-heterozygous CT in position 1236 and 3435 within 25 people (35%).

1. Introduction

Breast cancer is the second most frequent cancer experienced by women in both well developed and developing countries with the number of newly diagnosed cases being 1.7 million women in the world.[1] In Indonesia, breast cancer has an incidence rate of 26 per 100,000 adult women and breast cancer ranked first in hospitalized patients in all hospitals in Indonesia with a percentage of 16.86%.[2]

The administration of chemotherapy as one of the important therapy in the management of breast cancer patients, not only increased life expectancy but also cause various side effects. One of the riskiest side effects of chemotherapy is bone marrow suppression that can be by measuring absolute neutrophil...
count (ANC), leukocyte, and platelet counts. Neutropenia, leukocytopenia, and thrombocytopenia may increase infection risk and delay chemotherapy outcome.[3]

The presence of genetic polymorphisms in the ABCB1 C1236T (rs1128503) gene in exon 12 and C3435T (rs1045642) in exon 26 which encoded P-glycoprotein (P-gp) was suspected to be associated with the increased of bone marrow suppression risk due to chemotherapy administration. P-gp is a transporter protein mainly expressed in bone marrow and peripheral leukocytes, that acts as an active effluent pump for various toxins including carcinogens and medicines such as anthracyclines, taxan, and vincaalkaloids. The ABCB1 gene encoded a P-gp, the polymorphism of ABCB1 gene could indicate a change in the function and structure of P-gp. The most common ABCB1 gene polymorphisms are synonymous C3435T polymorphism (Ile1145Ile) and synonymous C1236T polymorphism (Gly412Gly). The single nucleotide polymorphism (SNP) on C3435T is known to have a linkage with SNP at C1236T.[4,5]

Sissung et al.[6] showed that the homozygous variant (TT) C3435T had 1.5 times decreased in the absolute number of neutrophils count lower than CC group. It is contrary to some previous studies that reported there was no association between the C3435T polymorphism and the incidence of neutropenia in cancer patients.[7] Although ABCB1 polymorphisms that include C1236T and C3435T have been widely investigated in pharmacological studies, limited studies have sought to link the ABCB1 gene polymorphism to the incidence of bone marrow suppression in breast cancer patients. Therefore, we evaluated the relationship between SNP C3435T and C1236T both individually and in haplotypes with the incidence of bone marrow suppression in breast cancer patients treated with anthracycline-based chemotherapy. The results of this study are expected to provide pharmacogenomics role against drug response.

2. Method
As many as 72 Indonesian women who met the inclusion criteria were included in the study. Protocol of study has been approved by Medical Ethics Committee Universitas Sumatera Utara (No.379/TGL/FK/KEPK FK USU-RSUP HAM/2017). The isolated DNA samples were amplified using the PCR method.[1,6] DNA amplification of Gen ABCB1 C1236T using GoTag® Green Master Mix (Promega) of 12.5 μl, a primer of exon forward 12 ABCB1 5'-GCCACAGTCTGGCCACTC-3' and reverse exons 12 ABCB1 5'-CCCATCGAAAAGAAATTAAG-3', respectively 1 μl, nuclease-free water as much as 7.5 μl and 3 μl DNA with final volume is 25 μl. The amplification process is based on an initial denaturation stage of 5 min at 94°C, followed by a 30-second denaturation step at 94°C at 35 cycles, annealing stage for 30 seconds at 56°C, extension stage for 45 seconds at 72°C and elongation at 72°C for 10 minutes.[8]

The analysis process of SNP ABCB1 C1236T and C3435T washy by thePCR-RFLP method. For SNP ABCB1 C1236T analysis, 5 μl of PCR product was digested by 1 unit of restriction enzyme HaeIII (Promega), incubated at 37°C for 1 hour. Digested products were separated by 4% agarose gel electrophoresis for 60 min at 90 mV and analyzed after staining with Ethidium Bromide under UV light. The electrophoresis pattern showed two bands (250 bp, 22 bp) for homozygous CC, two bands (272 bp and 250 bp) for heterozygote CT genotype and one band (272 bp) for homozygote TT variant. Data on neutropenia, leukocytopenia and thrombocytopenia will be classified according to Common Terminology and Criteria of Adverse Events v.4.0 (CTCAE).[9]

Statistical analysis was performed by applying IBM SPSS ver.23.0, kolmogorov-Smirnov test was performed to analyze the relation between ABCB1 polymorphism with neutropenia, leukocytopenia and thrombocytopenia grading. Values of p<0.05 were considered statistically significant. The frequency distribution of alleles and genotypes was calculated by Hardy-Weinberg Equilibrium.

3. Results and Discussions
Results and discussion will be discussed in the 3 subsections of allele frequencies and the genotype of ABCB1 polymorphism, linkage polymorphism ABCB1 gene at positions 3435 and 1236 and ABCB1 polymorphism relationship to bone marrow suppression.
3.1. Frequency of allele and genotype of ABCB1 polymorphism

Characteristics of subjects have been described in previous study.[10] The allele and genotype frequencies of ABCB1 C1236T and C3435T polymorphisms were shown in Table 1. In Table 1, it could be seen that homozygous CC, heterozygous CT and TT variants were present in both C1236T and C3435T. The genotype frequency of ABCB1 for both C1236T and C3435T in this study has a proportion similar to the proportion of C1236T and C3435T polymorphisms in Japanese and Chinese [11] but has different proportions with population in Russia, Serbia and Germany.[12]

The T allele frequencies tend to be higher in C1236T but C allele frequencies are higher at C3435T. Based on the distribution of polymorphism, the frequency of alleles and genotype in this study more closely related to Asian populations than Caucasians. In this study, p > 0.05 showed that there was no significant genotype and allele frequency deviation based on Hardy-Weinberg Equilibrium.

Table 1. The frequency of allele and genotype of ABCB1 gene polymorphism.

| Polymorphism       | Genotype | n (%)  | Allele | (%)  | Hardy-Weinberg p |
|--------------------|----------|--------|--------|------|------------------|
| rs1128503(C1236T)  | CC       | 11(15.28) | C     | 44.0 | 0.124            |
|                    | CT       | 42 (58.33) | T     | 56.0 |                  |
|                    | TT       | 19 (26.39) |       |      |                  |
| rs1045642(C3435T)  | CC       | 22 (30.6)  | C     | 57.1 | 0.409            |
|                    | CT       | 38 (52.7)  | T     | 42.9 |                  |
|                    | TT       | 12 (16.7)  |       |      |                  |

Electrophoresis pattern of ABCB1 C1236T polymorphism with the PCR-RFLP method can be seen in figure 1 below.

![Electrophoresis pattern](image)

Figure 1. ABCB1 C1236T polymorphism electrophoresis pattern analyzed by PCR-RFLP method for C1236T: CC genotype (column C, D), CT genotype (column E, F), TT genotype (column G, H, I) A (Marker, 50bp DNA Ladder), B (uncut PCR product).

3.2. Linkage of ABCB1 gene polymorphism at positions 3435 and 1236

The present study found that SNP C3435T in exon 26 linked with SNP C1236T in exon 12, this was particularly evident in the linkage between heterozygous CT-heterozygous CT within 25 people (35%) were shown in Table 2. However, it should be alinkage between the wild-type-wild type sequence and the sequence of variants in this position was incomplete and may occur independently. It is in line with the result of Milojkovic et al [12] study which suggested that linkage between wild-type and variant may occur independently. However, the results of the present study contradicted with the study of Erdelyi et al [13] that showed there was a linkage between wild type-wild type and variant in which the subject of research was dominated by haplotype 1236CC-3435CC and 1236TT-3435TT in the Caucasian population. The difference in these results, presumably caused by the differences in the study population in which the ABCB1 polymorphism pattern approached the pattern for the Asian population.
### Table 2. Linkage of ABCB1 polymorphism in 1236 and 3435 position.

| Polymorphism | 1236CC | 1236CT | 1236TT |
|--------------|--------|--------|--------|
| N            | f      | N      | f      |
| 3435CC       | 6      | 0.08   | 11     | 0.15   | 5      | 0.07   |
| 3435CT       | 5      | 0.07   | 25     | 0.35   | 8      | 0.12   |
| 3435TT       | 0      | 0      | 6      | 0.08   | 6      | 0.08   |

N: subjects number  
f: relative frequency

### 3.3 ABCB1 polymorphisms relation to bone marrow suppression

As shown in Table 3, 21 people (29.2%) had mild neutropenia (grade 1-2) and 5 people (6.9%) had severe neutropenia (grade 3-4). C3435T, C1236T polymorphisms either individually or in haplotype 1236TT-3435TT had no significant association with neutropenia (p > 0.05). The results of the present study were in line with a study by Cizmarikova et al. [14] that showed no significant association between C3435T polymorphism and bone marrow suppression events. A study conducted by Chang et al. in 121 cancer patients who received paclitaxel chemotherapy also showed that there was no association between C3435T polymorphism with the incidence of neutropenia grade 3 and 4.[15] The results of this study contradict the study conducted by Tran et al. [16] and Sissung et al. [17] which indicate that the homozygous variant of TT had a relationship to the incidence of severe neutropenia.

### Table 3. Relation of ABCB1 polymorphism with degree of neutropenia.

| Polymorphism | Neutropenia grading | Total | P   |
|--------------|---------------------|-------|-----|
|              | Normal              | Grade 1-2 | Grade 3-4 | n | % | n | % | n | % | n | % |
| 3435 CC+CT   | 40                  | 87      | 18    | 85.7 | 2  | 40 | 60 | 83.3 | 0.736 |
| 3435 TT      | 6                   | 13      | 3     | 14.3 | 3  | 60 | 12 | 18.7 |
| Total        | 46                  | 100     | 21    | 100  | 5  | 100 | 72 | 100 |
| 1236 CC+CT   | 36                  | 78.3    | 16    | 76.2 | 1  | 20 | 53 | 73.6 | 0.683 |
| 1236 TT      | 10                  | 21.7    | 5     | 5.5  | 4  | 80 | 19 | 26.4 |
| Total        | 46                  | 100     | 21    | 100  | 5  | 100 | 72 | 100 |
| 1236TT-3435TT| 2                   | 4.3     | 1     | 4.8  | 3  | 60 | 6  | 8.3  | 0.177 |
| Non 1236TT-3435TT | 44        | 95.7    | 20    | 95.2 | 2  | 40 | 66 | 91.7 |
| Total        | 46                  | 100     | 21    | 100  | 5  | 100 | 72 | 100 |

Kolmogorov-Smirnov Test

In the present study, the incidence of mild leukocytopenia (grade 1-2) occurred in 19 people (26.3%) and 1 person (0.01%) had severe leukocytopenia (grade 3-4) (Table 4). No linkage was between polymorphism C1236T and C3435T as well as its haplotype with the incidence of leukocytopenia (p > 0.05). It was in line with a study by Erdelyi et al. [13] which suggest that there was no association between ABCB1 C3435T polymorphism with severe leukocytopenia incidence.

### Table 4. Relation of ABCB1 polymorphism with leukocytopenia grading.

| Polymorphism | Leukocytopenia grading | Total | P   |
|--------------|------------------------|-------|-----|
|              | Normal                 | Grade 1-2 | Grade 3-4 | n | % | n | % | n | % | n | % |
| 3435 CC+CT   | 45                     | 86.5    | 14    | 73.7 | 1  | 100 | 60 | 83.3 | 0.944 |
| 3435 TT      | 7                      | 13.5    | 5     | 26.3 | 0  | 0  | 12 | 16.7 |
| Total        | 52                     | 100     | 19    | 100  | 1  | 100 | 72 | 100 |
| 1236 CC+CT   | 39                     | 75      | 13    | 68.4 | 1  | 100 | 53 | 73.6 | 0.912 |
| 1236 TT      | 13                     | 25      | 6     | 31.6 | 0  | 0  | 19 | 26.4 |
| Total        | 52                     | 100     | 19    | 100  | 1  | 100 | 72 | 100 |
The result also showed that only 2 (0.02%) and 1 (0.01%) patients had experienced thrombocytopenia (data was not shown). The results of this study were also in line with a study by Cizmarikova et al. [14] which suggest that the incidence of neutropenia and leukocytopenia were more common than with thrombocytopenia in the incidence of bone marrow suppression. There was no significant relationship between ABCB1 polymorphism and the incidence of thrombocytopenia (p > 0.05). The presence of a TT variant was known to cause lower P-gp expression and the presence of a C3435T haplotype with C1236T caused an effect on the more obvious P-gp function compared to only one SNP.[15] The absence of any association between ABCB1 C123T, C3435T and haplotype 1236TT-3435TT with the occurrence of neutropenia, leukocytopenia and thrombocytopenia may be due to other influencing factors; such as the presence of other gene polymorphisms [16] and the influence of ABCB1 G2677T polymorphism, it is known that the common haplotype in the ABCB1 gene was C3435T, C1236T and G2677T.[17]

### 4. Conclusions

All forms of CC, CT and TT polymorphisms in C1236T and C3435T were found in the present study. There was a linkage between heterozygous CT in positions 1236 and 3435. There was no significant association between ABCB1 C1236T, C3435T polymorphisms with bone marrow suppression events. Advanced research is needed with larger sample quantities to confirm gene polymorphisms relationship with response therapy.

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