Frequency of ABCB1 gene 3435 polymorphism on patients with coronary stent in Surakarta

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Abstract. The use of antithrombotic drugs on patients with coronary stent have reduced the risk of stent thrombosis. ABCB1 Gene 3435TT may limit the drugs availability and response. Frequencies of ABCB1 Gene 3435 Polymorphism varied among different populations. This research aimed to identify frequency of 3435 polymorphism on patients with coronary stent in Surakarta. All the patients (who underwent coronary stent, received antithrombotic therapy, and signed for informed consent forms on the period of May 7th – June 8th 2018 in doctor Moewardi Hospital) were included in this study. The single nucleotide polymorphism (SNP) was detected by PCR and sequencing. As a result, the frequencies of 3435 TT, 3435 CC, and 3435 TC on 30 patients were 50 % (15 subjects), 33 % (10 patients), and 17 % (5 subjects) respectively. In this study, the mutant T allele carriers was found in majority of the patients.

1. Introduction
Antithrombotic drugs, including clopidogrel, have become the standard therapy for protection against ischemic events and prevention of stent thrombosis. However, it was reported that around one third of patients were non-responsive to clopidogrel due to the interindividual genetic variability [1,2]. ATP-binding cassette, subfamily B, member 1 (ABCB1) gene polymorphisms might lead to increased P-glycoprotein (P-gp) expression and diminished clopidogrel absorption [3]. ABCB1 3435 polymorphism involved in Clopidogrel and its active metabolite absorption [1]. Frequencies of ABCB1 Gene 3435 Polymorphism differed among population groups. The mutant genotype frequency of ABCB1 gene 3435TT was observed in 28%, 32%, and 43% of Malays, Chinese, and Indians [4]. In pediatric patients with acute Lymphoblastic Leukemia (ALL) at Cipto Mangunkusumo Hospital Jakarta, the most common genotype was 3435TT (84%) [5]. This research aimed to identify frequency of the 3435 polymorphism on patients with coronary stent in Surakarta.

2. Experimental
The method of this study was approved by health research ethics committee of doctor Moewardi General Hospital – School of Medicine Universitas Sebelas Maret Surakarta Indonesia. All the patients (who underwent coronary stent coronary stent, received antithrombotic therapy, and signed for informed consent forms on the period of May 7th – June 8th 2018 in doctor Moewardi Hospital) were included in this study.
Deoxyribonucleic acid (DNA) was isolated from the subjects’ buffy coat with a standard salting out protocol. The primers to identify 3435 polymorphism were: 5’ACT CTT GTT TTC AGC TGC TTG 3’ (forward(F)) and 5’ AGA GAC TTA ACT TAG GCA GTG ACT 3’ (reverse (R)), yielding 206 base pairs (bp) polymerase chain reaction (PCR) product [6]. PCR amplification protocol was initiated with pre-denaturation (5 minutes - 94°C), then it was followed by 35 cycles of denaturation (90 seconds - 94 °C), annealing (60 seconds - 56 °C), and extension (60 seconds - 72 °C). The final extension was at 72 °C (10 minutes). The PCR products were determined by electrophoresis in agarose gel (1.5%) that was stained with ethidium bromide (0.5 μg/ml), and then were visualized under UV transilluminator. The rest of PCR products were sequenced. The nucleotide sequences were compared with ABCB1 wild-type (WT) sequence [7].

3. Results and Discussions

Thirty subjects were included in this research (Table 1 and 2). Heterozygous 3435TC had double peaks on sequencing chromatogram. Homozygous 3435TT and 3435CC had a single peak (Table 2). Frequencies of 3435 TT and 3435 TC were 50 % (15 subjects) and 17 % (5 subjects) respectively. The rest 10 patients were wild type (3435CC). The mutant T alleles carriers were observed in the majority of patients.

Table 1. Patients’ characteristic (N$_{total}$ = 30)

| Characteristic | N (%) |
|---------------|-------|
| Gender        |       |
| Female        | 9 (30%) |
| Male          | 21 (70%) |
| Age (years)   |       |
| 48-54         | 9 (30%) |
| 55-64         | 12 (40%) |
| 65-74         | 8 (27%) |
| >75           | 1 (3%)  |

ABCB1 3435 C>T is a silent single nucleotide polymorphism, but each allele that makes haplotype can give contribution and alter mRNA level, protein folding, and protein expression. Several studies reported the impact of 3435 polymorphism and its haplotype on MDR-1 structure / function. The mutation could alter the stability of MDR1 mRNA. The use of rare codon could influence the translation kinetics and alter the protein-folding dynamic. Silent polymorphism result ribosome-stalling. Therefore, it can affect drug disposition [8,9,10].

Many cardiovascular drugs, that interact with P-gp, were widely used, including clopidogrel, ticagrelol, digoxin, bepridil, verapamil, aliskiren dabigatran, apixaban, edoxaban, rivaroxaban, warfarin, celiprolol, diltiazem, labetolol, losartan, nadolol, propranolol, talinolol, timolol, atorvastatin, lovastatin, and ambrisentan. Interaction between drugs and P-gp transporter altered drugs absorption and elimination. It was reported that 3435 T allele carrier influenced oral digoxin pharmacokinetic by affecting duodenal P-gp transport of digoxin [3]. Clopidogrel absorption could be reduced by P-gp mediated efflux. P-gp was encoded by ABCB1 gene. The 3435TT polymorphism was significantly associated with lower Area Under Curve (AUC) and Maximum Concentration (C$_{max}$) of clopidogrel in patients who suffered from coronary artery disease [1]. In subjects who underwent coronary angiography and stent, Clopidogrel AUC was also significantly decreased in 3445 TT compared with CC and TT genotypes [11].

It was reported that blood tended to thrombose after coronary stent placement. Anti-thrombotic drug, including clopidogrel and aspirin, could reduce the risk of stent thrombosis and cardiovascular-cerebrovascular events [12]. However, a meta-analysis study showed that TT homozygous was
associated with the risk of major adverse cardiovascular events (MACE) in subjects who received clopidogrel 300, compared with CC genotype [13]. In other meta-analysis, 3435 TT might increase the risk of short term (≤ the first 30 days) recurrent ischemic events. On platelet reactivity, ABCB1 3435 variants had a higher impact at baseline, but the impact was decreased during follow up [14]. Therefore, further evaluation on the patients of this study are necessary in order to explore the risk of MACE.

| 3435TT | 3435CC | 3435TC (double peak) |
|--------|--------|----------------------|
| N (%)  | 15 (50%) | 10 (33 %) | 5 (17%) |

**Table 2. ABCB1 gene 3435 polymorphism (N_{total} = 30)**

**4. Conclusions**

Frequencies of 3435TT, 3435CC, and 3435TC were 50 % (15 subjects), 33 % (10 subjects), and 17 % (5 subjects) respectively. The mutant T alleles carriers were found in the majority of patients.

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