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Role of Cytochrome p450 in Clopidogrel Resistance in Indonesian Stroke Patients

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Abstract

BACKGROUND: Stroke is the second leading cause of death in the world. Clopidogrel is one of the recommended therapies for it. Disturbances in the metabolic stages of clopidogrel, such as dysregulation of cytochrome p450 (CYP450) and cytochrome P450 2C19 (CYP2C19), can cause unresponsiveness or resistance. However, studies on clopidogrel resistance related to CYP450 and CYP2C19 in Indonesia are limited.

METHODS: This cross-sectional study was conducted in two hospitals over a 1-year period. All patients had a minimum of one episode of ischemic stroke. Data on demographics, clopidogrel resistance, CYP450 concentration, and CYP2C19 polymorphism were collected. In total, 112 participants were enrolled in this study.

RESULTS: We found that the incidence of clopidogrel resistance was 14.3% and the bleeding risk was high (40.3 %). The number of mutations in the CYP2C19 allele *17 was 96.0%. We also found that sex, hemoglobin, and CYP450 were significantly correlated with clopidogrel resistance, and with hemoglobin and CYP450 as independent factors. Fasting blood glucose and CYP2C19 allele *3 were independent factors for CYP450.

CONCLUSION: The incidence of clopidogrel resistance in the Indonesian population is low; therefore, it is not an urgent issue. An important issue in this population is the high risk for bleeding. Patient treatment should be adjusted by considering this risk.

Introduction

Stroke is a medical emergency that ranks as the world's second-leading cause of death [1]. Based on the American Heart Association/American Stroke Association 2022, it is estimated that 795,000 people experience either a new stroke or recurrence per year. The most common type is ischemic stroke, which accounts for 87% of cases [2].

The Canadian Stroke Best Practice Recommendations in 2020 provided several recommendations for the prevention of recurrent stroke, such as administration of long-term antiplatelets. One of the recommendations was the administration of a 75 mg daily dosage of clopidogrel [3].

Clopidogrel is one of the recommended therapies since the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events study in 1996 that showed a better outcome of clopidogrel treatment on recurrent stroke than with acetylsalicylic acid. A previous study also showed that clopidogrel had a lower risk of bleeding [4].

Disturbances in the metabolic stages of clopidogrel cause a decreased response (unresponsiveness) condition, known as clopidogrel resistance. In various studies, clopidogrel resistance was also referred to as high on-treatment platelet reactivity [5]. Clopidogrel resistance correlates with race and individual factors [6]. In the West, its prevalence ranges from 5 to 44%, while in Asia, it reaches up to 70% [7].

Clopidogrel, in its metabolism, needs the help of cytochrome p450 (CYP450) to transform into an active form that has the function of an antiplatelet. CYP polymorphisms closely correlated with the decline in clopidogrel function include cytochrome P450 2C19 (CYP2C19) allele genes *2, *7, and *19 [7]. The clopidogrel resistances based on alleles *2, *3, and *17 from a study based on Asian population are 23.00%, 4.61%, and 15.18%, respectively [8]. Damage or loss of function (LOF) in CYP2C19 alleles *2 and *3 is closely correlated with the effectiveness

of clopidogrel [9], [10], [11]. Another CYP2C19 allele, the *17 allele, increases catalyst activity, but its effect on the pharmacodynamics of clopidogrel is not widely known [12], [13].

Various studies have examined the correlation between clopidogrel resistance and CYP2C19, but studies correlating with CYP450 are limited. Studies on clopidogrel resistance, focusing on the correlation between CYP2C19 and CYP450, are also minimal. The objective of this study was to determine the correlation between the two parameters within the scope of clopidogrel resistance.

Materials and Methods

This cross-sectional research was conducted at Universitas Indonesia Hospital and Dr. Cipto Mangunkusumo Hospital. The data were collected from January 2021 to January 2022. The inclusion criteria were occurrence of both primary and recurrent ischemic strokes and the patients should have consumed clopidogrel for at least 5 days. Meanwhile, the exclusion criteria were history of clopidogrel allergies; impaired renal function; blood clotting disorders; consumption of certain drugs such as omeprazole, esomeprazole, and atorvastatin; and incomplete data. The initial sample size in this study was 128, which was later reduced to 112 after the exclusion process.

Clopidogrel resistance examination was carried out by collecting a blood sample which was then checked using the VerifyNow system (VN). VN levels were measured using P2Y12 reaction units. There were three VN levels: non-response (>208), response (95–208), and bleeding risk (≤95).

CYP450 concentration was measured using enzyme-linked immunosorbent assay. The sample used was blood plasma content with anticoagulant EDTA or heparin.

Polymorphism of CYP2C19 was determined using the TaqMan hydrolysis probe method. The examination was carried out by taking the DNA from the blood sample, which was amplified based on the specific primers of alleles *2, *3, and *17, which were the most influential alleles in the clopidogrel resistance mechanism. This study used three classifications to categorize CYP2C19 polymorphisms in each allele. The first method categorized the mutants into homozygous wild type, heterozygous, and homozygous mutants, the second method into homozygous and non-homozygous, and the third into mutants and non-mutants.

The data obtained in this study were processed using IBM SPSS 26. Statistical tests were performed using the Chi-square and Kruskal–Wallis tests because the data collected were categorized specifically as nominal data. Statistical significance was set at $p < 0.05$. Logistic regression analysis was used to assess independent factors.

Ethics statement

The Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo Hospital, approved this study (approval number KET-658/UN2/F1/ETIK/PPM.00.02/2020). This study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Table 1 shows the characteristics of the 112 participants. The study used cutoff of age and body mass index (BMI) based on Stent Thrombosis in Belgium (STIB) scoring.

Table 1: The participant characteristics

Characteristic	n (%)	Mean ± SD/median (minimum–maximum)
Sex		
Male	66 (58.9)	
Female	46 (41.1)	
Age (years)		
> 68	13 (11.6)	57.1 ± 10.6/57.5 (25–80)
≤ 68	99 (88.4)	
BMI (kg/m^2)		
> 28	26 (23.2)	24.9 ± 4.7/24.3 (15.6–45.8)
≤ 28	86 (76.8)	
Hemoglobin (g/dL)		
≤ 13.9	57 (50.9)	13.8 ± 1.6/13.9 (7.9–18.4)
> 13.9	55 (49.1)	
HbA1c (%)		
≥ 6.5	37 (33.0)	6.5 ± 2.3/5.7 (4.0–15.0)
< 6.5	75 (67.0)	
FBG (mg/dL)		
≥ 100	70 (62.5)	129.7 ± 60.8/109.5 (73–449)
< 100	42 (37.5)	
LDL (mg/dL)		
≥ 100	86 (76.8)	127.0 (± 40.6)/125 (38-269)
< 100	26 (23.2)	
Hepatobiliary USG		
Fatty liver	60 (53.6)	
Non	52 (46.4)	

BMI: Body mass index, HbA1c: Hemoglobin A1c, FBG: Fasting blood glucose, LDL: Low-density lipoprotein, USG: Ultrasonography, SD: Standard deviation.

The clopidogrel resistance results were divided into three groups: non-response, response, and bleeding risk (Table 2). In this study, the results were not entirely different between the response and bleeding risk groups but differed from the non-response group. Based on these data, it can be concluded that the risk of resistance was low.

Table 2: The VerifyNow results of the participant

VerifyNow	n (%)	Mean ± SD/median (minimum–maximum)
Total participants		115.2 ± 71.3/105.5 (1–273)
Non-response	16 (14.3)	244.5 ± 18.8/241.5 (216.0–273.0)
Response	51 (45.5)	132.9 ± 27.4/134.0 (97.0–195.0)
Bleeding risk	45 (40.2)	49.2 ± 31.2/56.0 (1.0–93.0)

SD: Standard deviation.

CYP450 concentrations in each clopidogrel group are shown in Table 3. The mean and median scores of the groups differed. The median score was used as a cutoff value in the subsequent analysis.

Standard deviation (SD) scores that exceeded the mean score were likely due to an abnormal data distribution.

Table 3: Response to clopidogrel therapy correlated with CYP450 concentration

VerifyNow	CYP450 concentration	
	Mean ± SD	Median (minimum–maximum)
Non-response	1841.1 ± 2931.1	738.4 (112.8–10,234.3)
Response	825.1 ± 1075.3	458.9 (3.9–6946.0)
Bleeding risk	586.9 ± 872.8	335.6 (0.6–4845.4)

SD: Standard deviation.

Table 4 shows the CYP2C19 polymorphisms in alleles *2, *3, and *17. The wild-type homozygous allele dominated allele *2, heterozygous dominated allele *3, and mutant homozygotes dominated allele *17.

Table 4: The distribution of CYP2C19 by genotype

Genotype	Wild type, n (%)	Heterozygous, n (%)	Homozygous mutants, n (%)
CYP2C19*2	70 (62.5)	36 (3 2.1)	6 (5.4)
CYP2C19*3	28 (25.0)	77 (68.8)	7 (6.3)
CYP2C19*17	0	5 (4.0)	107 (96.0)

Table 5 shows the correlations between various factors and clopidogrel resistance. The table presents clopidogrel resistance as non-response and response. Significant correlations were obtained for sex and hemoglobin levels. Another significant correlation was obtained with clopidogrel resistance at a CYP450 concentration of 458.9 pg/mL. There was no significant correlation between clopidogrel resistance and CYP2C19 polymorphism. Mutations in CYP2C19 among the *2, *3, and *17 alleles showed no significant correlation with clopidogrel resistance. These results are in line with Table 2, which shows that the frequency of non-response participants was only 14.3%.

Table 5: The characteristics of participants correlated with CYP450 concentration

Characteristic	Non-response, n (%)	Response, n (%)	p	OR (95% CI)
Sex				
Female	10 (21.7)	36 (78.3)	0.06*,†	2.8 (0.931–8.288)
Male	6 (9.1)	60 (90.9)		
Age (years)				
> 68	1 (7.7)	12 (92.3)	0.47*	0.47 (0.056–3.860)
≤ 68	15 (15.2)	84 (84.8)		
BMI (kg/m^2)				
> 28	3 (11.5)	23 (88.5)	0.648*	0.73 (0.192–2.797)
≤ 28	13 (15.1)	73 (84.9)		
Hemoglobin (g/dL)				
≤ 13.9	15 (93.8)	46 (42.6)	<0.001*,†	19.29 (2.448–0.895)
> 13.9	1 (6.2)	62 (57.4)		
HbA1c (%)				
≥ 6.5	4 (10.8)	33 (89.2)	0.460*	0.636 (0.190–2.12)
< 6.5	12 (16.0)	63 (84.0)		
Hepatobiliary USG				
Fatty liver	10 (16.7)	50 (83.3)	0.439*	1.533 (0.516–4.554)
Non	6 (11.5)	46 (88.5)		
FBG (mg/dL)				
≥ 100	7 (10.0)	63 (90.0)	0.094*	0.407 (0.139–1.192)
< 100	9 (21.4)	33 (78.6)		
LDL (mg/dL)				
≥ 100	12 (14.0)	74 (86.0)	0.855*	0.892 (0.261–3.044)
< 100	4 (15.4)	22 (84.6)		
CYP450 (pg/mL)				
≥ 738.4	8 (23.5)	26 (76.5)	0.065*	2.692 (0.916–7.915)
< 738.4	8 (10.3)	70 (89.7)		
≥ 458.9	12 (24.0)	38 (76.0)	0.008*†	4.579 (1.375–15.252)
< 458.9	4 (6.5)	58 (93.5)		
≥ 335.6	12 (17.1)	58 (82.9)	0.265*	1.966 (0.590–6.547)
< 335.6	4 (9.5)	38 (90.5)		
CYP2C19*2				
Homozygous	9 (12.9)	61 (87.1)	0.620*	0.758 (0.252–2.277)
Non-homozygous	7 (16.7)	35 (83.3)		
Mutant	1 (16.7)	5 (83.3)	0.864*	1.21 (0.132–11.120)
Non-mutant	15 (14.2)	91 (85.8)		
CYP2C19*3				
Homozygous	4 (14.3)	24 (85.7)	1.0*	1.000 (0.319–3.132)
Non-homozygous	12 (14.3)	72 (85.7)		
Mutant	1 (14.3)	6 (85.7)	1.0*	1.000 (0.112–8.903)
Non-mutant	15 (14.3)	90 (85.7)		
CYP2C19*17				
Homozygous	0	0	0.709*	0.652 (0.068–6.239)
Non-homozygous	16 (14.3)	96 (85.7)		
Mutant	15 (14.0)	92 (86.0)	0.709*	0.652 (0.068–6.239)
Non-mutant	1 (20.0)	4 (80.0)		

*Chi-square, †p<0.05 . BMI: Body mass index, HbA1c: Hemoglobin A1c, FBG: Fasting blood glucose, LDL: Low-density lipoprotein, USG: Ultrasonography, CI: Confidence interval, OR: Odds ratio.

Based on bivariate analysis, a follow-up analysis was carried out through linear regression using the p-value limit of < 0.25 (Table 6). Multivariate analysis revealed significant results (p < 0.05) for hemoglobin values (p = 0.003) and a CYP450 cutoff of 458.9 pg/mL (p = 0.004). It can be concluded that hemoglobin values and CYP450 cutoff concentrations of 458.9 pg/mL were the independent factors associated with clopidogrel resistance.

Table 6: Multivariate analysis of clopidogrel resistance

Independent factor	p	95% CI
Sex	0.611	0.132–3.289
Hemoglobin	0.003†	3.635–487.346
FBG	0.208	0.107–1.629
CYP450 cutoff 738.4 (pg/mL)	0.608	0.267–9.533
CYP450 cutoff 458.9 (pg/mL)	0.004†	0.891–205.396

†p < 0.05. FBG: Fasting blood glucose, CI: Confidence interval.

Table 7 shows the correlation between CYP450 concentration and the characteristics of the various participants. CYP450 concentrations were divided into three groups based on clopidogrel resistance (non-response, response, and bleeding risk) using a median cutoff, as shown in Table 4. There was a significant correlation between fasting blood glucose (FBG) and CYP450 concentrations (458.9 pg/mL cutoff). There was also a significant correlation between CYP2C19 polymorphism and CYP450 concentration. A significant correlation was found between the CYP2C19 allele *3 homozygous group and each CYP450 group (non-response, response, and bleeding risk). In addition, there was also a significant correlation between the CYP2C19 allele *3.

From the finding in Table 7, multivariate analysis was performed using a p-value limit of < 0.25. The analysis revealed that FBG and CYP2C19 allele *3 were independent factors for CYP450 (Table 8).

Discussion

The total number of participants in this study was 112. The participants were predominantly men (58.9%), and a BMI >28 kg/m^2 was found in 23.2% of the participants. These results were different from those of Shiozawa *et al*., who found that the prevalence of stroke in men in the obesity group (BMI ≥30 kg/m^2) and the overweight group (≥25–29.9 kg/m^2) was 41.5 times and 38.7 times high, respectively, while women had a lower risk, which was 35.8 times and 34.7 times, respectively [14].

The number of participants resistant to clopidogrel in this study was 16 (14.3%). These results

Table 7: The participant's characteristics, CYP2C19 polymorphism, and CYP450 concentration

Characteristic	CYP450 concentration											
	Non-response				Response				Risk of bleeding			
	≥ 738.4 (pg/mL), n (%)	< 738.4 (pg/mL), n (%)	p	OR (95% CI)	≥ 458.9 (pg/mL), n (%)	< 458.9 (pg/mL), n (%)	p	OR (95% CI)	≥ 335.6 (pg/mL), n (%)	< 335.6 (pg/mL), n (%)	p	OR (95% CI)
Sex												
Female	13 (28.3)	33 (71.7)	0.687*	0.844	20 (43.5)	26 (56.5)	0.836*	0.923	26 (56.5)	20 (43.5)	0.275*	0.650
Male	21 (31.8)	45 (68.2)		(0.370–1.926)	30 (45.5)	36 (54.5)		(0.433–1.970)	44 (66.7)	22 (33.3)		(0.299–1.412)
Age (years)												
> 68	4 (30.8)	9 (69.2)	0.973*	1.022	6 (46.2)	7 (53.8)	0.907*	1.071	10 (76.9)	3 (23.1)	0.253*	2.167
≤ 68	30 (30.3)	69 (69.7)		(0.292–3.580)	44 (44.4)	55 (55.6)		(0.336–3.419)	60 (60.6)	39 (39.4)		(0.561–8.372)
BMI (kg/m^2)												
> 28	9 (34.6)	17 (65.4)	0.590*	1.292	12 (46.2)	14 (53.8)	0.860*	1.083	16 (61.5)	10 (38.5)	0.908*	0.948
≤ 28	25 (29.1)	61 (70.9)		(0.508–3.282)	38 (44.2)	48 (55.8)		(0.449–2.612)	54 (62.8)	32 (37.2)		(0.384–2.339)
Hemoglobin (g/dL)												
≤ 13.9	15 (26.3)	42 (73.7)	0.344*	0.677	24 (42.1)	33 (57.9)	0.582*	0.811	33 (57.9)	24 (42.1)	0.305*	0.669
> 13.9	19 (34.5)	36 (65.5)		(0.301–1.521)	26 (47.3)	29 (52.7)		(0.385–1.711)	37 (67.3)	18 (32.7)		(0.310–1.445)
HbA1c (%)												
≥ 6.5	13 (35.1)	24 (64.9)	0.440*	1.393	15 (40.5)	22 (59.5)	0.540*	0.779	25 (67.6)	12 (32.4)	0.437*	1.389
< 6.5	21 (28.0)	54 (72.0)		(0.600–3.234)	35 (46.7)	40 (53.3)		(0.351–1.730)	45 (60.0)	30 (40.0)		(0.606–3.182)
Hepatobiliary USG												
Fatty liver	19 (31.7)	41 (68.3)	0.746*	1.143	23 (38.3)	37 (61.7)	0.149*	0.576	34 (56.7)	26 (43.33)	0.171*	0.581
Not	15 (28.8)	37 (71.2)		(0.509–2.569)	27 (51.9)	25 (48.1)		(0.271–1.222)	36 (69.2)	16 (30.8)		(0.267–1.267)
FBG (mg/dL)												
≥ 100	20 (28.6)	50 (71.4)	0.596*	0.800	25 (35.7)	45 (64.3)	0.014*†	0.378	40 (57.1)	30 (42.9)	0.131*	0.533
< 100	14 (33.3)	28 (66.7)		(0.351–1.825)	25 (59.5)	17 (40.5)		(0.172–0.830)	30 (71.4)	12 (28.6)		(0.235–1.211)
LDL (mg/dL)												
≥ 100	26 (30.2)	60 (69.8)	0.958*	0.975	39 (45.3)	47 (54.7)	0.785*	1.132	53 (61.6)	33 (38.4)	0.729*	0.850
< 100	8 (30.8)	18 (69.2)		(0.377–2.525)	11 (42.3)	15 (57.7)		(0.466–2.745)	17 (65.4)	9 (34.6)		(0.340–2.128)
CYP2C19*2												
Homozygous	19 (27.1)	51 (72.9)	0.340*	0.671	29 (41.4)	41 (58.6)	0.377*	0.707	43 (61.4)	27 (38.6)	0.762*	0.885
Non-homozygous	15 (35.7)	27 (64.3)		(0.295–1.526)	21 (50.0)	21 (50.0)		(0.328–1.527)	27 (64.3)	15 (35.7)		(0.400–1.957)
Mutant	3 (50.0)	3 (50.0)	0.282*	2.419	4 (66.7)	2 (33.3)	0.265*	2.609	5 (83.3)	1 (16.7)	0.279*	3.154
Non-mutant	31 (29.2)	75 (70.8)		(0.463–12.650)	46 (43.4)	60 (6.6)		(0.458–14.687)	65 (61.3)	41 (38.7)		(0.356–27.965)
CYP2C19*3												
Homozygous	1 (3.6)	27 (96.4)	<0.001*†	0.057	3 (10.7)	25 (89.3)	<0.001*†	0.094	3 (10.7)	25 (89.3)	<0.001*†	0.030
Non-homozygous	33 (39.3)	51 (60.7)		(0.007–0.442)	47 (56.0)	37 (44.0)		(0.026–0.337)	67 (79.8)	17 (20.2)		(0.008–0.113)
Mutant	4 (57.1)	3 (42.9)	0.111*	3.333	5 (71.4)	2 (28.6)	0.141*	3.333	7 (100.0)	0	0.034*†	1.667
Non-mutant	30 (28.6)	75 (71.4)		(0.703–15.794)	45 (42.9)	60 (57.1)		(0.618–17.970)	63 (60.0)	42 (40.0)		(1.426–1.948)
CYP2C19*17												
Homozygous	0	0	-	-	0	0	-	-	0	0	-	-
Non-homozygous	34 (30.4)	78 (69.6)			50 (44.6)	62 (55.4)			70 (62.5)	42 (37.5)		
Mutant	32 (29.9)	75 (70.1)	0.631*	0.640	46 (43.0)	61 (57.0)	0.104*	0.189	66 (61.7)	41 (38.3)	0.408*	0.402
Non-mutant	2 (40.0)	3 (60.0)		(0.102–4.015)	4 (80.0)	1 (20.0)		(0.020–1.744)	4 (80.0)	1 (20.0)		(0.043–3.727)

*Chi-square, †p<0.05. BMI: Body mass index, HbA1c: Hemoglobin A1c, FBG: Fasting blood glucose, LDL: Low-density lipoprotein, USG: Ultrasonography, OR: Odds ratio, CI: Confidence interval.

were consistent with those of previous ones in Indonesia, with no difference in frequency (9 people, 15.8%) [5]. This differs from the results of the two studies. The STIB and Gauging Responsiveness with a VN P2Y12 Assay: Impact on Thrombosis and Safety GRAVITAS trials were conducted in Belgium and the US; the two studies found that the number of participants with clopidogrel resistance was 424 (50.2%) and 2,214 (40.8%), respectively. These differences were likely due to racial differences affecting genetic variants [15], [16].

The study found that clopidogrel resistance and sex were significantly correlated, with women having a 2.8 times higher risk than men. These results were supported by a preliminary study that showed that men had an 80% lower risk of resistance than women [5]. In addition, studies by Price *et al*. showed a significant correlation between sex and clopidogrel resistance [16]. However, Legrand *et al*. found a different result [15].

The study found no significant correlation between age and clopidogrel resistance (p = 0.47). Legrand *et al*. and Price *et al*. reported different results [15], [16]. This difference may be due to the average age of the study participants. In the study by Price *et al*., the average age was 57 years, whereas in that by Legrand *et al*., the average age was 66 years [15]. The study by Price *et al*. included an average age of 61 and 64 years in the response and non-response groups, respectively [16].

BMI in the study had no significant correlation with clopidogrel resistance (p = 0.648). Again, studies conducted by Legrand *et al*. and Price *et al*. reported different results [15], [16]. Legrand *et al*. reported an average BMI of 27 kg/m^2 in the response group and 28 kg/m^2 in the non-response group [15]. Price *et al*. reported a median score of 29 kg/m^2 in the response group and 31 kg/m^2 in the non-response group [16]. The BMI value was different from that in this study, which

Table 8: Multivariate analysis of CYP450 concentrations

Independent factors	CYP450 cutoff 738.4 pg/mL		CYP450 cutoff 458.9 pg/mL		CYP450 cutoff 335.6 pg/mL	
	p	95% CI	p	95% CI	p	95% CI
Age					0.435	0.303–16.117
Hepatobiliary USG			0.209	0.205–1.414	0.280	0.140–1.765
FBG			0.031†	0.130–0.906	0.509	0.168–2.424
CYP2C19 allele *3 code homozygous	0.005†	0.007–0.402	0.001†	0.026–0.389	<0.001†	0.004–0.091
CYP2C19 allele *3 mutant codes	0.331	0.401–15.100	0.530	0.273–12.478	0.999	0.000
CYP2C19 allele *17 mutant codes			0.512	0.040–4.979		

†p<0.05 . FBG: Fasting blood glucose, USG: Ultrasonography, CI: Confidence interval

has an average value of 24.9 kg/m^2 and a median of 24.3 kg/m^2.

This study showed that Hb level was an independent factor in clopidogrel resistance. Hb levels ≤13.9 g/dL increased the risk of resistance by 19 times. Similar results were reported by Legrand *et al*. The study showed that the clopidogrel resistance group had an average Hb content of 13.6 g/dL [15].

Diabetes mellitus (DM) was indicated by HbA1c level in this study and showed no significant correlation with clopidogrel resistance. These results are supported by a previous study showing that a history of DM and HbA1c level did not significantly correlate with clopidogrel resistance [5]. The studies by Legrand *et al*. and Price *et al*. showed different results. Legrand *et al*. stated that history of DM and HbA1C level were correlated significantly [15]. The study by Price *et al*. also showed that history of DM was significantly correlated with clopidogrel resistance [16]. The difference in results may be due to the cutoff HbA1C level in this study, which was 6.5%. Legrand *et al*. showed that the average HbA1C level in the non-response group was 6.3% [15].

This study's FBG, low-density lipoprotein (LDL), and fatty liver condition levels did not significantly correlate with clopidogrel resistance. These results were consistent with those of previous studies that showed no significant correlation between clopidogrel resistance and FBG, LDL, or fatty liver conditions [5].

In this study, clopidogrel resistance (VN) has its SD score exceeded the mean score. This was due to the abnormal distribution of the data. The minimum CYP450 concentration was 0.6 pg/mL, while the maximum value was 10,234.3 pg/mL. Extreme data distribution was also observed in each clopidogrel resistance group. This study demonstrated that CYP450 concentration of 458.9 pg/mL was an independent risk factor for clopidogrel resistance. A 4.6 times increase in the risk of clopidogrel resistance was observed at this concentration.

This study showed a significant correlation between CYP450 concentration and the CYP2C19 allele *3 polymorphism. CYP2C19 is a platelet receptor that primarily performs the active metabolism of clopidogrel [17], [18]. The occurrence of LOF in CYP2C19 caused major adverse coronary events (HR, 2.3), and ischemic risk [19], [20]. In contrast, Wang *et al*. found no significant correlation with bleeding risk in the LOF and non-LOF groups [7].

Mutations in CYP2C19 are often found in alleles *2 and *3. Mutation of allele *2 causes the proteins to produce no enzymatic activity. Mutations in allele *3 prematurely stop protein production. CYP2C19 improves gene transcription and increases enzyme function. LOF mutation occurs in alleles *2 and *3, whereas the mutation in allele *17 is called gain of function [21].

This study showed that the CYP2C19 allele*2 had no significant correlation with either clopidogrel resistance or CYP450 concentration. These results differed from those of Gurbel *et al*., who reported a significant correlation [22]. This may be due to racial differences, as the study by Gurbel *et al*. was conducted in the US. The study was dominated by Caucasians, African-Americans, and Asian-Americans, with 155, 101, and 5 participants, respectively [22]. The notable CYP2C19 allele *2 in this study was the heterozygous type, which accounted for more than half of the wild type (62.5% and 2.1%, respectively). This number suggested that there were numerous participants who lacked a response to clopidogrel therapy. This condition can be considered for genetic examinations in high-risk groups, such as acute coronary syndrome (ACS), DM, and chronic kidney disease (CKD).

The CYP2C19 allele *3 in this study did not significantly correlate with clopidogrel resistance, but there was a significant correlation with CYP450 concentration. The CYP2C19 allele *3 was an independent factor for CYP450 concentration. The study found that the prevalence of CYP2C19 alleles *3 of wild type, heterozygous, and mutant type was 25.0%, 68.8%, and 6.3%, respectively. Zhong *et al*.'s study in southern China found a different result, showing that CYP2C19 allele *3 wild type, heterozygotes, and mutant type were present in 91.3%, 8.53%, and 0.34%, respectively [23].

The CYP2C19 allele *17 in this study showed no significant correlation with clopidogrel resistance or CYP450 concentration. These results may be because the allele *17 was present in a small part of the population. Gurbel *et al*. showed that the alleles were found in 21% of Caucasian, 17% of African-American, and 20% of Asian-American populations [22]. The CYP2C19 allele *17 did not show significant accuracy in clinical use, but it was estimated that the allele was closely related to bleeding risk [24]. Although no statistically significant correlation was found, the frequency of alleles *17 mutant-type occurrence was 96.0%. This indicates that the risk of bleeding in the Indonesian population is significant. Therefore, more research is required. These results provide clues for antiplatelet management. Dual antiplatelet administration can last for 21 days based on the CHANCE study and 90 days based on the POINT study [25], [26]. From this study, it can be concluded that the Indonesian population should use the CHANCE study as a guideline, considering that the risk of bleeding is relatively high. This study also showed that adjustment of clopidogrel dose in patients is required.

Conclusions

This study showed that Hb level was an independent factor in clopidogrel resistance. Hb

levels ≤13.9 g/dL increased the risk of resistance by 19 times.

The CYP2C19 allele *3 was the dependent factor for CYP450 concentration. In addition, CYP450 expression significantly correlated with the occurrence of clopidogrel resistance. These showed that resistance to clopidogrel in Indonesia was low. In contrast, the decreases in the clopidogrel metabolic processes were high. Therefore, the severity of genetic variations can be considered in high-risk cases such as DM, ACS, and CKD.

The number of CYP2C19 allele *17 mutations and the high number of bleeding risk groups indicated a need for special attention related to bleeding risk with clopidogrel administration in Indonesia. Therefore, it was necessary to evaluate the duration of antiplatelet administration in stroke management in Indonesia. It is possible that this condition also applies to other populations, especially Asian populations.

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