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Clopidogrel Resistance Among Ischemic Stroke Patients and Its Risk Factors in Indonesia

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Abstract

Objective. Clopidogrel is a common antiplatelet used as secondary prevention of ischemic stroke, known to have better efficacy than aspirin, with a equivalent safety profile. However, clopidogrel resistance is not uncommon but has not been widely studied in Asia. This study will further assess clopidogrel resistance and its risk factors. **Materials and Methods.** A cross-sectional study was conducted at Rumah Sakit Universitas, Indonesia, and Rumah Sakit Cipto Mangunkusumo, Indonesia in 2020-2021. All patients had had at least one episode of ischemic stroke. Clopidogrel resistance was assessed using a VerifyNow assay. **Results.** 57 subjects were enrolled in this study. We found 15.8% of subjects were clopidogrel resistant. Gender was significantly associated with clopidogrel resistance, with males having 80% lower clopidogrel resistance (OR 0.2 (95% CI 0.022 – 0.638); P=0.006). Meanwhile, smoking was not associated with clopidogrel responsiveness (P=0.051). We found no association between haemoglobin, blood glucose, HbA1c, cholesterol, liver enzymes, serum urea concentration or creatinine levels and clopidogrel resistance. **Conclusion.** Clopidogrel remains an effective treatment to prevent recurrent ischemic stroke in Indonesia. Further studies are needed to assess gene polymorphism and clopidogrel resistance, which may explain the findings of this study.

Key Words: Clopidogrel Resistance ▪ Ischemic Stroke ▪ Risk Factors.

Introduction

The American Heart Association/American Stroke Association (AHA/ASA) guidelines for the secondary prevention of ischemic stroke recommend the administration of antiplatelet therapy over anticoagulation, to reduce the recurrence of non-cardioembolic ischemic stroke. Among the recommended antiplatelet therapies are aspirin 50 to 325 mg daily, clopidogrel 75 mg, or a combination of aspirin 25 mg and extended-release dipyridamole 200 mg, twice daily (1). It is also said that in patients with a recent minor stroke (NIHSS ≤3) and high-risk of transient ischemic attack (TIA; ABCD2 score ≥4), the combination of aspirin and clopidogrel should be initiated early, within 90 days, followed by single antiplatelet therapy for the prevention of ischemic stroke (2). However, not all individuals respond to aspirin, leading to recurrent cardiovascular events, despite ongoing therapy. Aspirin resistance was shown in 22.5-26.4% of individuals with cardiovascular disease, and even more in Asia, with 27.3% showing aspirin resistance (3).

Clopidogrel is a widely used antiplatelet for secondary prevention of stroke and coronary heart disease. It works by irreversibly modifying the adenosine diphosphate (ADP) receptor (P2Y12 receptor) on platelets, thus inhibiting platelet activation (4). It is also commonly used in Indonesia since it is a widely available alternative to aspirin. The CAPRIE study showed that clopidogrel is superior

in efficacy to aspirin, with a relatively equivalent safety profile (5). While aspirin resistance is common and widely known, there have not been many studies assessing clopidogrel resistance, especially in Indonesia. This study aims to assess the prevalence of clopidogrel resistance among ischemic stroke patients in Indonesia, and its risk factors.

We hope that this study will present the characteristics of patients who have a higher risk of clopidogrel resistance, thus providing awareness and alternative treatments for such populations.

Methods

This is a cross-sectional study performed at Rumah Sakit Universitas, Indonesia, and Rumah Sakit Cipto Mangunkusumo in Indonesia. All stroke patients in 2020-2021 who fulfilled the criteria for this research, which included ischemic stroke, whether primary or recurrent, age between 40 to 80 years, no history of kidney disease, bleeding disease or atrial fibrillation, and who had never taken anticoagulants before, and did not consume omeprazole, esomeprazole, or atorvastatin. The patients were given clopidogrel for five days, and a blood sample was taken to be examined for VerifyNow levels, which indicated clopidogrel resistance by the P2Y12 reaction unit (PRU) value. VerifyNow results were categorized using two different methods: the first method classified clopidogrel resistance as unresponsive (PRU >235) and responsive (PRU ≤235), while the second method classified clopidogrel resistance as unresponsive (PRU >208), responsive (PRU ≤208) and a bleeding risk (PRU <95). Clopidogrel resistance or responsiveness was considered as high on-treatment platelet reactivity (HTPR). HTPR may reflect the pharmacodynamics of clopidogrel on platelets because the cause of HTPR is the same as the cause of low response and resistance.

Ethics Statement

This research was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital, approval number KET-658/UN2/F1/ETIK/PPM.00.02/2020.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS version 25. Statistical tests were performed using Chi-square, one-way ANOVA, student's T-test and Kruskal-wallis. A significant result was defined as $P<0.05$. Logistic regression analysis was used to assess the associations with responsiveness to clopidogrel.

Results

In this study, we analysed 57 subjects who had taken Clopidogrel for at least 5 days for ischemic stroke prevention. Using the first method (unresponsive with PRU >235), we found 9 subjects (15.8%) who met the HTPR criteria (clopidogrel resistance) and 48 subjects (84.2%) who responded to clopidogrel. Bivariate analysis showed that several risk factors are associated with clopidogrel resistance, such as female gender and height (Table 1).

Moreover, we found that females generally had higher PRU levels than males, while smoking history, diabetes mellitus, hypertension, dyslipidemia, stroke history, and fatty liver were not significantly associated with VerifyNow PRU levels (Table 2).

We further analysed several serum markers that may be associated with clopidogrel resistance using the second method classifying VerifyNow results as unresponsive (PRU >208), responsive (PRU ≤208), and bleeding risk (PRU <95). No association was found between laboratory results and clopidogrel resistance or bleeding risk.

A significant association between gender and clopidogrel resistance was identified, and it was also shown that female had a higher chance to be resistant to clopidogrel than men ($P=0.006$). Further, the data from VerifyNow showed that stroke patients who smoke had lower VerifyNow levels compared to people who do not smoke, which suggested they were still responsive to clopidogrel although not reaching the bleeding risk level.

Table 1. Baseline Characteristics

Characteristics	Unresponsive N=9; (%)	Responsive N=48; (%)	P-value	Mean difference/OR (95% CI)
Gender				
Male	2 (22.2)	34 (70.8)	0.006*	0.2 (0.022 - 0.638)
Female	7 (77.8)	14 (29.2)		
Smoking				
Yes	1 (11.1)	22 (45.8)	0.051*	6.76 (0.785 - 58.404)
No	8 (88.9)	26 (54.2)		
DM				
Yes	3 (33.3)	15 (31.3)	0.902*	0.909 (0.200 - 4.133)
No	6 (66.7)	33 (68.8)		
Hypertension				
Yes	7 (77.8)	37 (77.1)	0.964*	0.961 (0.174 - 5.311)
No	2 (22.2)	11 (22.9)		
Dyslipidemia				
Yes	7 (77.8)	30 (62.5)	0.378*	0.476 (0.089 - 2.546)
No	2 (22.2)	18 (37.5)		
Dyspepsia				
Yes	2 (22.2)	10 (20.8)	0.925*	0.921 (0.165 -5.138)
No	7 (77.8)	38 (79.2)		
History of stroke				
Yes	3 (33.3)	18 (37.5)	0.812*	1.2 (0.267 – 5.400)
No	6 (66.7)	30 (62.5)		
Fatty liver				
Yes	5 (55.6)	21 (43.8)	0.514*	0.622 (0.148 - 2.608)
No	4 (44.4)	27 (56.3)		
Age	53.89 (±9.82)	55.56 (±11.10)	0.675†	1.673 (9.631 - 6.284)
Blood pressure				
Systolic	141.88 (±20.18)	137.29 (±31.44)	0.675†	4.59 (-12.58 - 21.77)
Diastolic	78.55 (±13.70)	79.79 (±11.05)	0.768†	1.23 (-12.01 - 9.53)
Weight	59 (±11.78)	67 (±14.96)	0.137†	8 (18.64 - 2.64)
Height	156.67 (±6.55)	162.11 (±7.18)	0.040†	2.58 (10.63 - 0.25)

*Chi-square; †One-way ANOVA.

Table 2. VerifyNow Levels by Groups

Characteristics	VerifyNow	P-value*
Gender		
Male	120.72 (±63.35)	0.036
Female	164.76 (±79.06)	
Smoking		
Yes	116.34 (±61.82)	0.076
No	150.88 (±76.03)	
DM		
Yes	146.44 (±73.62)	0.504
No	132.56 (±71.94)	
Hypertension		
Yes	136.06 (±74.68)	0.867
No	139.92 (±74.68)	
Dyslipidemia		
Yes	141.97 (±78.88)	
No	127.65 (±58.3)	0.479
Stroke History		
Yes	141 (±65.03)	0.749
No	134 (±76.73)	
Fatty liver		
Yes	142.65 (±78.36)	0.589
No	132.16 (±67.36)	

DM=Diabetes mellitus type 2; *Independent T-test.

Table 3. Laboratory Findings and Clopidogrel Resistance

Laboratory findings	Unresponsive N=12	Responsive N =31	Bleeding risk N =14	P-value
Haemoglobin (g/dL)	12. 48 (±1.85)	13.8 (±2.83)	14.02 (±2.27)	0.269*
FBG (mg/dL)	94.5 (80 – 280)	113 (73.7 - 331)	94.6 (77 - 449)	0.389†
HbA1c (%)	5.6 (4.8 – 11.8)	5.7 (4 - 12.3)	5.35 (4.4 - 15)	0.715†
Total Cholesterol (mg/dL)	193 (±47.4)	183.77 (±55.24)	196.42 (±54.26)	0.730*
HDL Cholesterol (mg/dL)	56.08 (±23.7)	48.67 (±10.81)	47.92 (±14.66)	0.307*
LDL Cholesterol (mg/dL)	133 (±50.42)	125.35 (±45.15)	126.07 (±45.47)	0.882*
Triglyceride (mg/dL)	147.5 (61 - 503)	150 (57 – 497)	140.5 (57-199)	0.596†
AST (IU/L)	18.67 (±7.64)	19.5 (±6.86)	19.07 (±5.83)	0.933*
ALT (IU/L)	20 (6 – 35)	21 (9 - 50)	19.5 (8 - 58)	0.756†
Urea (mg/dL)	30 (16 – 54)	27 (7 -168.4)	27 (17 - 55)	0.657†
Creatinine (mg/dL)	0.9 (0.5 – 3)	1 (0.48 - 17.8)	0.85 (0.5 - 2.48)	0.679†

FBG=Fasting Blood Glucose; HbA1c=Haemoglobin A1c; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; *One Way ANOVA; †Kruskal-Wallis.

Discussion

This study found that 15.8% ischemic stroke patients were resistant to clopidogrel. A significant association was found between gender and clopidogrel resistance (p=0.006) showing that males have 80% lower chance of having clopidogrel resistance. These results were slightly different than previous studies, which found that clopidogrel resistance in stroke patients varied from 28% to 44%. Some possible mechanisms have been suggested for antiplatelet resistance. These are generally divided into two mechanisms, the first being due to inadequate inhibition of COX-1 or P2Y12, and the second antiplatelet resistance despite adequate inhibition of COX-1 or P2Y12 (6, 7).

The first mechanism may occur due to a decrease in bioavailability caused by poor compliance, inappropriate dosing, a decrease in absorption, drug interaction, and the high metabolism of clopidogrel. Aside from bioavailability, gene polymorphism related to the P2Y12 receptor gene and CYP3A4, CYP1A2, CYP2C19 may also cause clopidogrel resistance. As for the second mechanism, it may be due to the activation of bizarre platelet stimulation pathways, including an increase in epinephrine mediated platelet activation, COX-2 expression induced by stress in platelets, high ADP and collagen platelet sensitivity, high release of ADP, and excessive activation of platelets induced by red cells. Another possible aetiology includes high platelet turnover induced by stress, surgery, acute ischemic or inflammation.

Currently there are no exact standards or techniques for measuring antiplatelet resistance. Some platelet function examinations are used, such as VerifyNow, Platelet Function Analyser and Thromboelastography, which are used to assess clopidogrel resistance in clinical studies. However, there are limitations to the interpretation of the results. Meanwhile, susceptibility to clopidogrel resistance is extremely important, as a study by Xi et al. showed that stroke patients with clopidogrel resistance may exhibit early neurological degradation and more frequent recurrence of ischemic stroke, with poor recovery of neuron cells (3, 4).

The associations between several risk factors that cause clopidogrel resistance were studied by Patel et al., including age, gender, concomitant drugs, duration of antiplatelet therapy, and NSAID and statin consumption (8). On the basis of their study, it is known that there is no significant association between clopidogrel resistance and those factors. Our study also showed that there is no significant association between age and clopidogrel resistance. However, in contrast to Patel et al., our study found that there was a significant association between gender and clopidogrel resistance (P=0.006). Furthermore, we found that men

have an 80% lower chance of having clopidogrel resistance (OR 0.2; 95% CI 0.022 – 0.638). This finding is also supported by the fact that women tend to show higher VerifyNow levels than men (P=0.036). A plausible reason for these findings is that there is a relationship between clopidogrel resistance and the gene expressed in the sex chromosome. Therefore, further studies in gene polymorphism are needed to confirm these findings.

Kang et al. found that there was no significant relationship between smoking and clopidogrel resistance (9). That finding is in contrast to another study by Maruyama et al. that showed that ischemic stroke patients who were smokers had a lower chance of developing clopidogrel resistance compared to patients who were not smokers (10). To emphasize, this means that the responsiveness of clopidogrel is enhanced in patients who are smokers. Maruyama et al. also explained in their previous finding that smokers have a higher level of VerifyNow scores than people who do not smoke (10).

Our study had the same perspective as Kang et al.'s study that smoking was not significantly associated with the VerifyNow levels (9). Additionally, we observed that, contrary to Maruyama et al.'s study, smokers had a lower VerifyNow score compared to non-smokers (P=0.076) (10). The explanation of these findings is still inconclusive since there are not many studies that have explained this association. There were other factors that this study observed, such as diabetes mellitus, hypertension, and atrial fibrillation, which were found to be insignificant (10). Kang et al. showed that diabetes mellitus is an independent risk factor for clopidogrel resistance (9). However, in our study, we found no association between HbA1c or fasting blood glucose levels and clopidogrel resistance.

Limitations of the Study

The main limitation of this study is the relatively small number of patients.

Conclusion

In conclusion, our study showed that 15.8% of patients in the population studied suffered from clopidogrel resistance, where women had a higher risk of clopidogrel resistance. We also found no association between smoking and clopidogrel resistance (P=0.051). Overall, we can say that clopidogrel is still potent for ischemic stroke patients since our data found that the majority of patients (84.2%) showed responsiveness to clopidogrel, based on VerifyNow results.

What Is Already Known on This Topic:
The superiority of clopidogrel as a treatment for some diseases such as stroke and coronary heart disease since it demonstrates better safety than previous treatments, such as aspirin.

What This Study Adds:
Clopidogrel resistance and its association with some risk factors.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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