

Evaluating Resistance to Acetyl Salicylic Acid Using Platelet Function Test in Patients with Ischemic Stroke at Cipto Mangunkusumo Hospital

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ABSTRAK

Tujuan: mengetahui prevalensi resistensi laboratorik ASA dengan uji fungsi trombosit pada pasien stroke iskemik di RSCM dan faktor-faktor yang berhubungan. **Metode:** desain penelitian potong lintang melibatkan 50 penderita stroke iskemik yang hanya mendapatkan terapi ASA. Pemeriksaan resistensi ASA dengan uji fungsi trombosit Verifynow[®]. Resistensi ASA dinyatakan jika ASA reaction unit (ARU) ≥ 550 . **Hasil:** didapatkan 7 penderita resistensi ASA. Kelompok resistensi ASA memiliki rerata usia 51,3 \pm 9,2 tahun sementara kelompok respons ASA memiliki rerata usia 57,8 \pm 9,7 tahun. Pada kelompok resistensi ASA 85,7% berjenis kelamin laki-laki, 57,1% perokok aktif dan 100% menderita hipertensi. **Kesimpulan:** didapatkan prevalensi resistensi laboratorik ASA dengan uji fungsi trombosit pada penderita stroke iskemik di RSCM sebesar 14%. Terdapat kecenderungan prevalensi resistensi laboratorik ASA lebih banyak terjadi pada penderita laki-laki, perokok aktif, berusia lebih muda dan menderita hipertensi.

Kata kunci: stroke iskemik, resistensi ASA, uji fungsi trombosit.

ABSTRACT

Aim: to identify the prevalence of laboratoric ASA resistance using platelet function tests in patients with ischemic stroke at Cipto Mangunkusumo Hospital and its associated factors. **Methods:** this study was a cross-sectional study involving 50 patients with ischemic stroke who only received ASA treatment. Evaluation of resistance to ASA was performed using Verifynow[®] platelet function test. ASA resistance was defined as ASA reaction unit (ARU) ≥ 550 . **Results:** there were 7 patients with ASA resistance. The mean age of subjects in ASA resistance group was 51.3 \pm 9.2 years; while in ASA responsive group was 57.8 \pm 9.7 years. In ASA resistance group, there were 85.7% male patients, 57.1% active smoker and 100% subjects with hypertension. **Conclusion:** the prevalence of laboratoric ASA resistance in patients with ischemic stroke evaluated using platelet function test at Cipto Mangunkusumo Hospital is 14%. The prevalence is more likely to occur in male patients who were active smoker, at younger age and with comorbidity of hypertension.

Key words: ischemic stroke, ASA resistance, platelet function test.

INTRODUCTION

Stroke patients are at high risk of recurrent ischemic stroke, which leads to high mortality and morbidity rates.^{1,2} A study in Indonesia shows 20% incidence of recurrent stroke, particularly ischemic stroke.³

Antiplatelet agents are drugs that inhibit platelet aggregation and prevent thrombus formation, thus prevent recurrent ischemic stroke. Acetyl salicylic acid (ASA) is an effective antiplatelet agent widely used for secondary prevention of recurrent ischemic stroke.^{4,5}

Some studies found a group of patients that do not experience beneficial effects of ASA treatment. Many factors have been assumed to be the causes including non-optimal ASA dose, non-compliance and genetic polymorphism, which may lead to ineffective ASA treatment. Patients who do not respond to ASA treatment are called as ASA resistance.^{5,6} Some studies have demonstrated that patients with ASA resistance are associated with poorer prognosis compared to those who respond well to ASA treatment.^{5,7,8}

Until now, there is no data about the prevalence of ASA resistance in patients with ischemic stroke in Indonesia, especially at Cipto Mangunkusumo Hospital. Therefore, this study was aimed to demonstrate laboratoric ASA resistance using platelet function test in patients with ischemic stroke at Cipto Mangunkusumo Hospital and its associated factors.

METHODS

This is a cross-sectional study on patients with ischemic stroke conducted in the ward and outpatient unit of Cipto Mangunkusumo Hospital between April and May 2014. Subjects were recruited by consecutive sampling. The inclusion criteria were adult patients diagnosed as ischemic stroke with or without imaging features, those who had ischemic stroke for the first time and received 80 mg ASA treatment for at least 5 days. The exclusion criteria were patients who used antiplatelet/anticoagulants other than ASA and non-compliance to the treatment.

ASA resistance or response was evaluated based on Verifynow® platelet function test. The Verifynow® platelet function test is a simple, fast and accurate method which has been validated

with the gold standard of platelet function test using light transmittance aggregometry (LTA).⁷ The threshold level of ASA reaction unit (ARU) was used to define one's non-responsiveness (resistance) to ASA. ASA resistance was defined as $ARU \geq 550$.⁹⁻¹¹

RESULTS

There were 50 patients with ischemic stroke for the first time, who were using only ASA as their single antiplatelet treatment and they were taking 80 mg ASA regularly for at least 5 days. The mean age of all subjects was 56.8 ± 9.8 years. Twenty nine (58%) subjects were male patients. Most patients with stroke were at the age group of 40-65 years, i.e. at 39 years (78%). Of all subjects, sixteen subjects (32%) were categorized as patients with new ischemic stroke who were having or had hospitalization at the ward with range of stroke onset of 6 - 24 days; the remaining 34 (68%) subjects were patients with old ischemic stroke who were having routine visit to outpatient unit with range of stroke onset of 1.5 months to 9 years.

The highest percentage of concomitant disease was hypertension, i.e. 49 (98%) subjects, followed by dyslipidemia in 23 (46%) subjects, diabetes in 15 (30%) subjects, inflammation in 5 (10%) subjects; while CAD and infection had the lowest percentage, i.e. 4 (8%) subjects.

The majority of subjects received ASA in the form of non-enteric coating, i.e. 27 (54%) subjects. The most common side effect complained by the users was gastric pain. Twenty three (46%) subjects had short-duration ASA treatment with the range of 5-24 days. Ten (30%) subjects who were the new user of ASA treatment had a loading dose of 320 mg at the beginning of treatment, i.e. those with acute ischemic stroke with onset of <48 hours.

Seven (14%) subjects with ASA treatment, also had PPI treatment, but there was no subject receiving NSAID as anti-inflammatory treatment. Data of subject characteristics on sociodemography, concomitant disease and ASA treatment for all subjects can be seen in **Table 1**.

The majority of stroke ischemic patients with hypertension had controlled systolic and diastolic blood pressure, either with single

or combined anti-hypertensive agent(s). The majority of stroke ischemic patients with diabetes also had controlled glucose level, either with hypoglycemic agent alone or combined treatment with insulin. Data of clinical and laboratory characteristics of all subjects can be seen in **Table 2**.

The prevalence of laboratory ASA resistance in patients with ischemic stroke at Cipto Mangunkusumo Hospital in our study was 14%. The group responsive to ASA treatment had ARU score with median value of 421 (350-548); while the ASA resistance group had ARU score with median of 650 (556-666).

Higher prevalence of ASA resistance was also found in male patients compared to female patients, aged 40-65 years, with old ischemic stroke and active smoker compared to those with no / had stopped smoking (**Table 3**). However, further statistic tests were not performed due to a small sample size.

The description on the prevalence of ASA resistance with concomitant disease revealed that all patients with ASA resistance had hypertension, 3 patients (42.85%) had dyslipidemia and only 1 patient had diabetes or CAD or inflammation. The description on the prevalence of ASA resistance and the characteristics of concomitant disease can be seen in **Table 4**.

The description on prevalence of ASA resistance with clinical and laboratory characteristics demonstrated younger age of subjects in ASA resistance group. For other parameters, there was no difference between subjects in ASA resistance group and ASA responsive group. The description of ASA resistance with clinical and laboratory characteristics can be seen in **Table 5**.

The description on prevalence of ASA resistance with characteristics of ASA treatment (**Table 6**) revealed that there were 71.4% subjects in ASA resistance group received ASA treatment without enteric coating, 1 patient had received loading dose at the beginning of the treatment and subjects in ASA resistance group did not have drug interaction with NSAID or PPI.

Table 1. Characteristics of sociodemography, concomitant disease and ASA treatment of all subjects

Characteristics	N (%)
Sex	
- Male	29 (58%)
- Female	21 (42%)
Age group	
- < 40 years	3 (6%)
- 40 – 65 years	39 (78%)
- > 65 years	8 (16%)
Stroke Onset	
- New stroke	16 (32%)
- Old stroke	34 (68%)
Smoking	
- Active	9 (18%)
- No / stop smoking	41 (82%)
Hypertension	
- Yes	49 (98%)
- No	1 (2%)
Diabetes	
- Yes	15 (30%)
- No	35 (70%)
Dyslipidemia	
- Yes	23 (46%)
- No	27 (54%)
CAD	
- Yes	4 (8%)
- No	46 (92%)
Infection	
- Yes	4 (8%)
- No	46 (92%)
Inflammation	
- Yes	5 (10%)
- No	45 (90%)
Duration of ASA treatment	
- Short duration	23 (46%)
- Long duration	27 (54%)
ASA loading dose	
- Given	40 (80%)
- Not given	10 (20%)
Interaction with NSAID	
- Present	0 (0%)
- Absent	50 (100%)
Interaction with PPI	
- Present	7 (14%)
- Absent	43 (86%)

Table 2. Clinical and laboratory characteristics of all subjects

Characteristics	mean value
Age (years)	56.8 ± 9.8
Systolic blood pressure (mmHg)	130 (100-200)
Diastolic blood pressure (mmHg)	80 (60-110)
Fasting blood glucose (mg/dL)	95 (71-214)
Post-prandial blood glucose (mg/dL)	119 (72-238)
HbA1c	6 (4.4-9.7)
Total cholesterol (mg/dL)	166.5 (99-283)
HDL (mg/dL)	46.2 ± 11.7
LDL (mg/dL)	101.5 (46-300)
Triglycerides (mg/dL)	110 (46-300)
Hemoglobin (g/dL)	13.6 ± 1.6
Hematocrit (%)	40.6 ± 4.5
Leukocytes (10 ³ /πL)	7.76 (3.76 – 14.6)
Platelets (10 ³ /πL)	265 ± 53.4

Table 3. Prevalence of ASA resistance and socio-demographic characteristics

Characteristics	ASA resistance (n=7)	ASA response (n=43)
Sex		
- Male	6 (85.7%)	23 (53.5%)
- Female	1 (14.3%)	20 (46.5%)
Age group		
- <40 years	1 (33.3%)	2 (66.7%)
- 40-65 years	6 (15.4%)	33 (84.6%)
- >65 years	0 (0%)	8 (100%)
Stroke onset		
- New stroke	1 (14.3%)	15 (34.9%)
- Old stroke	6 (85.7%)	28 (65.1%)
Smoking		
- Active	4 (57.1%)	6 (14%)
- No/stop smoking	3 (42.3%)	37 (86%)

DISCUSSION

Subject characteristics in our study demonstrated that there were larger number of male subjects with ischemic stroke than female, i.e. 58% vs 42% (1.3 : 1). Based on the age group, the largest number of stroke patients was at the age of 40-65 years (78%). Regarding the risk factors, hypertension had the highest percentage of 98%, which was followed with dyslipidemia in 46% subjects, diabetes in 30% subjects, smoking habit in 14% subjects and CAD in 8%

Table 4. Prevalence of ASA resistance with concomitant disease

Characteristics	ASA resistance (n=7)	ASA response (n=43)
Hypertension		
- Yes	7 (100%)	42 (97.7%)
- No	0 (0%)	1 (2.3%)
Diabetes		
- Yes	1 (14.3%)	14 (32.6%)
- No	6 (85.7%)	29 (67.4%)
Dyslipidemia		
- Yes	3 (42.9%)	20 (46.5%)
- No	4 (57.1%)	23 (53.5%)
CAD		
- Yes	1 (14.3%)	3 (7%)
- No	6 (85.7%)	40 (93%)
Infection		
- Yes	0 (0%)	4 (9.3%)
- No	7 (100%)	39 (90.7%)
Inflammation		
- Yes	1 (14.3%)	4 (9.3%)
- No	6 (85.7%)	39 (90.7%)

Table 5. Prevalence of ASA resistance with clinical and laboratory characteristics

Characteristics	ASA resistance (n=7)	ASA response (n=43)
Age (years)	51.3 ± 9.2	57.8 ± 9.7
Systolic blood pressure (mmHg)	130 (110-200)	135.6 ± 17.6
Diastolic blood pressure (mmHg)	78.6 ± 12.1	80 (60-110)
Fasting blood glucose (mg/dL)	97 (83-130)	95 (71-214)
Post-prandial blood glucose (mg/dL)	109 (72-238)	122 (75-234)
HbA1c	5.5 ± 0.6	6 (4.4-9.7)
Total cholesterol (mg/dL)	140 (124-283)	173.9 ± 40.9
HDL (mg/dL)	46.7 ± 14.9	46.1 ± 11.3
LDL (mg/dL)	77 (58-197)	109.2 ± 37.8
Triglycerides (mg/dL)	111.3 ± 54.2	110 (46-300)
Hemoglobin (g/dL)	13.5 ± 1.7	13.6 ± 1.6
Hematocrit (%)	40.0 ± 5.5	40.7 ± 4.4
Leukocytes (10 ³ /πL)	7.61 ± 1.75	8.10 ± 1.93
Platelets (10 ³ /πL)	266 ± 73.9	265.3 ± 50.5

subjects. A study conducted by Misbach J,³ which evaluated 3723 patients with stroke in 44 hospitals in 7 countries of ASEAN, reported that

Table 6. Prevalence of ASA resistance and characteristics of ASA treatment

Characteristics	ASA resistance (n=7)	ASA response (n=43)
Enteric coated ASA		
- Coating	2 (28.6%)	21 (48.8%)
- No Coating	5 (71.4%)	22 (51.2%)
Duration of ASA treatment		
- Short duration	4 (57.1%)	19 (44.2%)
- Long duration	3 (42.9%)	24 (55.8%)
Loading dose of ASA		
- Not given	6 (85.7%)	34 (79.1%)
- Given	1 (14.3%)	9 (20.9%)
Interaction with NSAID		
- Present	0 (0%)	0 (0%)
- Absent	7 (100%)	43 (100%)
Interaction with PPI		
- Present	0 (0%)	7 (16.3%)
- Absent	7 (100%)	36 (83.7%)

55% patients with stroke are male, with mean age of 59.0 ± 13.8 years and 47% patients are at the age group of 45-64 years, with the highest risk factor of hypertension (67.5%), followed by TIA (26.1%), hypercholesterolemia (19%), smoking (18.8%), diabetes (17.6%) and coronary heart disease (3.5%). Characteristics of subject population in our study can represent the general characteristics of patients with ischemic stroke.

ASA has been used widely as the standard treatment for ischemic stroke, both for acute and chronic stroke. The treatment is effective for recurrent ischemic stroke prevention and has been proven to lower mortality rate in acute phase. The Chinese Acute Stroke Trial (CAST) and International Stroke Trial (IST) demonstrate that immediate ASA treatment in patients with ischemic stroke or suspected ischemic stroke can lower the risk of both fatal and non-fatal recurrent stroke significantly, reduce mortality rate in hospitals and increase functional recovery of patients with stroke.¹²

ATT Collaboration conducted a meta-analysis involving 16 studies on secondary prevention of stroke, which reported that ASA treatment can significantly lower the incidence of recurrent ischemic stroke by 20% ($p < 0.001$).⁶

Other than effective, the treatment is also cost friendly compared to other treatment of antiplatelet and it is covered by the national health insurance (JKN) for all Indonesian citizen. It becomes the first line treatment for all patients with ischemic stroke.

In reality, there are incidences of recurrent ischemic stroke in patients who have received regular ASA treatment. One of the causes is resistance to ASA treatment. ASA resistance is clinically defined as the inability of ASA treatment to prevent the development of recurrent ischemic stroke; while the laboratory definition of ASA resistance is the inability of ASA treatment to reduce the thromboxane A₂ level or the inability to inhibit platelet aggregation.^{13,14}

There are various laboratory methods for evaluating ASA resistance. However, regardless of its assessment method, laboratory ASA resistance is associated with increased risk of recurrent ischemic stroke. A study conducted by Grotmeyer et al.^{10,15}, evaluating 180 patients with ischemic stroke who received ASA treatment demonstrated that there were 33% of patients with ASA resistance with 10 fold higher increase of recurrent ischemic stroke, myocardial infarct and death due to vascular events. The most recent study conducted by Zheng et al.¹⁶ showed that ASA resistance is associated significantly with the degree of severity of acute stroke in ASA resistance group with a median of National Institutes of Health Stroke Scale (NIHSS) score of 11 compared to the responsive ASA group, which had median NIHSS score of 4 ($p < 0.001$).

In our study we found that the prevalence of laboratory ASA resistance in patients with ischemic stroke at Cipto Mangunkusumo Hospital was 14%, which is a relatively high, in which 1 of 7 patients with ischemic stroke receiving routine ASA treatment actually did not respond to the treatment (ASA resistance). The prevalence rate is equivalent to results of study conducted by Kim et al.¹⁷, which evaluated 178 patients with ischemic stroke and coronary artery disease (CAD) who received ASA treatment in Korea. The method of determining ASA resistance in our study is also similar to those in the study conducted by Kim et al.,¹⁷ i.e. by using Verify now method with cut-off point $ARU \geq 550$

is considered as ASA resistant.

The prevalence of ASA in our study is also almost similar to the results of study by Navarro et al¹⁸, that found ASA resistance of 10.4% in 77 patients with ischemic stroke in Philippine using Ultegra (which has similar principles on mechanism of action to the verify now method) with an equal cut-off point, i.e. $ARU \geq 550$ is defined as ASA resistant. The similarity of results on the prevalence of ASA resistance in those three studies may be caused by the similarity in method of assay, similar cut-off point for ASA resistance of $ARU \geq 550$, similar sociodemographic and clinical characteristics as well as concomitant risk factors in patients with ischemic stroke who had become the subjects of studies and may be also because of similar genetic factors.

Many factors have been assumed to be the cause of ASA resistance, including the factor of using enteric coating ASA, interaction with other drugs, duration of treatment, the presence of concomitant disease and smoking habit.

In our study, there was a tendency of greater ASA resistance in the group of male subjects compared to the female subjects, in the age group of <40 years compared to those of >65 years and in the group of active smoker compared to those who were not smokers or had stopped smoking. However, further statistical analysis is not possible to be done considering the limited number of samples. The tendency found in our study is consistent with the results of previous studies on the prevalence of ASA resistance. A study conducted by Zheng et al.¹⁶, evaluating the prevalence of ASA resistance in 90 patients with acute ischemic stroke at the Royal Melbourne Hospital, Australia found a significant correlation between the prevalence of ASA resistance and patients' smoking habit (OR 3.08; 95% CI, 1.17-8.13; $p=0.02$). It is assumed that smoking can increase the production of isoprostane (a prostaglandin-F₂-like substance) that can produce thromboxane from the alternative non-COX pathway, which is not inhibited by ASA.¹⁹

Regarding the correlation between ASA resistance and demographic, clinical, laboratory and treatment characteristics, various studies, including the studies conducted by Ozben et al.¹¹ and Kim et al.¹⁷ did not find a significant

difference of concomitant disease such as hypertension, diabetes, dyslipidemia and other laboratory parameters with characteristics of treatment between the group of ASA resistant and ASA responsive. In our study, although there is a tendency that all patients who have ASA resistance are also have hypertension, but an evaluation to study statistical correlation was not possible due to a small number of subjects.

CONCLUSION

Our study found a relatively high prevalence of laboratory ASA resistance in patients with ischemic stroke at Cipto Mangunkusumo Hospital, i.e. as many as 14%, which means that 1 out of 7 patients with ischemic stroke who receive ASA treatment still have the risk for recurrent stroke because of resistance to the treatment.

There is a tendency that the prevalence of laboratory ASA resistance is higher in male patients, active smoker, younger patients and those with hypertension; however, to confirm the statistical significance, further studies must be carried out with a larger sample size.

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