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Procoagulant Phospholipid Activity and MPV Values in Acute Ischemic Stroke

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ABSTRACT

Ischemic stroke can be caused by atherothrombosis or embolism. Atherothrombosis occurs due to the rupture of an atherosclerotic plaque causing platelet activation. There are several markers of platelet activation, including platelet microparticles. The levels of platelet microparticles can be measured by examining procoagulant phospholipid (PPL) activity. It is not yet known exactly what activates thrombopoiesis, which can be assessed by an increase in Mean Platelet Volume (MPV). This study aimed to know whether there is an increase in PPL activity and MPV values in acute ischemic stroke, whether platelet activation is influenced by differences in ischemic stroke subtypes, and whether there is a relationship between PPL activity and MPV values in acute ischemic stroke. The study design was cross-sectional and involved 60 subjects. PPL activity in acute ischemic stroke (65.14±13.35 seconds) tends to be higher (shorter clotting time) than in healthy individuals (68.59±8.56 seconds), however, this difference was not statistically and clinically significant. The MPV value in acute ischemic stroke (9.83±0.72 fL) compared to healthy individuals (9.65±0.86 fL) however this difference was not statistically significant. PPL activity in the SAO subtype (61.66±1.31 seconds) tends to be higher than LAA (68.62±14.57 seconds), however, this difference was also not statistically significant. There was a weak correlation between PPL activity (seconds) and MPV value (fL) in acute ischemic stroke (r =0.34, p-value=0.03).

Keywords: Acute ischemic stroke, PPL activity, MPV value

INTRODUCTION

The prevalence of stroke cases in Indonesia has increased from 7‰ in 2013 to 10.9‰ in 2018.[1] Stroke attacks often recur and result in permanent disability, morbidity, and mortality. Based on the cause, stroke is classified into two groups, ischemic stroke, which is the cause of the majority of stroke cases (85%), and hemorrhagic stroke, which occurs in 15% of cases. Ischemic stroke is a circulatory disorder in the brain caused by reduced or stopped blood flow in areas of the brain, ischemic stroke can be caused by thrombosis or embolism separated from atherosclerotic lesions in the heart or arteries in the brain.[2-7]

Based on the criteria of the Trial of Org 10172 in Acute Stroke Treatment (TOAST), acute ischemic stroke is classified into two subtypes, Large Artery Atherosclerosis (LAA) and Small Artery Occlusion (SAO) or lacunar stroke. About 25% of ischemic strokes are lacunar strokes. Large artery atherosclerosis consists of cortical strokes and posterior circulation strokes. Cortical stroke is about 60% of cases of ischemic stroke, the cause is thrombosis or embolism of cerebral blood vessels originating from the heart or carotid arteries and their branches.[8]

The underlying causes of cardiovascular disease and acute ischemic stroke have similarities, most are due to atherosclerosis. Unstable atherosclerotic plaques can tear, especially in the plaque shoulder area. Plaque tears will activate platelets and coagulation factors in the blood that cause thrombosis. When platelets are activated, microparticles are formed which are thrombogenic, thus facilitating thrombosis. Loose plaque can be carried into the bloodstream and cause an embolism. A thrombus or embolus causing occlusion of a cerebral artery will result in an ischemic stroke.

Platelets are known to have a role in atherosclerosis. Parameters that affect platelets are the Mean Platelet Volume (MPV), Platelet Count (PC), plateletcrit (PCT), and Platelet Distribution Width (PDW). One of the most studied markers of platelet

activation is MPV since large platelets are more reactive than small ones. Previous studies have found MPV significantly increased in ischemic stroke. Platelet activation causes changes in platelet quantity and morphology, PDW is used to determine the heterogeneity of platelet size. The number of platelets in the blood and PCT can be measured. These parameters are useful in evaluating platelet function, but whether they also have the potential to monitor the development of ischemic stroke still needs to be investigated.[8-10]

Research on platelet activation markers has been widely carried out in the world, but the results are still inconsistent. Research on platelet activation markers in Indonesia by examining the activity of platelet microparticles as assessed by examining PPL activity in patients with cardiovascular disease has been carried out, and there are significant differences compared to healthy individuals, but none have been carried out in acute ischemic stroke patients. Therefore, researchers want to prove whether there is an increase in PPL activity and MPV value as a marker of platelet activation in acute ischemic stroke patients and want to know whether there are differences in ischemic stroke subtypes and whether there is a relationship between PPL activity and MPV value in ischemic stroke patients at the National Brain Center Hospital (RSPON).

METHODS

This study used a cross-sectional design. The study inclusion criteria were patients diagnosed with acute ischemic stroke who were willing to participate in the study by signing an informed consent. The study exclusion criteria were patients who had taken anti-platelet drugs regularly. Sampling was done by consecutive sampling. The research subjects consisted of 20 acute ischemic stroke patients with LAA and SAO subtypes and 20 healthy individuals. The healthy group aged 40-65 years without bleeding symptoms, no smoking, not obese, not taking anti-platelet drugs, NSAIDs, or anticoagulant drugs, and no history of hypertension, dyslipidemia, diabetes mellitus, heart disease, and stroke.

Research materials for examining PPL activity were obtained from patient blood specimens. Specimens were taken with a 9:1 ratio of 3 mL of citrate anticoagulant, and 1 blood draw. Then PPL activity was examined using the STA Compact coagulometer from Stago. The research material for the MPV values was obtained from the patient's blood specimen. Specimens were taken with 3 mL of EDTA anticoagulant, and 1 blood draw. Then a complete hematological examination was carried out using the XN-1000 hematology instrument from Sysmex.

The analysis was carried out using the SPSS version 20. Tests were carried out for accuracy and within run accuracy and between day PPL activity accuracy and MPV values. To find out the distribution of data analyzed using the Shapiro-Wilk test. Numerical data with normal data distribution were analyzed using the parametric student T-test. Abnormal distribution was analyzed using the Mann-Whitney non-parametric test. Analysis of differences between PPL activity and MPV values with subtypes of acute ischemic stroke patients used the parametric two-way ANOVA test if the data distribution was normal or the non-parametric Mann-Whitney test if the data distribution was not normal. Analysis of the relationship between PPL activity and MPV value used a correlation test. This research has received ethical approval from the Research Ethics Committee of the National Brain Center Hospital with number UM.01.05/12/008/2020.

RESULTS AND DISCUSSIONS

The results of the within-run accuracy test for examining PPL activity showed that the Coefficient of Variation (CV) for control N and control P was 0.85% each. The results of the accuracy test obtained the deviation range (d) from the N control +2.8% and the P control -2.6%. The results of the within-run accuracy test for examining the MPV values obtained the CV from level 1, level 2, and level 3, respectively of 0.93%, 0.88%, and 0.48%. The results of the accuracy test obtained the range of deviations (d) from level 1, level 2, and level 3, respectively 1.10%, 1.05%, and -1.06%. The results of the between-day accuracy test were carried out once on each day working on the research sample. The results of the between-day accuracy test for examining the MPV value obtained the CV from level 1, level 2, and level 3, respectively 2.25%, 1.42%, and 1.25%.

Healthy individuals who met the criteria in this study were 20 subjects, consisting of 15 (75%) female and 5 (25%) male. The age group of 40-49 years is the largest, amounting to 13 (65%) subjects. Of the 40 study subjects, the most common risk factor was dyslipidemia with the highest degree of stroke in the mild category (Table 1).

In patients with acute ischemic stroke, the average PPL activity (seconds) was shorter and smaller in ratio than in healthy individuals, although this difference was not statistically significant (p=0.23) (Table 2).

Table 1. Characteristics of research subjects

Characteristics'	Healthy Individuals	Patients with Acute Ischemic Stroke	
		LAA	SAO
Gender			
Male	5 (25)	14 (70)	15 (75)
Female	15 (75)	6 (30)	5 (25)
Age (years old)			
40 -49	13 (65)	3 (15)	5 (25)
50 -59	5 (25)	7 (35)	7 (35)
≥ 60	2 (10)	10 (50)	8 (40)
Referral origin			
Home	-	20 (100)	20 (100)
Nursing home	-	0 (0)	0 (0)
Other hospital	-	0 (0)	0 (0)
NIHSS score			
Mild	-	7 (35)	13 (65)
Moderate	-	11 (55)	6 (30)
Severe	-	2 (10)	1 (5)
Smoking			
Yes	0 (0)	8 (40)	9(45)
No	20 (100)	12 (60)	11 (55)
Obesity			
Yes	0 (0)	8 (40)	7 (35)
No	20 (100)	12 (60)	13 (65)
Hypertension			
Yes	0 (0)	15 (75)	15 (75)
No	20 (100)	5 (25)	5 (25)
Diabetes mellitus			
Yes	0 (0)	7 (35)	8 (40)
No	20 (100)	13 (65)	12 (60)
Heart diseases			
Yes	0 (0)	1 (5)	0 (0)
No	20 (100)	19 (95)	20 (100)
Dyslipidemia			
Yes	0 (0)	3 (15)	6 (30)
No	20 (100)	17 (85)	14 (70)

Table 2. Differences in PPL activity results of healthy individuals and acute ischemic stroke patients

Group	PPL Activity		p-value
	Second	Ratio	
Healthy individuals	68.59±8.56	1.00±0.12	0.23
Acute ischemic stroke	65.14±13.35	0.95±0.19	

In the ischemic stroke of the SAO subtype,the average PPL activity (seconds) was shorter and smaller in ratio than the LAA subtype. The results showed no statistically significant difference (Table 3).

The results of PPL activity on the patient's risk factors showed that patients with hypertension had more statistically significant abnormal PPL activity than normal subjects (p=0.02) (Table 4) (Figure 1).

Table 4. PPL activity on risk factors in acute ischemic stroke patients

Risk Factors	PPL Category		p-value
	Normal	Abnormal	
Smoking	8 (47.1)	9 (52.9)	0.16
Obesity	12 (80)	3 (20)	0.15
Hypertension	3 (30)	7 (70)	0.02
DM	14 (56)	11 (44)	0.45
Dyslipidemia	20 (64.5)	11 (35.5)	0.70
NIHSS			
Mild	14 (70)	6 (30)	
Moderate	8 (47.1)	9 (52.9)	0.13
Severe	3 (100)	0 (0)	

In acute ischemic stroke, the mean MPV in fL was slightly higher than that of healthy individuals (p=0.41) (Table 5).

Table 3. Differences in PPL activity results between ischemic stroke subtypes

Ischemic Stroke Subtypes	PPL Activity	p-value	PPL Activity	p-value
	Second		Ratio	
LAA	68.62±14.57	0.23	1.00±0.21	0.10
SAO	61.66±11.31		0.90±0.16	

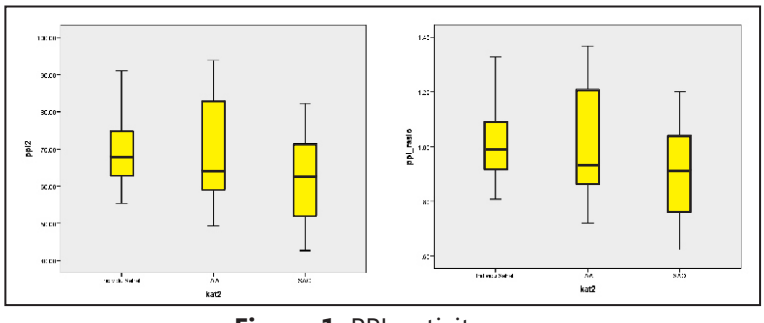

Figure 1. PPL activity

Table 5. Differences in the results of MPV activity (fL) in healthy individuals and ischemic stroke patients

Group	MPV Value fL	p-value
Healthy individuals	9.65±0.86	0.41
Acute ischemic stroke	9.83±0.72	

In the ischemic stroke of the LAA subtype, the mean MPV value in fL was higher than that of the SAO subtype (p=0.41) (Table 6).

Table 6. Differences in MPV (fL) values between ischemic stroke subtypes

Ischemic Stroke Subtypes	MPV Value fL	p-value
LAA	9.90±0.68	0.41
SAO	9.75±0.76	

It is noted that increased MPV acts as an indicator of increased platelet activation and is directly related to the severity of stroke, the larger the MPV, the worse the outcome.[11] This is in line with this study and other studies that found patients in the acute ischemic stroke group had significantly higher MPV (12.45 fL compared with the normal range of 6–11 fL in control) (Figure 2). The result also indicated an independent association between MPV and stroke therefore elevated MPV may be assessed as a risk factor for acute ischemic stroke.[12] An association of MPV with stroke is possibly due to higher platelet activity, aggregation, and turnover in patients with elevated MPV. It has been shown that increased MPV is linked to more hemostatically active platelets with higher avidity to aggregate.[13] Other study also found that MPV related to the severity of stroke with the result mean MPV of the study population with mRS 1, 2, and 3 were 5.7 fL, 6.5 fL, and 8.4 fL, respectively, while the patients having severe functional impairment with mRS 4 and mRS 5 had MPV of 10.1 fL and 10.6 fL, respectively with 0.818 Pearson correlation coefficient, which was statistically significant.[14] Other studies also found that MPV was a risk factor for ischemic stroke in patients with non-valvular atrial fibrillation. The ischemic stroke event rates were significantly increased in the highest MPV tertile when compared to the lowest MPV tertile (56.9% vs. 30.3%, p<0.001).[15] Nayyar *et al.* found that higher MPV and MPV/PC ratios were related to acute ischemic stroke compared to healthy individuals. Greater MPV and MPV/PC ratio values were associated with increased infarct size.[16] Other studies found that MPV values in ischemic stroke were higher than MPV in hemorrhagic stroke, although the difference was not statistically significant.[17]

The results of the correlation test between PPL activity (seconds) and MPV value (fL) in the ischemic stroke patient group showed a correlation coefficient (r) of 0.34 (p=0.03) (Figure 3). The correlation between PPL activity (seconds) and MPV value (fL) in the LAA subtype ischemic stroke patient group showed an r of 0.43 (p=0.06). Meanwhile, the correlation between PPL activity (seconds) and MPV value (fL) in the ischemic stroke patient group of the SAO subtype showed an r of 0.21 (p=0.37) (Table 7).

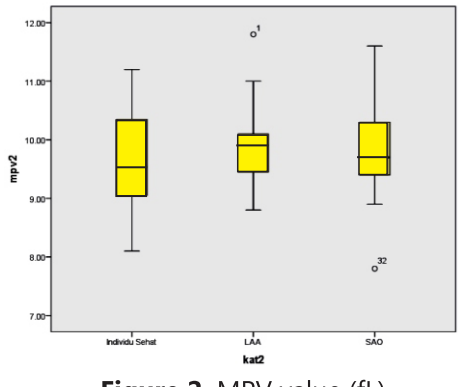

Figure 2. MPV value (fL)

Table 7. Correlation of PPL activity (seconds) and MPV values (fL)

Group	Correlation Coefficient (r)		p-value
Acute ischemic stroke	0.34	Weak	0.03
LAA	0.43	Weak	0.06
SAO	0.21	Very weak	0.37

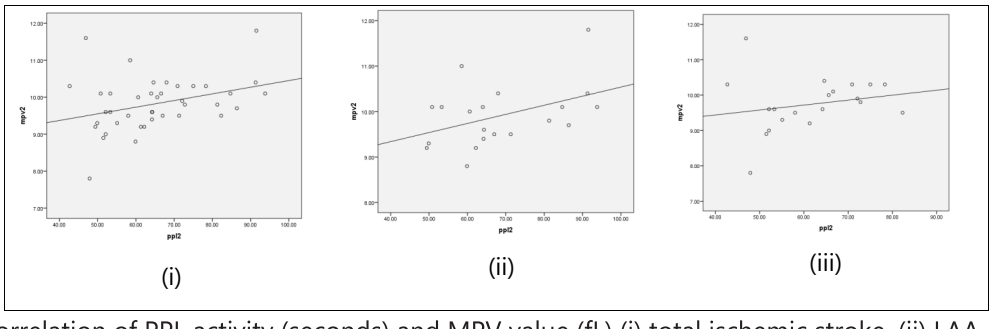

Figure 3. Correlation of PPL activity (seconds) and MPV value (fL) (i) total ischemic stroke, (ii) LAA, and (iii) SAO

CONCLUSIONS AND SUGGESTIONS

Procoagulant phospholipid activity in acute ischemic stroke patients was higher (shorter clotting time) than in healthy individuals, but this difference was not statistically or clinically significant. The results of MPV values in acute ischemic stroke patients compared to healthy individuals showed differences that were not statistically significant. Meanwhile, PPL activity in the SAO subtype was higher than in LAA but was not statistically significant. Patients with risk factors for hypertension showed that more patients had abnormal PPL activity than normal and this was statistically significant. Research on PPL activity as the underlying risk factor for atherosclerosis is needed.

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