The association of plasminogen activator inhibitor-1 level with ischemic stroke (preliminary study)

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Abstrak

Tujuan *Kadar plasminogen activator inhibitor-1 (PAI-1) yang tinggi menyebabkan penurunan aktivitas sistem fibrinolisis. Saat ini kadar PAI-1 yang tinggi diketahui merupakan faktor risiko penyakit jantung iskemik tetapi pada penderita stroke iskemik hal ini masih belum jelas. Pada penelitian ini ingin diketahui hubungan antara kadar PAI-1 dengan stroke iskemik.*

Metode *Dengan menggunakan desain kasus kontrol, kami melibatkan 38 subjek penderita stroke iskemik dan 38 subjek kontrol yang memenuhi kriteria penelitian. Kadar PAI-1 diperiksa dengan metode ELISA menggunakan reagen Asserachrom PAI-1 dari Stago.*

Hasil *Kadar PAI-1 yang tinggi ditemukan lebih sering pada penderita stroke iskemik daripada subjek kontrol (21.1% vs. 7.9 % dengan OR 3.1; 95 % CI 0.757 – 12.790). Analisa terhadap semua subjek yang diteliti menunjukkan adanya hubungan negatif yang lemah namun bermakna antara kadar PAI-1 dengan usia (r = - 0.4; P = 0.000). Kadar PAI-1 yang tinggi ditemukan lebih sering pada subjek berusia muda (40 – 58 tahun) daripada subjek berusia lebih tua (60 – 84 tahun) (20 vs. 9.8 %) (P = 0.004).*

Kesimpulan *Dari hasil penelitian pendahuluan ini diduga ada hubungan antara kadar PAI-1 dengan stroke iskemik pada usia muda. Penelitan lebih lanjut dengan jumlah subjek yang lebih besar diperlukan untuk memastikan keadaan ini.* ***(Med J Indones 2010; 19:158-63)***

Abstract

Aim Recently, increased plasminogen activator inhibitor-1 (PAI-1) has been known a risk factor for ischemic heart disease. However, the association of increased PAI-1 level with ischemic stroke remains unclear. The aim of this study was to analyze the association of PAI-1 level with ischemic stroke.

Methods By case control design we involved 38 ischemic stroke and 38 risky-matched control subjects who fulfilled the criteria. The PAI-1 level was determined by ELISA method using Asserachrom PAI-1 from Stago.

Results High PAI-1 level was found more frequent in ischemic stroke subjects than in control subjects (21.1% vs. 7.9 % with OR 3.1; 95 % CI 0.757 – 12.790). The analysis of all studied subjects showed that there was a weak negative correlation between PAI-1 level and age (r = -0.4; P = 0.000). High PAI-1 level was found more frequent in younger (40 – 58 years old) than in the older subjects (60 – 84 years old) (20% vs. 9.8 %) (p=0.004).

Conclusion The result of this preliminary study suggested an association between PAI-1 level and ischemic stroke in younger age. Further study with larger subjects is recommended to confirm this association. ***(Med J Indones 2009; 19:158 -63)***

Key words: *: ischemic stroke, plasminogen activator inhibitor-1, stroke risk factors*

Stroke is the second most common cause of death in the world, with almost 5.1 million people died annually due to stroke. In an attempt for stroke prevention, stroke risk factors should be recognized and controlled.[1] In the last decade several conditions associated with stroke Have revealed that plasminogen activator inhibitor-1 (PAI-1) was suspected to play a role in stroke incidence.[2]

Plasminogen activator inhibitor-1 is the main physiologic regulator in fibrinolytic system since it can inhibit the activity of plasminogen activator. Increased PAI-1 level results in decreased fibrinolytic activity and, therefore, increases the risk of thrombosis.2 It was reported that PAI-1 may influence the progression of atherosclerosis. Increased PAI-1 level causes atherosclerotic lesion susceptible to thrombotic complication.[3-6]

Increased PAI-1 level has been found in some myocardial infarct cases.[7] The risk for myocardial infarction is 3.35 times higher in subjects with high level of PAI-1 compared to those with the normal level of PAI-1.[8] The properties of vasculature in the heart and brain are different. Although increased PAI-1 level has been known as a risk factor for myocardial infarct, this issue remains unclear for stroke.[7] The association of PAI-1 with other stroke risk factors such as hypertriglyceridemia,[7,9] hyperglicemia,[10,11]

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hyperinsulinemia,[12] and hypertension[13] have been reported by many studies, although the mechanism is not fully understood.[14-16]

The main aim of this study was to analyze the association between PAI-1 level and ischemic stroke. If the association between PAI-1 and ischemic stroke was proved, then reducing the level of PAI-1 may help to prevent stroke. The secondary aim was to analyze the association of PAI-1 level with other stroke risk factors, such as age, gender, hypertension, glucose control, triglyceride level, and obesity.

METHODS

We performed this study by a case control design. The protocol of this study had been approved by the ethical committee of the Faculty of Medicine, the University of Indonesia. Subjects were Indonesian people who were admitted to the Ciptomangunkusumo National Hospital from February until April 2005. We recruited 38 subjects of each group by matching the age and gender. Informed consent was obtained from each patient before inclusion in this study.

Case subjects were taken by consecutive methods from patients who had suffered from ischemic stroke attack within 2 weeks to 3 months prior to inclusion. Subjects with acute inflammation (C-reactive protein/ CRP level was > 10 mg/l as stated in kit insert),[17] or known decreased systemic perfusion and history of myocardial infarction based on medical record were excluded.

Control subjects were subjects who had never had any stroke attack, with age-matched control of within + 2 years old and gender with case subjects, and had one or more stroke risk factors. The stroke risk factors that we included were uncontrolled blood glucose, hypertriglyceridemia, hypertension, and obesity. Based on Consensus for Type 2 Diabetes Mellitus Controlling in Indonesia, uncontrolled blood glucose was stated if HbA1c was > 6.5 %.[18] Based on the third report of the National Cholesterol Education Program, hypertriglyceridemia was stated if the triglyceride level was > 1.7 mmol/l.[19] Based on the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of high blood pressure, hypertension was stated if the systolic and or diastolic blood pressure was > 140 and / 90 mmHg or taking anti-hypertensive. We classified hypertension as uncontrolled if the blood pressure was > 140 / 90 mmHg after treatment. In the event of diabetes mellitus, we classified hypertension as uncontrolled if the blood pressure was > 130 / 80 mmHg.[20] Based on National Obesity Symposium in Indonesia, obese was stated if waist circumference was > 90 cm for male; > 80 cm for female.[21] Control subjects with acute inflammation or history of myocardial infarction were also excluded.

After 12 – 14 hours fasting, blood was drawn from cubital vein with minimal stasis, in less than 2 minutes.[22] Samples were taken between 8 – 9 a.m., then the blood was divided into heparin and 0.109 M citrate vacuum tube. Heparinized blood was used for HbA1c[23] analysis and heparin plasma was used for CRP and triglyceride analysis.[17,24] Citrated blood was immediately stored in a cooling box and was centrifuged within 30 minutes from blood collection, 3000 G for 15 minutes at 40C.[22,25] Citrated plasma was stored at -800C until analysis.[22]

C-reactive protein, triglyceride and HbA1c was measured using reagent from Roche Diagnostics (USA). PAI-1 level was measured by enzyme immunoassay method using Asserachrom PAI-1 (Stago, France). Based on the manufacturer's kit insert, we defined that PAI-1 level was high if the level was more than 43 μg/l.[25]

Data analysis was performed by Statistical Program for Social Sciences (SPSS) program. The association of PAI-1 level with ischemic stroke was analyzed by calculating the odds ratio (OR). The correlation of PAI-1 level and other risk factors for ischemic stroke was analyzed by multivariate analysis.[26,27]

RESULTS

Characteristic of the subjects was shown in Table 1. The mean age of case subjects was 58 years old, ranging from 40 to 83 years old, mostly were male. There was no statistically significant difference of age between control and case subjects *(P = 0.230)*. High PAI-1 level was found in 8 out of 38 (21.1%) stroke cases, while in control subjects it was only 3 out of 38 subjects (7.9 %). From several risk factors that we studied, we found that the proportion of uncontrolled risk factors was greater in control subjects than in case subjects.

Table 1. Subject Characteristic

	Case (N = 38)	Control (N = 38)
Age		
Mean ± SD (years)	58 ± 10	59 ± 11
Gender		
- Male	27 (71 %)	27 (71 %)
- Female	11 (29 %)	11 (29 %)
Risk factors associated with PAI-1*		
- Uncontrolled blood glucose	8 (21 %)	14 (36.8 %)
- Hypertriglyceridemia	21 (55.3 %)	21 (55.3 %)
- Obesity	27 (71.1 %)	30 (78.9 %)
- Hypertension	34 (89.4 %)	29 (76.4 %)
- Controlled	14 (36.8 %)	2 (5.3 %)
- Uncontrolled	20 (52.6 %)	27 (71.1 %)
High PAI-1 level	8 (21.1 %)	3 (7.9 %)

Table 2 showed the measured risk factors and PAI-1 level. Risk factors in case subjects were better controlled than control subjects although the difference was not significant ($p > 0.05$). The median value of PAI-1 level in both case and control subjects were within normal limit, but it was higher in case group than control group although the difference was not significant ($P = 0.280$).

If the 76 total subjects were classified into 4 quartiles according to age, we got 35 subjects were in the first and second quartile (40 – 58 years old) and 41 subjects were in the third and fourth quartile (60 – 84 years old) (Figure 1A and Table 3). High PAI-1 level was found in 11 out of 76 total subjects, 7 subjects were in the first and second quartile (20 %) and 4 subjects were in the third and fourth quartile (9.8 %).

Table 2. Measurement result of risk factors and pai-1 level in case and control subjects

	HbA_{1c} (%)	Triglyceride (mmol/l)	Waist circumference (cm)	Systolic (mmHg)	Diastolic (mmHg)	PAI-1 (µg/l)
Case	5.7 (4.6-13.3)	1.9±0.8	92±10	135 (90-200)	80 (60-140)	23 (6-95)
Control	5.9 (5.0-12.3)	2.2±1.2	96±8	140 (110-220)	90 (60-120)	21 (7-85)
P	0.347#	0.216£	0.057£	0.801#	0.349#	0.316#

#) Mann Whitney test £) T-test

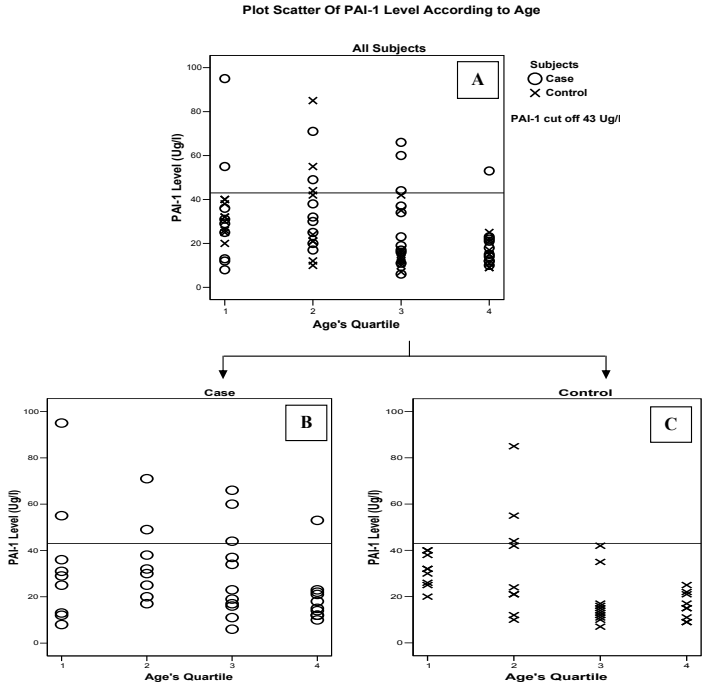

Figure 1. Plot Scatter of PAI-1 Level According to Age. Figure 1A represents all subjects. Figure 1 B represents case subjects. Figure 1C represents control subjects. The age was divided into 4 quartiles: first quartile (40 – 49 years old), second quartile (50 – 58 years old), third quartile (60 – 64 years old), and fourth quartile (65 – 84 years old).

Among all case subjects, there were 17 ischemic stroke subjects in the first and second quartile and 21 ischemic stroke subjects in the third and fourth quartile. In these ischemic stroke subjects, high PAI-1 level was found in 4 out of 17 subjects in first and second quartile (23.5 %) and in 4 of 21 subjects in third and fourth quartile (19 %) (Figure 1B).

Table 3 described the number of subjects who had high and normal PAI-1 level in both case and control groups. The odds ratio was 3.1 with 95 % confidence interval (CI) of 0.757 - 12.790 *(P = 0.103)*.

Table 3. 2x2 table of PAI-1 level and ischemic stroke

		Ischemic stroke		Total
		Case	Control	
High PAI-1 level	Yes	8	3	11
	No	30	35	65
	Total	38	38	76

PAI-1 level was compared according to age's quartile, gender, blood glucose controlling, hypertriglyceridemia, hypertension and obesity (Table 4). There was only a significant difference of PAI-1 level according to age's quartile and hypertension. PAI-1 level was higher in younger subjects than in the older subjects *(P = 0.004)*.

Table 4. Comparison of PAI-1 level median according to age's quartile, gender, and risk factor control

Stroke Risk Factor		PAI-1 Level (µg/l) Mean (Range)	*p* value
Age's quartile (years old)	I (40–49)	30.5 (8–95)	0.004¥
	II (50–58)	30 (10 – 85)	
	III (60–64)	16.5 (6–66)	
	IV (65–84)	15 (9–53)	
Gender	Female	17.5 (9–85)	0.076*
	Male	25 (6–95)	
Uncontrolled blood glucose	No	22 (6–71)	0.886*
	Yes	21 (9–95)	
Hypertriglyceridemia	No	18.5 (6–55)	0.073*
	Yes	24.5 (8–95)	
Hypertension	No	34 (13–66)	0.015*
	Yes	21 (6–95)	
Obesity	No	17 (6–71)	0.235*
	Yes	23 (7-95)	

¥ Kruskal Wallis One Way Anova Test
*Mann Whitney Test

The result of multivariate analysis indicated that PAI-1 level only correlated significantly with age (*P = 0.000, r = -0.4)*.

DISCUSSION

Several studies had evaluated the association of PAI-1 level and myocardial infarct, but not all of the studies showed positive correlation. These inconsistent results might be due to intra individual and inter individual variation of PAI-1 level, variation in sampling method and analysis method.[13] In van Goor's study median PAI-1 level was found higher in control subjects than in ischemic stroke subjects and there was no correlation between high PAI-1 level and ischemic stroke (OR 0.73; 95% CI 0.4 – 1.4).[28] Lindgren also showed there was no significant correlation between PAI-1 level with ischemic stroke (*P = 0.05*).[7]

The result of our study was different with previous study by van Goor[28] and Lindgren[7] who studied with more subjects than ours. Unlike the previous studies, we first screened the conditions that cause false high PAI-1 level, such as acute inflammation by CRP assay and unstable stroke condition that usually takes place within first two weeks of stroke.[2,11] We also considered carefully on blood collection and handling procedures for assessment of PAI-1. Activation of coagulation system was avoided by drawing the specimen with a minimal stasis. In order to overcome the diurnal variation and short half life of PAI-1 level,[22,25] the sampling time was standardized between 8 a.m. until 9 a.m. and then the specimen was immediately stored in a cooled box and centrifuged in a 4^0C within 30 minutes after blood collection.[11,22,25] We kept the sample in -80^0C until the sample was analyzed.[22]

The occurrence of ischemic stroke was influenced by many factors, such as age, gender, and stroke risk factors. We avoided the influence of these factors by matching the characteristic of subjects in case and control. The case and control characteristics were matched by age and gender, also by including risky-subjects as control.

More risk factors were found in control subjects than in the ischemic stroke subjects (Table 1). The explanation of this condition might be that the subjects of stroke cases got more intensive treatment than the control subjects**.** However, the risk factors in ischemic stroke subjects were more controlled, high PAI-1 level was still found more frequent in case subjects (21.1 %) than in control subjects (7.9 %) (Table 1) and the level of PAI-1 was still higher in ischemic stroke subjects than in control subjects (Table 2). Therefore, by considering the subjects selection criteria and pre analytical phase that were different with previous studies, we suggested that

high PAI-1 level was a risk factor of ischemic stroke *(OR = 3.1; 95 % CI = 0.757 – 12.790; P = 0.103)* (Table 3).

Although there was a significant difference of PAI-1 level between younger and older subjects *(P = 0.004);* and between hypertension and normotension *(P = 0.015)* (Table 4), the result of multivariate analysis indicated that PAI-1 level only correlated significantly with age (*P = 0.000, r = -0.4)*. The risk of thrombosis was higher in elderly because of endothelial dysfunction. In Margaglione's study that was performed in general population without clinical atherosclerosis evidence, there was a weak but significant positive correlation between PAI-1 level and age *(r = 0.174, p < 0.001)*.[9] In this study PAI-1 level was significantly higher in younger than older subjects (*P = 0.004)* (Table 4.). Unlike Margaglione's study that was performed in general population without clinical evidence of atherosclerosis, the subjects of our study were selected from ischemic stroke cases and control group consisted of subjects who had one or more risk factors of stroke. The result of our study indicated that high PAI-1 level was found more frequent in younger age (20 %) instead of the older age (9.8 %) (Figure 1A).

High PAI-1 level results in decreased fibrinolytic activity.[2] Among ischemic stroke subjects, high PAI-1 level was found a bit more frequent in younger subjects (23.5 %) than in older subjects (19 %) (Figure 1B). Up to now there is no study about PAI-1 level in Indonesian population, but the result of this study was rather similar to Northwick Park Heart Study's which showed that younger (40 – 54 years old) subjects with coronary heart disease had lower fibrinolytic activity compared to older subjects (55 – 64 years old).[29] According to Hamsten, high PAI-1 level was an independent risk factors for myocardial infarct on younger subjects (less than 45 years old).[30]

This preliminary study concluded that high PAI-1 level might be a risk factor of ischemic stroke and there was a tendency of high PAI-1 level in younger subjects. In order to confirm if PAI-1 plays a role in ischemic stroke, especially in younger, we recommend this study to be continued by further study with larger sample size and prospective design.

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