

Association Between PAI-1 Activity Levels and t-PA Antigen with Glycemic Status in Prediabetic Population

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ABSTRAK

Tujuan: mengevaluasi hubungan antara defek fibrinolisis dengan status glikemik pada populasi prediabetik dengan menilai kadar antigen t-PA dan aktivitas PAI-1. **Metode:** penelitian ini merupakan penelitian observasional dengan pendekatan potong lintang. Sebanyak 72 orang, usia 30-50 tahun yang memenuhi kriteria inklusi, dimana diagnosis diabetes mellitus (DM) dan status glikemik ditegakkan berdasarkan kriteria American Diabetes Association (ADA). Kadar PAI-1 dan kadar antigen t-PA diukur secara kuantitatif dengan metode enzyme-linked immunosorbent assay (ELISA). Analisis antara kadar antigen t-PA dan aktivitas PAI-1 menggunakan uji ANOVA. **Hasil:** kadar antigen t-PA secara bermakna lebih tinggi pada subyek toleransi gula terganggu (TGT), subyek glukosa darah puasa terganggu (GDPT) dan glukosa darah puasa terganggu (GDPT) dibanding toleransi glukosa normal (TGN) ($p=0.047$). Aktivitas PAI-1 secara bermakna lebih tinggi pada subyek TGT, GDPT dan subyek GDPT dibanding TGN ($p=0.024$). Ada hubungan bermakna antara status glikemik pada subyek prediabetik dengan aktivitas PAI-1 ($p=0.04$). **Kesimpulan:** kadar antigen t-PA dan aktivitas PAI-1 secara bermakna lebih tinggi pada subyek prediabetik dibanding TGN, dan didapatkan hubungan bermakna antara status glikemik subyek prediabetik dengan aktivitas PAI-1.

Kata kunci: prediabetik, efek fibrinolisis, plasminogen activator inhibitor-1 (PAI-1), antigen tissue-type plasminogen activator (t-PA).

ABSTRACT

Aim: to evaluate an association between fibrinolysis defect and glycemic status in prediabetic population by assessing the levels of t-PA antigen and PAI-1 activity. **Methods:** it was an observational study with cross-sectional approach. There were 72 subjects aged 30-50 years who had met the inclusion criteria. The diagnosis of diabetes mellitus (DM) and glycemic index were determined based on the American Diabetes Association (ADA) criteria. The PAI-1 and t-PA antigen levels were measured quantitatively using enzyme-linked immunosorbent assay (ELISA). Analysis between the levels of t-PA antigen and PAI-1 activity was performed using ANOVA. **Results:** the t-PA antigen level was significantly higher in subjects with impaired glucose tolerance (IGT) and impaired fasting blood glucose (IFBG) as well as subject with impaired fasting blood glucose (IFBG) than those with normal glucose tolerance (NGT) ($p=0.047$). The PAI-1 activity was significantly higher in subjects with IGT, IFBG and subjects with IFBG than NGT ($p=0.024$). There was a significant association between glycemic status in prediabetic subjects and PAI-1 activity ($p=0.04$). **Conclusion:** the level of t-PA antigen and PAI-1 activity were significantly higher

in prediabetic subjects than those with NGT; and there was a significant association between glycemic status in prediabetic subjects and PAI-1 activity.

Keywords: *prediabetic, fibrinolytic defect, plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (t-PA) antigen.*

INTRODUCTION

The mechanism of the atherothrombosis development in prediabetic population has not been fully understood. It is assumed that it occurs due to insulin resistance in addition to hemostatic disorders, which involve hypercoagulability and fibrinolysis defect.^{1,2} The effectiveness of fibrinolysis mainly depends on the activity of tissue-type plasminogen activator (t-PA), which is released by damaged vascular wall in order to alter plasminogen into active plasmin on thrombus surface that leads to the development of thrombolysis. Afterwards, it will be rapidly inhibited by Plasminogen Activator Inhibitor-1 (PAI-1) activity in circulation.¹ Numerous prospective studies have demonstrated that fibrinolysis defect also has contribution in the development of vascular event, particularly atherothrombosis in various populations including patients with diabetes mellitus (DM).²⁻⁵

It has been proven that DM patients have higher risk for developing cardiovascular disease, peripheral arterial disease and stroke than non-diabetic population.⁶ The morbidity and mortality rate due to cardiovascular events in general population has been decreased in the last few years. However, the phenomenon has not been demonstrated in diabetic population.⁷ The high prevalence of DM will have various impacts on morbidity and mortality due to its potential complications that eventually will cause increased health care cost.⁸ The severity of hyperglycemia and the duration of suffering DM type 2 have been consistently demonstrated to bring some effects on the development of microvascular complications. However, similar issue has not been found for macrovascular complications.^{9,10} It is assumed that the differences may occur due to a metabolic disorder in prediabetic phase and in turn, it is believed that the differences will play a role in the development of macrovascular

complications.⁹ Prediabetic population consists of subjects with impaired glucose tolerance (IGT) and subjects with impaired fasting blood glucose (IFBG). Both groups are considered as those who are in intermediate metabolic state, which is between the phase of normal glucose tolerance and DM.¹¹

A study conducted by Rewers et al¹² in a population in Colorado, United States found that the prevalence of CHD in subjects with IGT has reached 1.5-2.6 folds compared to subjects with normal glucose tolerance (NGT). Results from Framingham Offspring Study (FOS) showed that there is a significant association between the levels of fasting insulin and the levels of PAI-1, t-PA antigens, factor VII, factor vWF and fibrinogen in a population with normal glucose levels; while in prediabetic population, only the marker of fibrinolysis defect (PAI-1 levels and t-PA antigen) have a significant association with hyperinsulinemia state.³

Almost all studies have demonstrated consistent evidence on the presence of coagulation disorder and fibrinolysis defect in DM population; while similar studies on prediabetic population have inconsistent results. Many studies which are conducted in Indonesia have demonstrated the role of inflammatory factors on cardiovascular events. However, studies on the role of hemostatic factors, particularly the fibrinolysis defect are still limited. The aim of our study was to identify the role of fibrinolysis defect on various prediabetes status by assessing the levels of t-PA antigen and PAI-1 activity on various status of prediabetes status.

METHODS

Our study was an observational study with cross-sectional approach. The population of study was 72 subjects who were obtained by consecutive sampling when the patients visited

the outpatient clinic at Department of Internal Medicine in Wahidin Sudirohusudo Hospital, Labuang Baji Hospital and Prodia Laboratory in Makassar. Subjects participated in the study were the study population who had met the inclusion criteria, i.e. male or female subjects aged between 30 and 50 years who were willing to participate in the study and signed the informed consent form. The exclusion criteria were history of diabetes mellitus, coronary heart disease, stroke, hypertension, history of taking anti-hypertensive agents, anti-thrombotic and lipid modifying agent. The study was conducted with the approval by the Ethical Committee on Biomedical Research in Human, Faculty of Medicine, University of Hassanudin with the number of approval of 0047/H04.8.4.5.31/PP36-KOMETIK/2008.

The diagnosis of DM is established based on the American Diabetes Association (ADA) criteria, i.e. those with classical symptoms of diabetes (polyuria, polyphagia and unexplainable weight loss) as well as random blood glucose levels (RBG) of >200 mg/dL; or fasting blood glucose levels (FBG) of >126 mg/dL or 2 hours post prandial blood glucose levels (2hPPBG) of >200 mg/dL after the loading.¹³ Normal glucose tolerance (NGT) was defined when the fasting blood glucose levels was <100 mg/dL and after 2 hours, the post oral glucose tolerance test levels was <140 mg/dL.¹³ Prediabetics are patients with IFBG (Impaired Fasting Blood Glucose) and/or Impaired Glucose Tolerance (IGT) as defined by the modified ADA criteria (2004). The subjects were considered as those with IFBG when the fasting blood glucose levels range was 100-125 mg/dl and 2hPPBG after loading was <140 mg/dl. They were considered as subjects with IGT when their 2hPPBG levels after glucose loading ranged between 140 and 199 mg/dl and their FBG <100 mg/dl.¹⁴ PAI-1 activity was defined as the levels of active free PAI-1. The reference value was 1-7 IU/ml; while the levels of t-PA antigen was t-PA antigen calculated quantitatively in the plasma with a reference value of 1-20 ng/ml.¹⁵

Anthropometric evaluation, blood pressure examination, ECG, evaluation on lipid fraction, fasting blood glucose levels and 2-hour post prandial blood glucose levels using hexokinase

method of Roche were carried out after the subjects had their fasting (no calorie intake) for 12 hours and 2 hours after glucose loading. Oral glucose tolerance test (OGTT) was performed based on WHO criteria (1994).

In the evaluation of PAI-1 activity and plasma t-PA antigen, 6 ml of blood was withdrawn after the subjects had 8-12 hours of fasting between 7.00 and 10.30 a.m. and put into a tube containing citrate with 9 : 1 ratio to prevent diurnal variation of PAI-1 levels. Furthermore, the blood was immediately processed within <120 minutes. After 15 minutes of centrifugation (3000 rpm) at 2-8°C, the plasma and serum layers were separated and was subsequently put into a cup fisher as much as 0.5 cc each and the sample could be preserved at -80°C for 6 months. PAI-1 levels was measured quantitatively from citrate plasma by using the sandwich technique or enzyme-linked immunosorbent assay (ELISA) utilizing the Technozym® PAI-1 kit. The levels of t-PA antigen was measured quantitatively from the citrate plasma by using Biopool's TinElize® t-PA, an enzyme immunoassay instrument.

The obtained data was analyzed using Statistical Package for Social Science (SPSS) software program version 17. Afterwards, univariate and bivariate analysis were carried out. The significance levels of statistic test was determined as 5%. Analysis on difference between the levels of t-PA antigen and PAI-1 activity among prediabetic subjects was performed using ANOVA (analysis of variance); while the association between PAI-1 and prediabetic subjects was evaluated using the unadjusted Pearson chi square test.

RESULTS

There were 72 subjects who fulfilled the inclusion criteria with age range of 30-50 years and there were female subjects as many as 52%. Subject characteristics were categorized based on FBG levels, 2hPPBG, lipid profile, PAI-1 activity, t-PA antigen levels and waist circumference as has been presented in **Table 1**.

In our study, we found 18 subjects (25%) with NGT and 54 subjects (75%) who were prediabetics. An analysis on t-PA antigen levels on each glycemic status found that there was

Table 1. Data on subject characteristics (n=72)

Variables	Mean (SD)
Age (years)	42.23 (5.52)
FBG (mg/dl)	95.91 (14.34)
2hPPBG (mg/dl)	137.65 (33.58)
Total cholesterol (mg/dl)	216.67 (47.23)
LDL cholesterol(mg/dl)	129.51 (33.04)
HDL cholesterol (mg/dl)	48.39 (15.66)
Triglycerides (mg/dl)	143.43 (35.49)
Waist circumference (cm)	84.52 (10.03)
PAI-1 activity (IU/ml)	7.87 (6.48)
The level of t-PA antigen (ng/ml)	9.02 (4.32)

a higher t-PA antigen levels in prediabetic subjects than subjects with NGT; however, the difference of t-PA antigen levels was not significant ($p=0.253$). When the prediabetic subjects were categorized separately as the subjects with IGT, IFBG and IGT + IFBG and then they were compared with the subjects with NGT, we subsequently found that the mean t-PA antigen levels in prediabetic subjects was significantly higher than subjects with NGT ($p=0.047$) (**Table 2**).

Analysis of PAI-1 activity for each glycemic status found that there was a higher level of PAI-1 activity in prediabetic subjects than those with NGT; however, the difference of PAI-1 activity was not significant ($p=0.094$). When the prediabetic subjects were categorized separately as the subjects with IGT, IFBG and IGT + IFBG and compared with the subjects with NGT, we found that the mean levels of PAI-1 activity in prediabetic subjects was significantly higher than subjects with NGT ($p=0.024$) (**Table 3**).

By using the Pearson chi square test, we found supporting result in line with the findings, which was a significant association between glycemic status of prediabetic subjects and PAI-1

activity ($p=0.044$). The risk of having increased PAI-1 activity of more than 7 IU/ml in subjects with IGT+IFBG was 4.32 folds than subjects with NGT; while in subjects with only IFBG, the risk was 2.02 folds compared to the NGT subjects (**Table 4**).

Table 3. The mean level of PAI-1 activity in the group of subjects with NGT, IGT, IFBG and IGT + IFBG

Subject group	Mean (SD)	p value
IGT+IFBG (n=15)	11.96 (7.94)	0.024
IFBG (n=16)	8.86 (5.88)	
IGT (n=23)	6.40 (6.57)	
NGT (n=18)	5.67 (3.92)	

Table 4. The correlation between PAI-1 activity and glycemic status in prediabetic subjects

Glycemic status	PAI-1 Activity (IU/ml)		OR (95% CI)
	n (%)		
	>7	≤7	
IGT+IFBG	11 (32.35)	4 (10.53)	4.32 (1.88-17.56)
IFBG	9 (23.53)	7 (21.05)	2.02 (1.45-7.20)
NGT	9 (23.53)	14 (39.47)	1.91 (1.25-3.25)
NGT	7 (20.59)	11 (28.95)	1

* p value = 0.044 (Pearson correlation)

DISCUSSION

In our study, we found higher t-PA antigen levels in prediabetic subjects than NGT subjects although the difference was not significant ($p=0.253$). Nevertheless, when the prediabetic subjects were categorized as the subjects with IGT + IFBG, IFBG and IGT, we found high mean value of t-PA antigen levels, which was 11.56 (4.34), 9.60 (5.50), and 7.83 (2.89), respectively; while the difference of t-PA antigen levels was statistically significant ($p=0.047$).

There are limited studies investigating the consistent association between increased t-PA antigen levels and the course of illness of diabetes mellitus. It may be caused by the difficulty in interpreting the standard reference value and the measurement may be affected by the levels of PAI-1, which is bound in complex form with t-PA.¹⁶ Lowe et al.¹⁷ stated that in type

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IFBG (n=16)	9.60 (5.50)	
IGT (n=23)	7.83 (2.89)	
NGT (n=18)	8.01 (4.06)	

2 DM patients, increased t-PA antigen levels is only one of markers for cardiovascular events, which is different from PAI-1 that have been demonstrated and proven by various large-scale studies as one of independent risk factors for cardiovascular events and type 2 DM.^{4,5,18,19}

Eliasson et al.² in The Northern Sweden MONICA Study have performed a follow-up study for 9 years in normal subjects and they found that there was a significant increase of t-PA antigen levels ($p < 0.001$), which was correlated with DM incidence. It has shown that increased t-PA antigen has an independent predictive value on DM incidence in the future. The study also demonstrated that fibrinolysis defect characterized by increased t-PA antigen and PAI-1 activity and reduced t-PA activity has been occurred in prediabetic population before there is a further increase in blood glucose levels.²

In our study, we found higher PAI-1 activity in prediabetic subjects than subjects with NGT; however, the difference was not significant ($p = 0.094$). When the prediabetic subjects were categorized as the subjects with IGT + IFBG, IFBG and IGT, they found high mean value of PAI-1, which was 11.96 (7.94), 8.86 (5.88) and 6.40 (6.57), respectively in which the difference of PAI-1 activity was significant statistically ($p = 0.024$).

Pearson chi square test also demonstrated that there was a significant association ($p = 0.044$) between PAI-1 activity, which had been categorized based on the limit value of PAI-1 activity and the prediabetic glycemic status. The results of our study showed that the PAI-1 activity was >7 IU/ml in subjects with IGT + IFBG, which was four times higher than subjects with NGT; while in subjects with IFBG, it was twice higher than subjects with NGT and subjects with IFBG had higher PAI-1 activity of >7 IU/ml than subjects with NGT. In the literature, it has been explained that the prediabetic population has higher risk on the development of DM and the future thromboembolic phenomenon.^{20,21}

Our study is consistent with the results of other studies. Meigs et al.³ in The Framingham Offspring Study showed a higher PAI-1 levels in prediabetic group compared to the NGT group and the difference was statistically significant.

Leurs et al.²² also demonstrate a significantly higher PAI-1 levels in subjects with IGT and DM compared to subjects with NGT. The mechanism of how hyperglycemia can induce PAI-1 production has not been fully understood; however, in vitro studies have found that hyperglycemia state causes stimulation on PAI-1 gene in the vascular smooth muscle cells.^{19,23}

After a more detail categorization on prediabetic population, we found that the mean PAI-1 activity was higher in subjects with IFBG than those with IGT. The condition can be explained by the rational that in IFBG subjects, there is insulin resistance, which predominantly occur in the liver; while the insulin sensitivity in the muscle is usually normal; on the contrary, in subjects with IGT, the insulin resistance occurs mainly in the muscle and there is only few of them that experience changes of insulin sensitivity in the liver. A combination of insulin resistance in the liver and impaired secretion of basal insulin has caused increased production of glucose in the liver or it is known as the endogenous glucose production (EGP) in subjects with IFBG; while the combination of the second phase impaired insulin secretion and the presence of insulin resistance in the muscle will be found in subjects with IGT.²⁴ In fasting state, the blood glucose levels is determined by the amount of glucose produced by the liver (EGP), particularly through gluconeogenesis or glycogenolysis and the insulin levels produced by the pancreas.²⁵ A study by Wayer et al.²⁶ also explains that increased EGP is found in subjects with IFBG; however, increased EGP is not found in subjects with IGT and NGT. In the same population, it is also said that there is a strong association found between PAI-1 levels and insulin resistance that occurs in the liver compared to insulin resistance that occurs in the muscles.²⁷

Gluconeogenesis, which is derived from breakdown of lipids will cause release of various active substances such as free fatty acids and harmful adipokines such as proinflammatory cytokines and PAI-1.²⁸ It is consistent with the results of The Insulin Resistance Atherosclerosis Study, which demonstrates that fasting glucose has more role in developing future diabetes and

its association with increased PAI-1 levels.⁴ A multicenter epidemiological study, the Insulin Resistance Atherosclerosis Study (IRAS) by Festa et al.⁵ investigates the association of dynamic change of PAI-1 and fibrinogen on type 2 DM incidence in healthy non-DM individuals. The study concludes that increased PAI-1 that occurs continuously in subjects with initially high PA-1 levels is correlated with the incidence of type 2 DM.

There is a association between increased PAI-1 and the incidence of diabetes, which is based on various reasons.^{4,5} First, it is assumed that there is an equal genetic background between PAI-1 expression and the risk of developing type 2 DM; Second, PAI-1 is assumed to have a role in the pathophysiology of type 2 DM causatively; and third, there are various similar and affecting factors, either on the PAI-1 expression or on the development of type 2 DM such as weight, insulin resistance, glucocorticoid, triglycerides and free fatty acids. Various in vitro and in vivo studies have demonstrated that PAI-1 not only has a role in the process of atherothrombosis of vascular wall, but also has a role in the development of adipose tissues and it can control the insulin signaling in the adipocytes.²⁸

CONCLUSION

The levels of t-PA antigen is significantly higher in subjects with IGT + IFBG and subjects with IFBG than those with NGT; while the levels of t-PA antigen in IGT is not significantly different than those with NGT. The activity of PAI-1 is significantly higher in subjects with IGT + IFBG and subjects with IFBG than those with NGT; while the activity of PAI-1 in subjects with IGT is not significantly different from those with NGT.

In prediabetic subjects, treatment that has been demonstrated to reduce the levels of t-PA antigen and PAI-1 activity should be considered. An evaluation on markers of fibrinolysis defect (t-PA antigen and/or PAI-1) in prediabetic subjects is necessary to identify one of the risk factors for cardiovascular events.

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