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RESEARCH

MOLECULAR ASPECT CORRELATION BETWEEN GLYCATED HEMOGLOBIN (HBA1C), PROTHROMBIN TIME (PT) AND ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) ON TYPE 2 DIABETES MELLITUS (T2DM)

(Aspek molekuler Hubungan Kadar Hemoglobin Terglikasi (HbA1c), Prothrombin Time (PT) dan Activated Partial Thromboplastin Time (APTT) di Diabetes Melitus Tipe 2)

Indranila KS

ABSTRAK

Diabetes Melitus (DM) memerlukan pengendalian glikemia yang dapat diketahui dengan melakukan pemeriksaan hemoglobin terglikasi (HbA1c). Semakin tinggi kadar hemoglobin terglikasi (HbA1c), semakin tidak terkendali kadar gula darah pasien DM tipe 2. Hal ini dapat menyebabkan terjadinya proses hiperkoagulasi dan gangguan mikrovaskular maupun makrovaskular. Pemeriksaan Protrombin Time (PT) dan Activated Partial Thromboplastin Time (APTT) diharapkan dapat mendeteksi secara dini adanya gangguan koagulasi di pasien DM tipe 2. Penelitian potong lintang terhadap 72 orang pasien DM tipe 2 yang berusia diatas 18 tahun diperiksa kadar HbA1c dan dikaji koagulasi (PT dan APTT). Pasien dengan penyakit penyerta seperti anemia dan kelainan hemoglobin, keganasan atau kelainan hematologis, pasca bedah, hipertiroid, perempuan hamil, riwayat penyakit hati dan pasien yang mengkonsumsi obat-obatan yang mengganggu fungsi koagulasi dikeluarkan dari penelitian ini. Uji normalitas data menggunakan Kolmogorov-Smirnov dan analisis hubungan menggunakan uji Pearson. Analisis kenasaban terdapat hubungan antara kadar hemoglobin terglikasi dengan Prothrombin Time negatif lemah (r= -0,179; p=0,132) dan dengan Activated Partial Thromboplastin Time positif sangat lemah (r=0,016; p=0,892). Berdasarkan telitian ini terdapat hubungan negatif lemah yang bermakna antara kadar hemoglobin terglikasi dengan PT dan hubungan positif sangat lemah yang tidak bermakna dengan Activated Partial Thomboplastin Time.

Kata kunci: *Aspek molekuler, HbA1c, PT, APTT, T2 DM*

ABSTRACT

Diabetes mellitus (DM) requires glycemic control which can be determined by performing a glycated hemoglobin (HbA1c). The higher levels of glycated hemoglobin (HbA1c), the more uncontrolled blood sugar levels of patients with type 2 diabetes This can lead to a hypercoagulable process, microvascular and macrovascular disorders. Examination Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) is expected to detect at an early stage coagulation disorders in patients with diabetes mellitus type 2. The cross-sectional study of 72 people with type 2 diabetes patients aged over 18 years, to be assessed HbA1c levels and biomarkeragulation (PT and APTT). Patients with concomitant diseases such as anemia and hemoglobin disorders, malignancy or haematological disorders, post-surgical, hyperthyroidism, pregnant women, history of liver disease and patients taking drugs that interfere with coagulation function were excluded from this study. Test data normality using the Kolmogorov-Smirnov test and analysis correlation using the Pearson test. Correlation analysis of the relationship between glycated hemoglobin levels with the Prothrombin Time shows a weak negative (r = -0.179; p = 0.132), and with Activated Partial Thromboplastin Time a very weak positive (r = 0.016; p = 0.892). Based on this results the conclusion were a weak negative correlation significantly between glycated hemoglobin levels with PT and very weak positive correlation insignificant with Activated Partial Thomboplastin Time.

Key words: *Molecular aspect, HbA1c, PT, APTT, DMT2*

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INTRODUCTION

Diabetes Mellitus (DM) is a syndrome with disruption of carbohydrate, protein and fat metabolism caused by reduced insulin secretion or a decrease in tissue sensitivity to insulin, and are commonly found in Type 2 diabetes for approximately 90% of all cases DM.[1] There were 171 million people worldwide with diabetes in 2000 and will rise to 366 milions by the year 2030.[2] Diabetes mellitus currently affects 29.1 million peoples in United States.[3] In Indonesia has now been ranked fourth for the highest number of people with diabetes after the United states, China and India.[4] Based on data from the Central Statistics Agency or "Badan Pusat Statistik", the number of people in Indonesia suffering from diabetes in 2003 was 13,7 millions and it will be 20.1 million peoples in 2030 with a prevalence rate of 14.7% in urban areas and 7.2% in rural ones.[5]

Glycemic control is one of the important things in evaluating patients with DM, which can predict the complications that will happen, and also can predict the prognosis of the patients.[6] Glycemic control which is often used as one of laboratory diagnostics parameters for diabetes mellitus is glycated hemoglobin level (HbA1c). HbA1c as glycated hemoglobin is a fraction of hemoglobin in the human body that binds to glucose enzymatically.[7] Measurement of HbA1c level reflects average glucose level in three months, prior in accordance with the age of erythrocyte cells. According to American Diabetic Association (ADA), the level of HbA1c more than or similar to 6.5% can be categorized into diabetes mellitus.[8]

The highest causes of disability and death in patients with diabetes mellitus is Cardiovascular disease. Moreover, a hypercoagulabe condition often occurs in people with diabetes mellitus, especially in uncontrolled DM, is considered as one

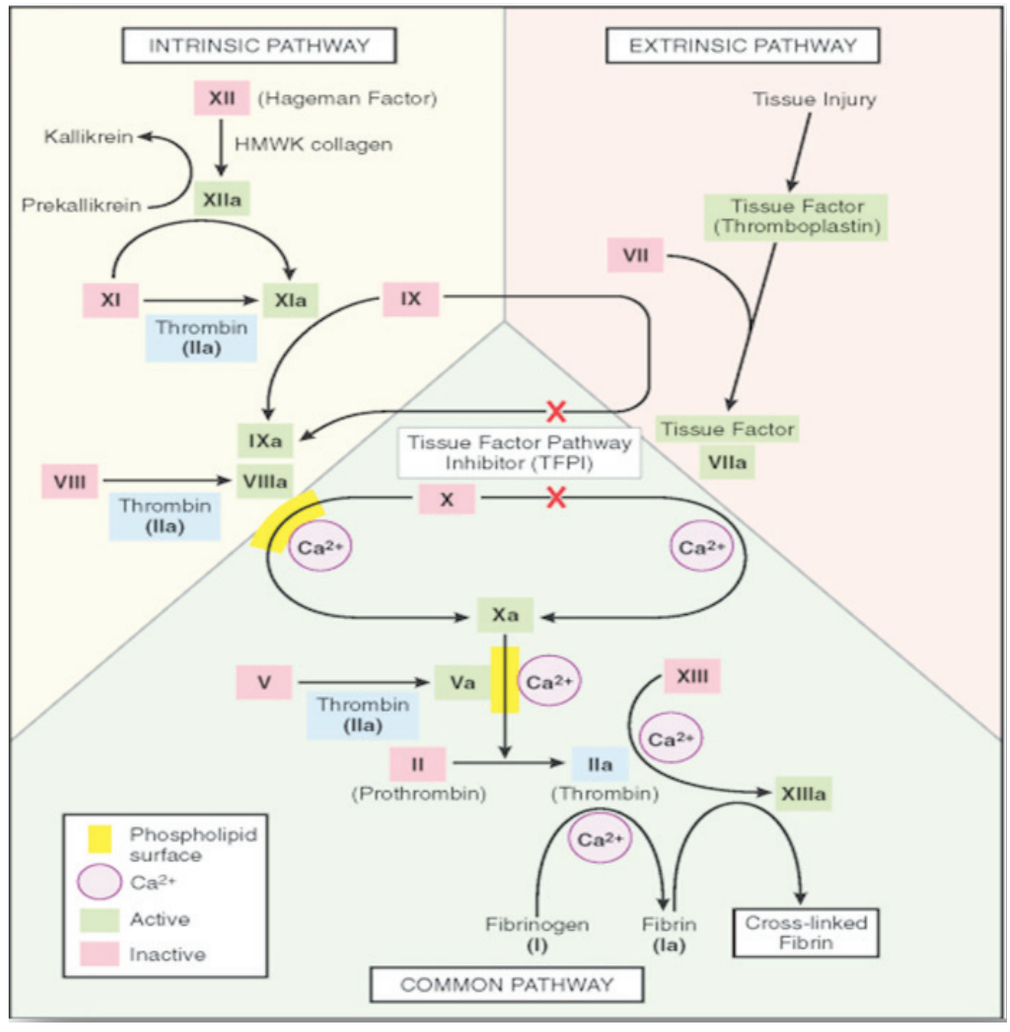


Figure 1. Coagulation cascade system, PT dan APTT test function.[13] (modification).

of the factors causing cardiovascular disease. This hypercoagulable condition then triggers DM patients to suffer from thromboemboli and various hemostasis abnormalities.[9]

Coagulation screening standards, such as Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT), as a result, are still considered as the important basic checks in the clinical laboratory.[10] As a markers of intrinsic and extrinsic pathway activations, PT and APTT can also considered to be used to assess suspected hypercoagulable condition characterized by shortening PT and APTT, consequently, shortening PT and APTT values can be a risk factor for thromboembolism of cardiovascular disease in patients with type 1 DM (see Figure 1).[11,13]

Intact and normal endothel cells help maintain blood flow by inhibiting the activation of platelets and clotting factors. The endothelial cells are stimulated by injury or inflammatory cytokine, will increase coagulant factor expression, facilitate clots, and reduced anti-clotting factor expression. The loss of endothelial integrity causes subendothelial vWF and collagen basement membrane exposure, stimulates platelet adhesion, platelet activation and clot formation.[12]

This research aimed to determine whether there was a correlation between hemoglobin glycated (HbA1c) and biomarker coagulation as PT and APTT in patients with type 2 diabetes. This research was also expected to optimize the management of DM with detected hypercoagulable biomarker as PT and APTT. This research is to detected the correlation between parameters of glycemic control checks (HbA1c) and the parameters of coagulation (PT and APTT). This research was also expected to be a prognosis marker of coagulation disorders and used in the management of patients with type 2 DM and this markers are inexpensive, easy and often used in hospital and laboratories.

METHODS

This research was a cross-sectional study conducted at the Dr. Kariadi Hospital Semarang (May-July 2016). Data were taken from the medical records of patients diagnosed with type 2 diabetes based on the 1998 WHO guidelines[2] and hospitalized at the Dr. Kariadi Hospital Semarang. This research conducted 72 patients, aged over 18 years and consisted of 35 males and 37 females. The HbA1c level was examined using elecktrophoresis instrument from Sebia. Biomarker PT and APTT level were examined using a Sysmex coagulation analyzer, Sysmex Cs-2100. Patients with concomitant disease, such as anemia and hemoglobin disorders, malignancies and other hematological disorders, post-surgery, hyperthyroidism, pregnancy, a history of liver disease and taking medications which interfere the coagulation function, were excluded from this research.[11,14-16] Ethical clearance was obtained from the Institutional Medical and Health Research Ethics Committee of Faculty of Medicine, University of Diponegoro in Semarang, No 150/EC/FK-RSDK/2016.

Data obtained were analyzed using computer. Normality test data was performed using Kolmogorov-Smirnov because the sample size more than 50 samples indicate normal distribution data. Data then was displayed in mean. Finally, Pearson test was conducted to analyze the correlation with a significant p-value of less than 0.05.[17]

RESULTS AND DISCUSSION

The number of the research subjects in this research was 72 patients, consisted of 35 males (46.6%) and 37 females (53.33%), aged a minimum of 23 years old and a maximum of 75 years old. Data on the overal characteristics of the research subjects can be seen in Table 1.

Table 1. Characteristics of research subjects

Characteristics	n (%)	Mean (Min-Max)
Males	35 (48.61%)	
Females	37 (51.38%)	
HbA1c		8.05 (4.4–18.6)
PT		10.19 (8.9–13.3)
APTT		31.45 (20.4–37.8)

The distribution of HbA1c, PT and APTT data in this research was presented in a boxplot graph as seen in Figure 2.

The correlation among the data obtained in this research was tested by Pearson correlation analysis as shown in Table 2. There was very weak negative correlation between HbA1c and PT in patients with type 2 diabetes. Thus there was an significant correlation between HbA1c and PT in patients with type 2 diabetes (r= -0.179; p=0.132). Therefore, it can be said that the higher level of HbA1c, the more shorter the PT's value.

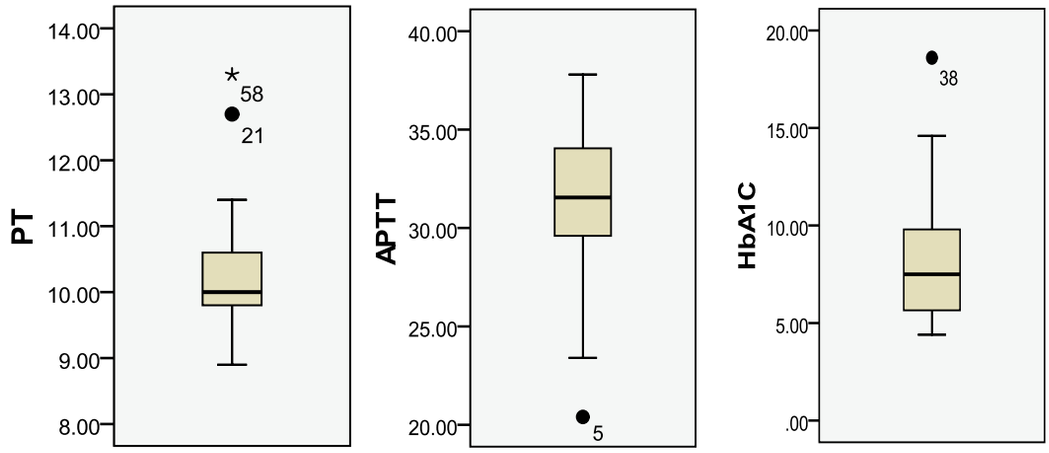


Figure 2. Distribution of PT, APTT and HbA1c in this research.

Tabel 2. The correlation between HbA1c, PT and APTT in patients with type 2 DM

Variable	HbA1c	
	r	P
PT	-0.179	0.132
APTT	0.016	0.892

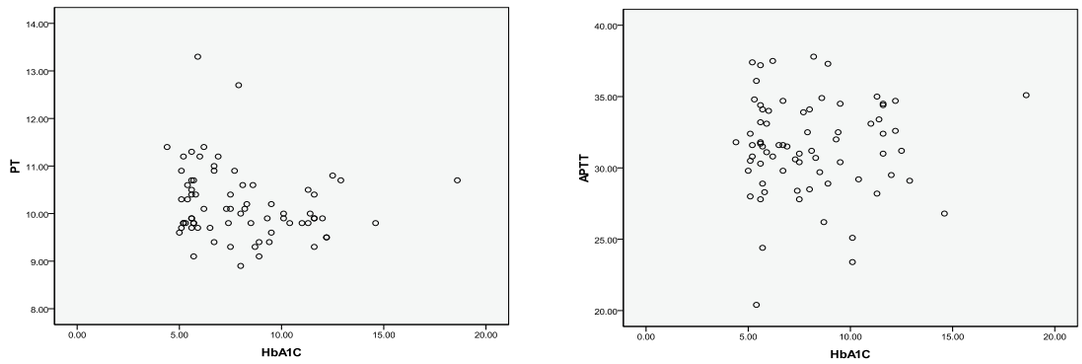
*significant, $p < 0.05$

On the other hand, there was a very weak positive correlation between HbA1c and APTT. Thus indicating that there was an insignificant correlation between HbA1c and APTT in patients with type 2 diabetes ($r=0.016$; $p=0.892$). Thus, it can be said that the higher the level of HbA1c, the more longer APTT's value.

The correlation between HbA1c parameter and coagulation parameters (PT and APTT) were also presented in a scatter graph (see Figure 3).

Patients with type 2 diabetes have a high risk for atherothrombosis or thromboemboli. Several studies even have shown abnormalities in hemostasis and thrombosis associated with type 2 DM patients. 80% of patients with diabetes die of thrombosis, while 75% of them die because of cardiovascular and cerebrovascular complications.[18] Patients with type 2 diabetes are expected to be in a hypercoagulable state if there are abnormalities on coagulation examination. This hypercoagulable state is associated with an increased incidence of thromboemboli which can increase the risk of cardiovascular disease leading to death.[19] Thus, it is important for patients with type 2 diabetes realize if a hypercoagulable state emerges, so those patients with type 2 diabetes can prevent further atherothrombotic state to control blood sugar or with the aid of anti-coagulation drugs.[10,12]

Coagulation studies on PT and APTT actually are relatively inexpensive and easy to perform in both hospitals and private laboratories. Test on



a) b)

Figure 3. Scatter Graph of the correlation between HbA1c and a) PT as well as b) APTT.

PT and APTT was performed both as a standard screening examination to determine the function of the coagulation system and as a means of monitoring anticoagulation therapy that has already be accepted widely.[20] PT is a screening test for intrinsic factor in coagulation pathway, initiated by tissue factors that are very sensitive to levels of factor VII. Coagulation activity derived from factor VII is higher in patients with diabetes mellitus or in patients with metabolic syndrome.[21] In vivo prothrombin activation occurs on the surface of platelets, the addition of platelets occurs in the plasma and platelet activation increases thrombin production.[22] When thrombin is formed from prothrombin fragment, prothrombin activation is released and its level increases in diabetes mellitus.[23] APTT is used to identify abnormalities in tissue contacts (factor XII, prekalikrein and high–molecular weight kininogen), intrinsic factors (factors VIII, IX and XI) and common pathway (factors II, V, X and fibrinogen) on coagulation pathway.[24] Lengthening of APTT value has a clinical relevance as an indicator of deficiency of coagulation factors or the presence of coagulation inhibitors.[9] Meanwhile, shortening of APTT is often regarded as a laboratory finding caused due to errors in venipuncture.[25] However, in some cases, shortening of APTT could reflect hypercoagulable states, which could increase the risk of thrombosis and cardiovascular disease. Shortening of APTT is also considered as a result of the accumulation of activation of coagulation factors that circulate in the plasma as a result of increased coagulation activity.[26,27]

Hypercoagulability secondary (acquired) found on various conditions in patients with underlying diseases or clinical conditions known to be associated with an increased risk of trombosis such as malignancy, pregnancy,heart failure, trauma, stasis (injury) vascular, oral contraceptive use, hiperestrogenisme, hiperlipidema, diabetes mellitus and abnormalties of blood vessels and rheology.[28]

Vascular endothelium, the primary defense against thrombosis, is abnormal in diabetes. Endothelial abnormalities undoubtedly play a role in the enhanced activation of platelets and clotting factors seen in diabetes. Coagulation activation markers, such as prothrombin activation fragment 1+2 and thrombin-anti-thrombin complexes, are elevated in diabetes.[26,29] The plasma levels of many clotting factors including fibrinogen, factor VII, VIII, XI, XII, kallikrein and von Willebrand factor are elevated in diabetes.[29,30] The fibrinolytic system, the primary means of removing clots, is relatively inhibited in diabetes due to abnormal clot structures that are more resistant to degradation and an increase in plasminogen activator inhibitor type 1(PAI-1).[31] Increased platelet reactivity, increased circulating platelet aggregates, platelet aggregation in response to platelet agonists, Platelet Contractile Force (PCF) and presence of higher plasma levels of platelet release products, such as beta-thromboglobulin, platelet factor 4 (PF4) and thromboxane B (2), demonstrate platelet hyperactivity in diabetes patients.[22,32]

In this research, there was a weak negative, and significant correlation between HbA1c and PT in patients with type 2 diabetes (r=0.016; p=0,892). Therefore, it can be said that the higher HbA1c, the shorter PT value. Otherwise, the higher HbA1c the longer APTT value. However, the results of this research were not consistent with the results of the research conducted from recent studies, which showing that fibrinogen level was higher and APTT value was shorter in patients with diabetes mellitus than those in non-diabetic patients.[33,34] This may occur because of other factors affecting coagulation, such as administration of anticoagulant drugs or foods containing anticoagulants that were not detected during sampling in patients with diabetes mellitus.[35]

CONCLUSION AND SUGGESTION

There is a tendency for hypercoagulation in patients with type2 diabetes mellitus indicated by shortening of Prothrombin Time (PT) and the lengthening of Active Partial Thromboplastin Time (APTT) as increasing of HbA1c level. Therefore, further examination of other biomarkers influencing hypercoagulation and hypocoagulation screening is needed, with much more amount of sample.

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