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RESEARCH

CORRELATION OF COAGULATION STATUS AND ANKLE BRACHIAL INDEX (ABI) IN DIABETES MELLITUS PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

(Hubungan Status Koagulasi terhadap Nilai Ankle Brachial Index (ABI) Pasien Penyakit Arteri Perifer dengan Diabetes Melitus)

Lany Anggreani Hutagalung[1], Adi Koesema Aman[2], Syanti Syafril[3]

ABSTRAK

Diabetes Melitus (DM) sering dihubungkan dengan komplikasi mikrovaskular dan makrovaskular. Hiperglikemia merupakan faktor kebahayaan aterosklerosis dan penyakit vaskuler yang menyebabkan kerusakan pembuluh darah serta menyebabkan terjadinya glikasi terhadap hemoglobin, protrombin, fibrinogen dan protein lain yang terlibat dalam mekanisme pembekuan. Diabetes melitus merupakan salah satu faktor kebahayaan Penyakit Arteri Perifer (PAP). Tujuan penelitian ini adalah untuk mengetahui hubungan status koagulasi terhadap nilai ABI pasien penyakit arteri perifer dengan diabetes melitus. Penelitian ini bersifat analitik observasional yang dilakukan di Departemen Penyakit Dalam RSUP. Adam Malik Medan masa waktu bulan April-Oktober 2015. Pasien DM dilakukan pemeriksaan Ankle Brachial Index (ABI) dan status koagulasi seperti PT, APTT, fibrinogen dan D-dimer. Pada Penelitian ini menunjukkan perbedaan bermakna antara kadar fibrinogen dan D-dimer dengan PAP, yaitu kadar fibrinogen dan D-dimer di pasien DM dengan PAP lebih tinggi dibandingkan dengan pasien DM non-PAP (333,35±127,49 vs 244,95±83,96; p=0,001) dan (648,40±443,96 vs 302,45±108,41; p=0,008). Didapatkan perbedaan bermakna antara kadar fibrinogen dan D-dimer dengan derajat PAP, yaitu kadar fibrinogen dan D-dimer di derajat PAP berat lebih tinggi, dibandingkan dengan PAP ringan (374,00±114,94 vs 327,14 ± 136,45; p=0,012) dan (1170,67 ± 398,72 vs 537,36 ± 348,08; p=0,012). Setelah dilakukan uji kenasaban Spearman, Didapatkan adanya hubungan bermakna antara kadar D-dimer dengan PAP, yaitu peningkatan kadar D-dimer berhubungan dengan rendahnya nilai ABI (r -0,577; p=0,000). Kadar Fibrinogen dan D-dimer di pasien DM dengan PAP lebih tinggi dibandingkan di pasien DM non-PAP. Peningkatan kadar D- dimer berhubungan dengan rendahnya nilai ABI.

***Kata kunci**: Diabetes melitus, penyakit arteri pertifer, status koagulasi, ankle brachial index*

ABSTRACT

Diabetes Mellitus (DM) is commonly associated with both microvascular and macrovascular complications. Hyperglycemia, a well-defined risk factor for accelerated atherosclerosis and vascular disease, may cause vessel damage and resulting in glycation of hemoglobin, prothrombin, fibrinogen and other proteins involved in clotting mechanisms. The glycation results in the incomplete activation and function of the clotting cascade. Diabetes mellitus is a risk factor for Peripheral Arterial Disease (PAD). This research is aimed to know the correlation between coagulation status, Ankle Brachial Index (ABI) and PAD in patients with DM. This study was an observational analytical study that was performed in the Adam Malik Hospital Medan, from April to October 2015. All samples were examined for Ankle Brachial index (ABI) and coagulation parameters such as PT, APTT, fibrinogen and D-dimer level. There was a significantly difference between fibrinogen and D-dimer level with PAD. DM patients with PAD had significantly higher fibrinogen and D-dimer levels compared with DM patients without PAD (333.35±127.49 vs 244.95±83.96; p=0.001) and (648.40±443.96 vs 302.45±108.41; p=0.008). There was a significantly difference between fibrinogen and D-dimer level with severity of PAD, whereas severe PAD had significantly higher fibrinogen and D-dimer levels compared with mild PAD (374.00±114.94 vs 327.14±136.45; p=0.012) and (1170.67±398.72 vs 537.36±348.08; p=0.012). Also there was a negative correlation between D-dimer level and ABI

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values (r -0.577; p=0.000). Diabetes mellitus patients with PAD had significantly higher fibrinogen and D-dimer levels compared with DM patients without PAD. There was a negative correlation between D-dimer level and ABI values.

Key words: Diabetes mellitus, peripheral arterial disease, coagulation status, ankle brachial index

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia and impaired metabolism of carbohydrates, proteins and lipids caused by a defect in insulin secretion and/or insulin work.[1] In 2013, Indonesia was the seventh largest country of diabetes mellitus sufferers in the world with 8.5 million people and the number of DM sufferers will be 14.1 million by 2035.[2] Diabetes mellitus is often associated with microvascular and macrovascular complications, in which hyperglycemia is a risk factor for atherosclerosis and vascular disease, causing damage to blood vessels.[3]

Peripheral Arterial Disease (PAP), moreover, is a disease with reduced blood flow to the extremities characterized by typical ischemic pain, non-typical pain, or even no symptoms depending on the severity degree of the disease. Peripheral arterial disease in diabetes is different in biology, clinical description, and management. Therefore, this disease often does not cause any symptoms or unclear complaints, different from PAP with classic symptoms, intermittent claudication. Thus, as a consequence of neuropathy, PAP and diabetes sufferers often arrive late and already show certain symptoms, from rest pain, ulcers, to gangrene.[4]

Some researches show a change in hematologic status in patients with DM. In patients with diabetes, chronic hyperglycemia will trigger glycation of hemoglobin, prothrombin, fibrinogen and other proteins involved in the clotting mechanism. Glycation then stimulates activation and function of the clotting to become incomplete.[5]

Fibrinogen has atherogenic effects on blood vessels, indicated by both fibrin deposition on the endothelium and a change in endothelial permeability by fibrin. Fibrinogen levels actually will increase in the presence of microvascular or macrovascular disease, indicating that this protein plays a role in the pathogenesis of diabetic complications.[6] Most researches even suggest that high fibrinogen levels are independently associated with a lower Ankle Brachial Index (ABI), but there are also some researches suggesting otherwise.[7]

D-dimer, furthermore, is a fibrin degradation product. D-dimer levels in the blood circulation are not only dependent on the formation of fibrin, but also on fibrinolytic activity. As a result, D-dimer levels can be associated with atherosclerosis.[3] Therefore, this research aimed to determine differences in levels of fibrinogen and D-dimer towards the severity degree of peripheral arterial disease in patients with diabetes mellitus. Similarly, a research suggests that increased D-dimer is associated with a rapid decline in physical activity of patients with peripheral arterial disease.[8]

METHODS

This research was approved by the Ethics Committee for Health Research in Faculty of Medicine, University of North Sumatra. This research was an analytical observational research with a cross sectional design. The research population comprised patients with a diagnosis of diabetes mellitus in the Department of Medicine of the Adam Malik Hospital from April to October 2015.

In addition, sampling was conducted consecutively in all reasonable population that met the research criteria. Inclusion criteria were patients at the age of >18 years suffering from DM. Meanwhile, exclusion criteria were using anticoagulant medications in the past one week and having impaired liver function, hypertension as well as smoking habits.[5]

Next, Ankle Brachial Index (ABI) of the research samples was measured by using a handheld Doppler device 8 MHz Doppler probe to assess the severity degree of PAP, namely mild PAP (0.70 to 0.90), moderate PAP (0.40 to 0.69), severe PAP (<0.40). Citrate blood samples then were taken for examination of fibrinogen and D-dimer levels.

Afterwards, fibrinogen examination was conducted using Coatron A4 tool (Automated Coagulation Analyzer) with end point method. The principle used was that the formation of fibrin could cause turbidity in the samples detected by the photometer.

On the other hand, D-dimer examination was performed using JR Dimex instrument with latex agglutination method. Immunoturbidimetri principles were used, in which the intensive rays could penetrate into the turbid solution, such as latex suspension used in the measurement of D-dimer.

And the last, analysis of data was conducted to assess differences in parameters of coagulation status towards the value of ABI using unpaired t test. Next, to assess differences in parameters of coagulation status

towards the severity degree of PAP based on the value of ABI, One Way Anova test was performed. To see the correlation of the coagulation parameters and PAP, Spearman's test then was conducted. The test results were considered statistically significant if P was less than 0.05 with a confidence level of 95%.

RESULTS AND DISCUSSION

Table 1 showed that of the 40 patients with DM, there were 20 DM patients also suffering from PAP. Of the 20 DM patients with PAP, there were 14 females (70%) and 6 males (30%). According to Teodorescu *et al.*[9], the incidence of PAP is more common in females than in males due to a positive relationship between waist-to-thigh ratio, waist circumference and PAP in females.[9] In contrary, a research conducted by Mascarenhas *et al.*[10] showed that the prevalence ratio of PAP in males and females was 2:1 due to the protective effects of estrogen in premenopausal females.[10]

Based on the ABI value obtained, PAP was divided into three, namely mild PAP as many as 14 people (35%), moderate PAP as many as three people (7.5%) and severe PAP as many as 3 people (7.5%). The mean age in DM patients with PAP was younger than in DM patients without PAP (54.20±7.84 vs 57.60±9.46). Fasting blood sugar levels, blood sugar two hours post-prandial levels and HbA1c levels in DM patients with PAP were higher than in DM patients without PAP. Some researches even reported a correlation of HbA1c levels and the incidence of macrovascular complications in patients with diabetes, such as coronary heart disease, stroke and PAP.[11]

Table 1. Basic characteristics of the research samples

Variables	PAP (n=20)	Non-PAP (n=20)
Sex		
Female	14 (70%)	10 (50%)
Male	6 (30%)	10 (50%)
Age (years)	54.20±7.84	57.60±9.46
FBSL	171.29±57.31	170.20±57.13
BSL 2PP	269.15±115.15	259.85±84.42
HbA1c	8.56±1.92	8.43±2.30
PAP Levels		
Mild PAP	14 (35%)	
Moderate PAP	3 (7.5%)	
Severe PAP	3 (7.5%)	

Note: FBSL: Fasting Blood Sugar Levels, BSL 2PP: Blood Sugar Levels 2 Hours Post-prandial, HbA1c: Hemoglobin A1c, PAP: Peripheral Arterial Disease

Based on Table 2, there were significant differences between fibrinogen levels and PAP. Thus, the levels of fibrinogen in DM patients with PAP were higher than in DM patients without PAP (333.35±127.49 vs 244.95±83.96; p=0.001). Similarly, a research conducted by Paraskevas *et al.*[6] reported that there was a correlation between fibrinogen levels and PAP in DM patients, so DM patients with PAP had higher fibrinogen levels than DM patients without PAP (10.9±2.3 vs 10.2±2.2 mol/l, p<0.0001).

Like fibrinogen levels, there was also a significant correlation between D-dimer levels and PAP. As a result, the levels of D-dimer in DM patients with PAP were higher than in DM patients without PAP (648.40±443.96 vs 302.45±108.41; p=0.008). Similarly, a research conducted by McDermot *et al.*[8] revealed that the levels of D-dimer in patients with PAP were higher than in patients without PAP (0.62 ug/mL (0.45 to 0.99) vs. 0:46 ug/mL (0.33 to 0.73).

However, there was no difference in the values of the prothrombin time (15.22±2.90 vs. 15.33±2.43; p=0.829), INR (1.10±0.22 vs 1.11±0.18; p=0.745) and APTT (31.13±4.02 vs. 31.16±4.78; p=0.986) between in DM patients with PAP and in DM patients without PAP. Similarly, a research conducted by Chavan *et al.*[12] reported that the value of APTT in patients with type 2 DM was shorter than in healthy individuals (26.71 vs. 29.90; p<0.0001), but there was no significant difference in the value of PT between in patients with type 2 DM and in healthy people (13.24 vs 13.59; P=0.260).

Based on Table 3, there was a significant difference between the levels of fibrinogen and D-dimer and the severity degree of PAP. Consequently, the levels of fibrinogen and D-dimer in the severe PAP patients were higher than in the mild PAP patients. Nevertheless, there was no significant difference between the levels of PT, INR, as well as APTT and the severity degree of PAP.

In Table 4, based on the results of Spearman correlation test, there was no significant correlation between PT, INR, APTT, as well as fibrinogen and PAP, namely (r 0.21; p=0.194), (r 0.109; p=0.503), (r 0.155; p=0.34) and (r -0.140; p=0.389). There was also a significant negative correlation between D-dimer levels and PAP. Therefore, the elevated levels of D-dimer could be associated with the decreased or low value of Ankle Brachial Index (ABI) (r -0.577; p=0.000). In a

Table 2. Differences in hemostasis parameters between patients with PAP and patients without PAP

Variables	PAP (n=20)	Non-PAP (n=20)	Nilai P
PT	15.22±2.90	15.33±2.43	0.829
INR	1.10±0.22	1.11±0.18	0.745
APTT	31.13±4.02	31.16±4.78	0.986
Fibrinogen	333.35 ± 127.49	244.95±83.96	0.001
D-dimer	648.40 ± 443.96	302.45±108.41	0.008

Note: PT: Prothrombin Time, INR: International Normalized Ratio, APTT: Activated Partial Thromboplastic Time, PAP: Peripheral Arterial Disease

Table 3. Differences in hemostasis parameters based on the severity degree of PAP

Variables	Mild PAP (n=14)	Moderate PAP (n=3)	Severe PAP (n=3)	P
PT	15.06±2.36	13.97±3.93	17.20±4.44	0.511
INR	1.09±0.17	1.01±0.30	1.26±0.33	0.520
APTT	31.20±4.10	31.30±4.30	30.63±5.05	0.975
Fibrinogen	327.14±136.45	321.67±131.23	374.00±114.94	0.012
D-dimer	537.36±348.08	644.33±656.08	1170.67±398.72	0.012

Note: PT: Prothrombin Time, INR: International Normalized Ratio, APTT: Activated Partial Thromboplastic Time, PAP: Peripheral Arterial Disease

Table 4. Correlation between hemostasis parameters and PAP based on the values of ABI

Variables	PAP
PT	r 0.21
	p 0.194
INR	r 0.109
	p 0.503
APTT	r 0.155
	p 0.34
Fibrinogen	r - 0.140
	p 0.389
D-Dimer	r - 0.577
	p 0.000

Note: PT: Prothrombin Time, INR: International Normalized Ratio, APTT: Activated Partial Thromboplastic Time, PAP: Peripheral Arterial Disease

research, the increase in D-dimer levels even could also be associated with the rapid decline in physical activity in patients with PAP.[8] Similarly, in another research, the increased levels of D-dimer could be associated with the low value of Ankle Brachial Index.[13]

There was a significant negative correlation between the levels of D-Dimer and the values of Ankle Brachial Index (ABI). Therefore, the elevated levels of

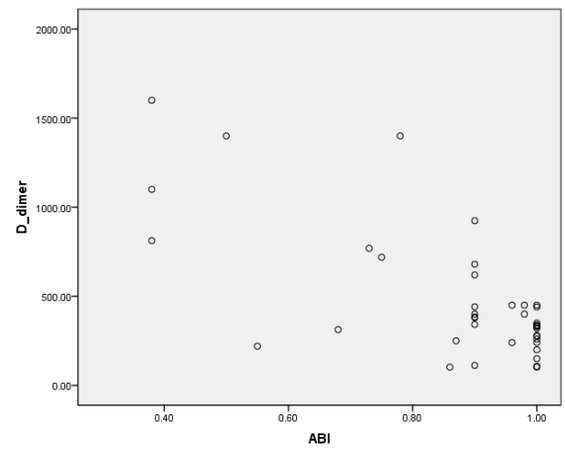

Figure 1. Scatterplot correlation between the levels of D-dimer and the values of ABI.

D-dimer could be associated with the decreased or low value of Ankle Brachial Index (ABI) (see Figure 1).

CONCLUSION AND SUGGESTION

Based on the results of this research, it can be concluded that there were significant differences in the levels of fibrinogen and D-dimer between in DM patients with PAP and in DM patients without PAP. The levels of fibrinogen and D-dimer in DM patients with PAP were higher than in DM patients without PAP. It is also known that there was no significant difference in Prothrombin Time values, INR values, and APTT values between in DM patients with PAP and in DM patients without PAP.

However, there was a significant difference between the levels of fibrinogen and D-dimer and the severity degree of PAP. The levels of fibrinogen and D-dimer in severe PAP were higher than in mild PAP. But, there was no significant differences in Prothrombin Time value, INR value and APTT value among the severity degrees of PAP.

Moreover, fibrinogen can affect all aspects of hemostasis, including influencing blood viscosity, plaque formation, and platelet activation. Blood and plasma viscosity then will increase because of increased levels of fibrinogen in the blood, so the shape of red blood cells will also be influential. In the end, this process becomes more relevant to stenosis that occurs in DM patients with PAP. D-dimer, on the other hand, is a fibrin degradation products, so D-dimer levels in the blood circulation are not only dependent on the formation of fibrin, but also on the activity of fibrinolytics.[6] In this research, it was known that there was a significant negative correlation between the levels of D-dimer and ABI. Thus, the elevated levels of D-dimer could be associated with the lower value of Ankle Brachial Index (ABI). For those reasons, the level of D-dimer can be used as a biomarker for predictors of peripheral arterial disease in patients with diabetes mellitus.

REFERENCES

1. Dhule S, Gawali S. Platelet aggregation and clotting time in type II diabetic males. National Journal of Physiology, Pharmacy & Pharmacology; 2014; 4(2): 121–123.
2. International Diabetes Federation (IDF) consensus. Managing older people with type 2 diabetes. Brussels, 2013; 1–96.
3. Bosevski M, Kostoska S, Tosevi S, Borozanov V. Prognostic importance of haemostatic parameters in polyarterial disease. Sec. Biol. Med. Sci; 2005; 26: 81–92.
4. ADA Consensus Statement. Peripheral Arterial Disease in People with Diabetes. Diabetes Care 2003; 26: 3333–3341.
5. Abdulrahaman Y, Dallatu MK. Evaluation of Prothrombin Time and Activated Partial Thromboplastin in Patients with Diabetes Mellitus. Nigerian Journal of Basic and Applied Science, 2012; 20(1): 60–63.
6. Paraskevas KI, Baker DM, Vrentzos ZE. The role of fibrinogen and fibrinolysis in peripheral arterial disease. Thrombosis research, 2008; 122: 1–12.
7. Khawaja FJ, Kullo IJ. Novel markers of peripheral arterial disease. Vasc Med, 2009; 14(4): 381–392.
8. McDermott MM, Ferrucci L, Guralnik JM. Elevated Levels of Inflammation, D-Dimer and Homocysteine Are Associated With Adverse Calf Muscle Characteristics and Reduced Calf Strength in Peripheral Arterial Disease. JACC, 2007; 50: 897–905.
9. Teodorescu VJ, Vavra AK, Kibbe MR. Peripheral arterial disease in woman. J Vasc Surg, 2013; 57: 18S–26S.
10. Mascarenhas JV, Albayati MA, Shearman CP. Peripheral arterial disease. Endocrinol Metab Clin N Am, 2014; 43(1): 149–166.
11. Muntner P, Wilman RP, Reynolds K. Relationship between HbA1c level and peripheral arterial disease. Diabetes care, 2005; 28(8); 1981–1987.
12. Chavan PS, Afroz S, Jadhav S. A comparative study of coagulation tests in type 2 diabetes mellitus individuals and healthy individuals. International journal of medical and applied sciences, 2014; 3: 290–298.
13. Unlu Y, Karapolat S, Karaca Y, Kiziltunc A. Comparison of level inflammatory markers and hemostatic factors in the patients with and without peripheral arterial disease. Thomres, 2005; 03–19.