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Comparison of Protein-C levels in type 2 Diabetes Mellitus patients with and without diabetic foot ulcers at Haji Adam Malik Hospital, Medan, Indonesia from May to July 2017

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ABSTRACT

Introduction: People with diabetes mellitus (DM) have an increased risk for thrombosis compared with non-diabetic patients. Several studies showed contradicting in data on levels of protein C in people with type 2 DM and diabetic ulcers.

Methods: This is a descriptive and analytical cross-sectional study on protein C concentration of patients with type 2 DM with and without foot ulcers. The control group was the type 2 DM patients without foot ulcers, whereas the case group was the type 2 DM patients with a rigid diabetic foot ulcer based on the Wagner criteria. Blood samples are then taken after 8 to 10 hours of fasting to check for protein C concentration and hemostasis examination including platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen levels, and D-dimers.

Results: Of a total of 20 samples, there was no statistically significant difference in protein C levels of diabetic foot ulcers with hypercoagulation compared with no diabetic foot ulcers with hypocoagulation and no significant correlation between grade diabetic foot ulcers and protein C concentration.

Keywords: C-protein, type 2 diabetes mellitus, diabetic foot, diabetic ulcer

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INTRODUCTION

The blood physiological condition can be obtained by a balanced hemostasis system between coagulation and fibrinolysis activity involving vascular endothelial, platelets, clotting proteins, anticoagulant proteins, and fibrinolytic enzymes. Any defects in one or more of these components will cause disturbance of hemostasis balance and cause complications of bleeding or thrombosis.[1,2]

Evidence from various studies revealed that people with diabetes mellitus (DM) are in a state of hypercoagulation. The condition of hyperglycemia, hyperinsulinemia, and insulin resistance can trigger changes in the components of hemostasis that cause increased tendency to experience thrombosis compared with the non-DM patient.[3,4,5]

Foot ulcers were found in 28.4% of diabetic foot patients who underwent treatment in Jakarta from 1999 to 2004, and 72.1% of the patients had gangrene.[6] Research conducted on diabetic foot population in Taiwan found 26.9% diabetic foot ulcers eventually develop into gangrene.[7] A study in Sweden found that diabetic foot ulcer patients with peripheral artery disease (PAD) experience elevated levels of several hemostasis parameters suggesting hypercoagulation and there was a relationship between the density of the fibrin gel structure formed by hemostasis function.[8]

In the group of patients receiving dalteparin and aspirin, there was an improvement in the function of skin microcirculation and lower amputation rates compared with the aspirin and placebo groups.[9] According to a study,[10] there was a decreased protein C concentration in type 2 DM patients. In another study, there was no difference in protein C levels in patients with DM compared to patients without type 2 DM.[11] In contrast to these results, other studies have increased levels of protein C in patients with type 2 DM[12], according to a study,[13] patients with type 2 DM who have ulcers in the lower extremities have lower protein C levels when compared with patients with type 2 DM without ulcers in the extremities.

Due to these contradicting findings, this research focused on the status of protein C in the group of patients with type 2 DM, with and without diabetic foot ulcers. This study assessed the ratio of decreased levels of protein C in patients with type 2 DM with ulcers and without diabetic foot ulcers.

METHODS

This is a descriptive and analytical cross-sectional study conducted in May - July 2017 at RSUP Haji Adam Malik Medan. The inclusion criteria of this study were men or women who were over the age of 18 year-old and willing to attend the study. The control group was the type 2 DM patients without foot ulcers, whereas the case group was the type 2 DM patients with a rigid diabetic foot ulcer based on the Wagner criteria. Patients with type 2 DM with other hematologic disorders other than type 2 DM such as heart failure, kidney failure, liver failure, pregnancy, malignancy, and ulcers from a major surgery were excluded.

All participants who completed the written informed consent form were requested to fill in their basic personal information about age, gender, and comorbid diseases. Blood samples are then taken after 8 to 10 hours of fasting to check for protein C concentration and hemostasis examination including platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen levels, and D-dimers. Quantitative data are presented in mean ± SD. Categorical data was displayed in numbers and percentages. Data processing was done using Statistical Package for the Social Sciences (SPSS) program. Chi-square test was used for comparison of categorical data. Unpaired t-test was used for comparison of parametric data. Pearson test was used for correlation. The results of statistical analysis said to have significance if the value of p is less than 0.05.

RESULTS

Characteristics of Research Subjects

A total of 40 samples are obtained, 20 samples for each diabetic foot ulcers and non-ulcers group. In the diabetic foot ulcers group, 13 patients (65%) were male and 7 patients (35%) were female. While in the non-diabetic foot ulcers group, the number of male and female patients was 10 patients each, there was no statistically significant difference in the gender between the two study groups (p> 0.05). The majority of the diabetic foot ulcers subjects were 50 - 59 years-old (11 patients (55%)) and the least frequent age group was 60 - 69 years-old with only 4 patients (20%) in this category. While in the non-diabetic foot ulcers subjects, 50 percent of the subjects were in the 50 - 59 years-old age group. Only 3 patients (15%) fell into the 40 - 49 years-old group. With Fisher exact test, no statistically significant difference in the age between the two study groups (p> 0.05) was found.

The most commonly found grade of diabetic foot ulcers was grade 4, which were constituted from 10 patients (50%), 3 patients (15%) were grade 5, and only 7% patients with a grade 3 ulcer.

The results of this study showed that the mean hemoglobin (Hb) level of diabetic foot ulcers was 10.70 ± 1.66 g/dL, which was lower than the mean Hb of the non-ulcer group level of 13.05 ± 1.74 g/dL. A significant difference was found between the concentration of Hb between the diabetic ulcer groups and the non-ulcer group (p <0.05) using t-test.

The mean platelet level of the diabetic foot ulcers group was 381200 ± 122599.734 mm^3, which was higher than the mean platelet level of the non-ulcer group of 303350 ± 110211.412 mm^3. Statistically, there was a significant difference in the platelet value between the diabetic foot ulcers group and the non-diabetic foot ulcers group (p <0.05) using t-test.

The mean fibrinogen level of the diabetic foot ulcers group was 445.40 ± 214.78 mg/dL, which was higher than the mean Fibrinogen level of the group without diabetic foot ulcers of 290.80 ± 90.16 mg/dL. There was a statistically significant difference between the fibrinogen level of diabetic foot ulcers group and the group without diabetic foot ulcer (p <0.05) using t-test or Mann-Whitney test (?).

The mean D-dimer concentration of the diabetic foot ulcer group was 647 ± 313 (ng / mL), which was higher than that of diabetic foot diabetic ulcer group i.e. 404 ± 301 (ng/mL). Using t-test, there was a statistically significant difference between the diabetic foot ulcers group and the group without diabetic foot ulcers (p <0.05).

The coagulation status in patients with diabetic foot ulcer revealed that there were six people with hypocoagulation (30%) and fourteen people with hypercoagulation (70%).

Protein C content in Type 2 DM patients with diabetic foot ulcer compared with without diabetic foot ulcer

The median for protein C concentration in diabetic foot ulcers group were 22.20 (18.6-525.27) ng/mL was lower than in the group without diabetic foot ulcer that was 30.66 (5.79-189.74) ng/mL. Mann-Whitney test showed no significant decrease in the protein C of diabetic foot ulcers group compared with the non-diabetic foot ulcers (p> 0.05). In addition, the median value of protein C concentration in the diabetic foot ulcer group with hypercoagulation was 20.79 (15.89-519), which was lower than the protein C concentration in the diabetic foot ulcer group with hypocoagulation that was 33.13 (5.79-525). However, no statistically significant differences in protein C levels of patients with diabetic foot ulcers and hypercoagulation group

Table 1. Characteristics of research subjects

	With diabetic foot ulcer (=20) Mean ± SD	Without diabetic Foot Ulcer(=20) Mean ± SD	*p-value*
Age (year old)			
40-49	5 (25)	3 (15)	
50-59	11(55)	10 (50)	0.493
60-69	4 (20)	7 (35)	
Gender			
Male	13 (65)	10 (50)	0.337
Female	7 (35)	10 (50)	
Grade of Ulcer			
0	0	20 (100)	
3	7 (35%)	0	
4	10 (50)	0	
5	3 (15)	0	
Complete Blood Count			
Hb (g/dl)	10.70 ± 1.66	13.05 ±1.74	0.001*
Platelet (mm^3)	381 ± 122	303± 110	0.041*
Hemostasis Physiology			
Fibrinogen	445 ± 214	290± 90	0.009*
D-Dimer	647 ± 313	404 ± 301	0.017*
Coagulation status			
Hypercoagulation	14(70)		
Hypocoagulation	6 (30)		

* Statistically significant (p<0.05).

compared with no ulcers and hypocoagulation group using Mann-Whitney test (p> 0.05).

Correlation between Protein C Concentration and Grade of Diabetic Foot Ulcers

Pearson test was used to assess the correlation between diabetic foot ulcers grade and the protein C level. No significant correlation between diabetic foot ulcers grade and protein C (r = 0.34; p = 0.144 (p> 0.05)).

DISCUSSION

The results of this study showed no significant differences between the lifespan of patients with type 2 DM with diabetic foot ulcers and patients without diabetic foot ulcers (p <0.05). This finding is supported with a previous research[13] where no significant differences were found between the lifespan of patients with type 2 DM and diabetic foot ulcers or patients without diabetic foot ulcers. However, unlike the results of a study conducted by Yekta,[14] where there was a significant difference between the lifespan of patients with type 2 DM and diabetic foot ulcers with no diabetic foot ulcers, with the older age suffering more diabetic foot ulcers complication than the younger age.

The study also found that male who suffered diabetic foot ulcers were as many as 13 (65%) patients, and there were 10 patients without diabetic foot ulcers (50%). The number of female subjects suffering from diabetic foot ulcers was 7 (35%), and patients without diabetic foot ulcer were 10 (50%) patients. No significant differences were found regarding the gender of the participants, this was

Table 2. Concentration of protein C (ng/mL) in patients with or without diabetic foot ulcers and its relationship with the coagulation status

	With ulcer n=20	Without ulcer n=20	*p*	Hypercoagulation with ulcer n=14	Hypocoagulation with ulcer n=6	*P*
Protein C (ng/mL)	22.200 (18.6-525.27)	30,66 (5.79-189.74)	0.665	20.79 (15.89-519)	33.13 (5.79-525)	0.61

Table 3. Protein C level based on diabetic foot ulcers grade and the correlation between diabetic foot ulcers grade and protein C level

	Grade 3	Grade 4	Grade 5	*P*	R
Protein C (ng/mL)	19.54 (18.09-42.75)	65.02 (17.75-519.95)	21.39 (18.75-525.27)	0.144	0.34

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consistent with a study obtained by Whitmont,[13] but different results were obtained from a study conducted by Al-Rubeaan[15] in which diabetic foot ulcers were significantly higher in men since the group had a more restricted joint mobility and higher foot pressure when compared to women.

The lower mean hemoglobin values of diabetic foot ulcers patients in this study when compared with patients without diabetic foot ulcers was in accordance with the results of another research[16] where hemoglobin values were significantly lower in patients with diabetic foot ulcers than patients without diabetic foot ulcers, but the platelet values did not differ significantly. Similarly, a research conducted in London[17] found 14 patients with anemia in 27 diabetic foot ulcers patients. Vascular complications of diabetes neuropathy, nephropathy, and retinopathy are associated with anemia that can affect wound healing and macrovascular disease. Low levels of oxygen in the blood followed by low levels of hemoglobin can cause the ischemic condition of the lower limbs worsened. Various studies found that the anemia is more common in people with DM compared with those without DM. The association between anemia and diabetic foot ulcers remains unexplained, but the role of inflammation and chronic disease in diabetic foot ulcers has an important role in the occurrence of anemia.[17,18] Regarding the significantly higher mean platelet values in patient with diabetic foot ulcer were than the patients without diabetic foot ulcers, a study conducted in China[19] achieved higher platelet grade values in 152 patients with diabetic foot ulcers (255800 ± 79.5 mm^3) compared with 52 control patients (233500 ± 62.1 mm^3), although the difference was not statistically significant. However, these higher platelet values could not serve as a reference to the severity of diabetic foot ulcers because platelet values may change with the degree of inflammation.[19]

In this study, we found 14 diabetic foot ulcers patients (70%) with hypercoagulation and six patients (30%) with hypocoagulation. A previous study in Medan[20] have resulted in 67% of diabetic foot ulcers patients were in hypercoagulation state. The results also found that the mean fibrinogen level of diabetic foot ulcer group of 445 ± 214 mg/dL was higher than the non-ulcers group's mean fibrinogen i.e. 290 ± 90 mg/dL. Statistical tests with Mann-Whitney test showed no significant difference in Fibrinogen concentration between diabetic foot ulcers group with the non-diabetic foot ulcers ($p < 0.05$). This result is consistent with the research by Madan,[11] which revealed an increased fibrinogen levels in patients with DM with microvascular complications. Another research in China[19] also found elevated fibrinogen levels in 152 patients with diabetic foot ulcers. This study explained that there is a change in hemostasis component in diabetic foot ulcers patients which resulted in patients with type 2 DM and diabetic foot ulcers to experience higher tendency to be in hypercoagulation state. Fibrinogen has an important role in endothelial damage as a marker of inflammation, coagulation agent of major proteins in the blood, and determinants of blood viscosity and platelet aggregation. The study also described fibrinogen concentration association with severity of diabetic foot ulcers and amputation.[19]

Protein C has several important functions to regulate the hemostasis system and as a defense system against vascular and inflammatory injury. Anticoagulant protein C is designed to regulate coagulation, maintain smooth flow of blood in the blood vessels, and prevent thrombosis.[21-25] According to a study,[10] there was a decrease in protein C level in type 2 DM patients. However, according to another study,[11] there was no difference in protein C level between patients with type 2 DM and patients without. In contrast to the above results, a study by Yano[26] obtained a significant increase in protein C levels in type 2 DM. In this study, the median of protein C concentration in diabetic foot ulcers patients was 22.20 (18.6-525.27) ng/mL, which was lower than the median of protein C in the non-ulcers group i.e. 30.66 (5.79-189.74) ng/mL. Statistically, however, the reduction in protein C of diabetic foot ulcers patients was not significant ($p > 0.05$). A study by Whitmont,[13] suggested that patients with type 2 DM who have ulcers in the lower extremities have significantly lower protein C levels when compared with type 2 DM patients without ulcers in the extremities.

In this study, the statistically insignificant decrease may be due to the protein C measurement was not immediately done when the patient went to the hospital, initial treatment of DM has been started before the protein C concentration measurement was done. This was because RSUP Haji Adam Malik Medan is a regional referral hospital, where all patients that were referred to this hospital had previously been treated for type 2 DM and diabetic foot ulcers.

The aim of this study to find a relationship between the coagulation statuses of diabetic foot ulcer patients with protein C concentration yielded in no statistically significant difference even when the level of protein C in patients with hypocoagulation state is higher when compared with the value of protein C in hypercoagulate patients. This may be due to the fact that other factors that influence coagulation disorders such

as hypoalbuminemia were not examined which are also common in patients with chronic illnesses.

The limitations of this study were the involvement of only one center and lack of data on the duration type 2 DM suffered by the patients. In a previous study by Al-Rubeaan,[15] it was found that the duration of DM and HbA1c was significantly higher in patients with diabetic foot ulcers and amputations compared with in patients without diabetic foot ulcers. Therefore, the longer duration DM increases the risk for chronic complications. This study did not take into account any previously given DM treatment before the study was conducted. This study also did not measure the level of albumin as a factor that may give clue to certain coagulation disorders.

CONCLUSION

The protein C concentration in patients with diabetic foot ulcers was lower than those without diabetic foot ulcers, but the lower value was not statistically significant. There was no significant difference between the value of protein C and the degree of diabetic foot ulcers.

SUGGESTION

Information from the patient's medical history can be used to complement any missing data before the examination was done. Further studies with bigger samples size and involving more than one center will be useful to examine other factors that may influence coagulation factors.

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