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Relationships between the Microvascular Complications of Diabetes Mellitus (DM) and Natural Coagulation Inhibitors and Anticardiolipin (ACA) Antibodies

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ABSTRACT

Objective: *Changes in the coagulation system in patients with diabetes mellitus draw interest because of their potential role in pathogenesis of great and small vessel diseases. Therefore, in this study we aimed to investigate the relationship between microvascular complications and natural coagulation inhibitors and anticardiolipin antibodies (ACA) in patients with Type 2 DM.*

Methods: *This study included a total of 50 Type 2 DM patients with 15 being male (30%) and 35 female (70%) and 22 healthy persons as the control group. The mean age was 50.06±8.77 in patients and 44.91±4.13 in the control group. Protein S (PS), Protein C (PC), Antithrombin 3 (AT3) and ACA levels were measured in the patient and control groups. Microalbuminuria, retinopathy and neuropathy conditions were determined and compared between the patient and control groups.*

Results: *Protein C and AT3 levels were significantly higher in the patient group than in the controls (P<0.05). Whereas protein S was significantly higher in the control group (P<0.05). ACA positivity was increased in the patient group, but no statistically significant differrence found compared to the control group. Protein C and AT3 were significantly higher in microalbuminuric patients compared to normoalbuminuric patients. PC and AT3 were higher in patients with retinopathy than the patients without retinopathy, but the difference was not statistically significant. No significant correlation was found between retinopathy and PS and ACA. PC was higher in patients with neuropathy compared to those without retinopathy, and the difference was statistically significant.*

Conclusion: *High levels of PC and AT3 contibutes to microvascular complications in diabetes mellitus, while PS and ACA seem to not have much effect. Increased levels of PC and AT3 may be indirect indicators of previously existing disease. Further studies are warranted in order to evaluate effects of various therapeutic strategies hemostatic abnormalities in early period of Type 2 DM patients.*

Keywords: *Anticardiolipin antibodies, Antithrombin 3, Diabetes mellitus, microvascular complications, Protein S, Protein C.*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which occurs with absolute or relative deficiency of insulin secretion or insulin resistance and is characterized by chronic hyperglycemia resulted from the impairment of carbonhydrate, fat and protein metabolism (1).

DM causes acute metabolic complications as well as macrovascular and microvascular complications in long term. Studies have demonstrated a significant correlation between microangiopathic complications of DM and poor metabolic control (2). Activation of coagulation system has been considered in the pathogenesis of vascular complications in patients with DM. Abnormalities in anticoagulant system may be a potential triggering factor for hemostatic activation seen in diabetic patients (3). Two studies have found that antigen or activity of protein C decreases in patients with insulin dependent DM (IDDM) patients and returns to normal with improvement of hyperglycemia. There are also studies that have found higher than normal or normal levels of protein C in patients with IDDM or non-insulin dependent DM (NIDDM). Several studies reporting normal, low or high

levels of protein S levels in DM have been published in the literature (4-6). Similar to protein C-S system, there are studies reporting decrease or increase or no change in Antithrombin III (AT3) activity (7,8). Ceriello et al. argued that hyperglicemia disrupts AT3 activity by non-enzymatic glycation both in diabetics and normal persons, and thus while antigenic level of AT3 is normal in DM patients, its activity is impaired (9). Increased frequency of anticardiolipin (ACA) has been reported in DM patients, but there are only a few reports about its potential relationship with diabetic complications. ACA has been argued to be correlated with diabetic nephropathy and macroangiopathy (10). Changes in the coagulation system in patients with diabetes mellitus draw interest because of their potential role in pathogenesis of great and small vessel diseases. Therefore, in this study we aimed to investigate the relationship between microvascular complications and natural coagulation inhibitors and anticardiolipin antibodies in patients with Type 2 DM.

METHODS

This study was designed as a prospective controlled study. A total of 50 patients diagnosed with Type 2 DM who presented to Ankara Diskapi Yildirim Beyazit Training and Research Hospital, Internal Medicine outpatient clinic over 2 years period. Patients who had only microvascular complications (nehropathy, retinopathy and neuropathy) as diabetic complications were included in the study. 22 healthy volunteers who had no history of vascular and systemic disease and no liver, renal disease and no infections were included in the study as the control group. Patients with a known cardiovascular disease, HT and hyperlipidemia were excluded. According to the medical history, physical examination and laboratory outcomes, none of the patients had macrovascular disease. Anamnesis and physical examination of the patients were carefully carried out considering persistence of chronic complications at the first admission and follow-up. Clinical evaluation of the chronic complications and risk factors was carried out as follows:

Blood pressure of the patients was measured after resting for 10 minutes with 6 hours intervals at sitting position. Persons with a high pressure of 140/90 mmHg or lower at least at two measurements were included in the study. Body mass index (BMI) of the patients was calculated (kg/m2). Patients with a BMI between 18.5-25 were considered as normal weight, 25.1-30 as over weight, 30.1-40 as obese and > 40 morbid obese. For diabetic nephropathy, microalbuminuria and quantitave protein amount in 24-hour urine were measured with nephelometric method (Dade Behring, BN II; Germany) and existence of nephropathy was determined. For creatinine clearance values; lower limit was considered as 75 mL/min, upper limit as 135 mL/min and microalbuminuria as ≥ 30 mg/24 h. Values of 75 mL/min or lower and 135 mL/min or higher were considered as pathologic for creatinine clearance, and 30 mg/24h was considered as pathologic for microalbuminuria. Accordingly, patients were grouped as the patients with and without miccroalbuminuria.The necessary investigations for detection of existence and stage of diabetic retinopathy were performed in ophtalmology outpatient clinic by specialists. Existence of neuropathy was evaluated in consultation with neurology clinic of our hospital by neurologists.

STATISTICAL ANALYSIS

The results are expressed as mean, ±SD and median value. Significance between two means (t test) was used in the independent groups. Mann-Whitney *U* test, Student's t test and Chi-square test were used in the comparisons. One way Anova test was used for the comparison of patients with and without retinopathy and neuropathy, while post-hoc Dunnet and Tuckey tests were used in the comparisons with the control group in the case of difference. In our study statistical analysis was carried out with SPSS for Windows 17 software. $P<0.05$ values were considered as statistically significant.

RESULTS

Out of 50 patients, 35 were female (70%) and 15 were male (30%). Ages of the patients varied between 29-72 (mean 50±8.7). Durations of known diabetes were found to be between 5 and 25 years (mean 9.78±4.6). Of the control group, 15 were female (68.2%) and 7 were male (31.8%). Ages of the controls varied between 37-53 years (44.9±4.1). Age, fasting plasma glucose, duration of diabetes, height, weight, body mass index (BMI), glycosylated hemoglobin, systolic and diastolic pressure values were recorded in the patient and control groups. PC, PS, AT3, ACA, hematocrit and hemoglobin counts, blood glucose, HbA1c, total cholesterol level, and triglycerides levels were measured. Of all patients, 43 were receiving oral

antidiabetics (OAD) (86%), and 7 (14%) were receiving insulin therapy. Demographics and laboratory outcomes of the patient and control groups are summarized in Table 1. A statistically significant difference was found between the two groups in terms of the mean PC, PS and AT3 values (P<0.05). ACA was positive in 3 patients, while none of the controls was ACA positive. There was no statistically significant difference between them (P>0.05). Whereas 32 patients had at least one complication (64%), 18 patients had no complications (36%). Distribution of the patients according to DM complications is shown in Table 2. PC was found as 96.23±13.01 in patients without complication and 124.94±20.41 in the patients who had at least one complication. AT2 was found as 97.33±12.24 in patients without complication and 111.69±13.16 in the patients who had at least one complication. There was a statistically significant difference between the two groups in terms of PC and AT3 values (P<0.05). No statistically significant difference was found between the patients without complication and the patient who had at least one complication in terms of PS and ACA (P>0.05). There was a statistically significant difference between the groups in terms of PC, PS and AT3 (P<0.05). However, there was no significant difference in terms of ACA (P>0.05). No statistically significant difference between the groups in terms of PC, PS and ACA values (P>0.05). There was a significant difference between the two groups in terms of AT3 (P<0.05).

Table1. *Demographics and laboratory outcomes of the patient and control groups*

	DM	CONTROL	P
Age (Years)	50±8,7	44,9±4,1	Ø
DM Period (Years)	9,7±4,6	--	
HbA1C(%)	9,05±2,68	4,85±4,34	Ø
SBP(mmHg)	122,40±8,75	123,45±7,08	Ø
DBP(mmHg)	75,22±6,05	73,55±4,90	Ø
T.Cholesterol(mg/dl)	207,86±49,82	204,00±25,36	Ø
Trigliseride(mg/dl)	147,92±58,48	153,36±23,01	Ø
Glucose(mg/dl)	223,18±78,67	86,77±6,73	P<0,05
Hb	14,11±1,31	14,25±0,77	Ø
Htc	41,75±3,90	42,77±2,40	Ø
PC(%)	114,61±22,71	92,22±18,38	P<0,05
PS(%)	75,14±23,02	87,22±14,32	P<0,05
AT3(%)	106,70±14,38	89,13±16,40	P<0,05
ACA	Pozitive for 3 patients	Negative	Ø
BMI(kg/m2)	27,63±4,71	26,77±4,34	Ø

SBP: Sistolic Blood Pressure, DBP: Diastolic Blood Pressure

Table2. *Distribution of the patients according to DM complications*

	Microalbuminuria	Retinopathy	Neuropathy
No	25(%50)	35(%70)	31(%62)
Yes	25(%50)	15(%30)	19(%38)

n(%)

When patients with microalbuminuria were compared with the controls in terms of mean PC, PS and AT3 values, the difference between them was statistically significant (P<0.05). No significant difference was found between the two groups in terms of PS and ACA (P>0.05).

When patients with normoalbuminuria were compared with the controls in terms of mean PC, PS and AT3 values, the difference between them was statistically significant (P<0.05). No significant difference was found between the two groups in terms of ACA (P>0.05).

Comparison of diabetic retinopathy and PC, PS, AT3 and ACA levels is given in Table 3. Comparison of diabetic neuropathy and PC, PS, AT3 and ACA levels is determined in Table 4.

Table3. *Comparison of diabetic retinopathy and PC, PS, AT3 and ACA levels*

	Retinopathy	**No retinopathy**	**Control**	**P**
PC	120,24±20,73	112,19±23,38	92,22±18,38	**P<0,05**
PS	71,72±19,08	76,61±24,62	87,22±14,32	P>0,05
AT3	113,56±11,40	103,76±14,65	89,13±16,40	**P<0,05**
ACA	1	1	0	P>0,05

Table4. *Comparison of diabetic neuropathy and PC, PS, AT3 and ACA levels*

	Neuropathy	No Neuropathy	Control	P
PC	126,81±22,19	107,12±19,88	92,22 ±18,38	**P<0,05**
PS	70,18±21,67	78,18±23,63	87,22±14,32	**P<0,05**
AT3	112,92±11,26	102,89±14,90	89,13 ±16,40	**P<0,05**
ACA	1	0	0	P>0,05

In the patient group, duration of DM was 5-9 years in 31 (62%) patients, 10-14 years in 9 (18%) and 15 years or longer in 10 (20%). The correlation between duration DM and incidence of microalbuminuria was not statistically significant (P>0.05). The correlation between duration DM and incidence of retinopathy was statistically significant in the patient group (P<0.05). The correlation between duration DM and incidence of neuropathy was statistically significant in the patient group (P<0.05). Comparison of HbA1c levels and PC, PS, AT3 and ACA levels is summarized in Table 5.

Table5. *Comparison of HbA1c levels and PC, PS, AT3 and ACA levels*

	HbA1C≤7,5	HbA1C ≥7,6	Control	P
PC	105,81±20,75	119,14±22,64	92,22±18,38	**P<0,05**
PS	76,60±22,83	74,39±23,43	87,22±14,32	P>0,05
AT3	102,83±14,06	108,70±14,34	89,13±16,40	**P<0,05**
ACA	1	2	0	P>0,05

DISCUSSION

Changes in the coagulation system in patients with DM are important in the pathogenesis of great and small vessel diseases. Elevated procoagulant activity in these patients is thought to be associated with increased cardiovascular morbidity and mortality. Because this could not be fully explained with arterial HT, smoking and hypercholesterolemia that are major risk factors. Impaired hemostasis is also important in development of complications such as nephropathy, retinopathy and increased atherogenesis (2). However, mechanism of the increased activity of coaagulation system is not clear for DM. Studies about natural anticoagulant proteins (PC, PS) in diabetic patients have found different results.

Although two studies have found that antigen or activity of protein C decreases in patients with insulin dependent DM patients and returns to normal with improvement of hyperglycemia, there are also studies that have found higher than normal or normal levels of protein C in patients with IDDM or non-insulin dependent DM. There are studies reporting normal, low and high levels of protein S (3, 5-8, 10, 11). In our study also PC and AT3 were significantly higher in the patient group compared to the controls. Whereas PS was significantly lower in the patient group than in the control group. Our results were consistent with the literature. Different results can be explained with types of diabetes, and difference in glycemic and lipidemic controls (12).

Some studies have showed increased PC in microalbuminuric patients (3, 13). In our study also there was a statistically significant difference when microalbuminuric patients were compared with normoalbuminuric patients. PC was higher in microalbuminuric patients. This result supports previous studies. Whereas low plasma levels of PS were found in patients with IDDM, PS was found to be increased in patients with NIDDM (13). In the present study also PS level was lower compared to the control group. This was not consistent with the literature. In addition, no statistically signifficant difference was found between the patients with microalbuminuria and normoalbuminuria and the control group. Some studies have found increase in AT3 together with PC and PS (12, 13). In our study, AT3 level was significantly higher in microalbuminuric patients compared with normoalbuminuric patients. Similarly, AT3 level was significantly increased in these patients compared with the control group. Impairment of the anticoagulant system components may be associated with hyperglycemia. Hyperglycemia has been demonstrated to cause decrease in biological activity of anticoagulant protein AT3 in diabetic and non-diabetic persons (14). Structural changes due to nonenzymatic glycation are thought as a causal factor of AT3 dysfunction (15).

In our study, retinopathy and neuropathy that are among the other microvascular complications were also evaluated. PC and AT3

levels were higher in the retinopathy group than in the group without retinopathy. However, the difference between them was not statistically significant. PS level was similar with the literature in the groups with and without retinopathy (16). Our results suggest that PC and AT3 may have contribution to the pathogenesis of retinopathy, while PS has no contribution. PC was higher in the patients with neuropathy and the difference was statistically significant. However this correlation could not be shown for PS and AT3. Therefore, it is thought that increased PC level may be a risk factor for the development of neuropathy.

In a study, a positive correlation was reported between microalbuminuria and macroalbuminuria and anticoagulant proteins in diabetic patients (13). PC and PS levels were found to be high and it was stated that this may indicate reactive anticoagulator compensation (5). Another possible mechanism is that thrombomodulin (TM) is a physiological receptor and coactivator of PC and it is normally expressed from the surface of endothelial cells. Endothelial cell dysfunction plays a role in the pathogenesis of atherosclerosis and diabetic vascular diseases and it can be formed with the effect pd nonenzymatic glycation or lipid peroxides in diabetic patients (13). TM releases to the circulation following endothelial damage (11). Therefore, decrease of surface concentration of TM may lead to decrease and clearing of PC activation. Therefore, increased levels of PC and PS may lead to acceleration of procoagulant activity with endothelial membrane related decrease of TM which is an indirect indicator of pre-existing disease together with poor PC anticoagulant response.

Even decrease in PC activation due to endothelial cell damage may reduce inactivation rate of plasminogen activator inhibitor -1 and this leads to inhibition of fibrinolytic activity, resulting in thrombogenic trends (17). In our study also PC levels were increased, but PS levels were found to be lower. Abnormal hemostatic values may be a result of the activation of cellular defense mechanisms. For example, adhesion of monocytes on the damaged endothelial cells in diabetic angiopathy region leads to increased secretion of cytokines and hepatic synthesis of acute phase reaction agents such as fibrinogen and other proteins increases. Since there is a close relationship between immunological systems, long term DM may be effective at a few points in these systems through cytokines, endothelial cells, complement and hemostasis (13).

Risk factors of micro and macro complications are not clear in patients with DM. Some studies have reported increased ACAs in diabetic patients with macrovascular complications (10). Existence of ACA was also reported in patients with end stage kidney disease who received hempdialysis and after myocardial infarction (18). In a study it was found that existence of ACA was an independent risk factor for diabetic macrovascular angiopathy and ACA are not found in diabetic patients without complications, but it is associated with diabetic nephropathy and macroangiopathy. It has been argued that ACAs may create an environmental medium which triggers the events leading to vascular diseases (10). The role of ACAs in thrombotic diseases is unclear. Possible pathophysiological mechanisms of ACA related thrombotic diseases include unrepaired endothelial cell function, platelet activation, change in Prostacyclin/Thromboxane balance, increased endothelial cell procoagulation activity with TNF-α related potentialization (19, 20). It has been reported that endothelial dysfunction and platelet activation leads to a procoagulant condition in diabetic patients. Abnormalities in expression of TNF-α have been found in diabetic patients (14). Thus, it can be concluded that, ACAs may interact with previously damaged eendothelial cells, leading to macrovascular damage (10). In our study, ACA IgG was found positive at low titer in 3 patients. Two of them were in microalbuminuric patient group and one was in normoalbuminuric patient group. There was no statistically significant difference between them. Similarly no significant correlation found between retinopathy and neuropathy and ACA. We probably could not find a correlation, because our patient number was limited, only patients with microvascular disease were included, and also there were no patients without cardiac and renal pathologies in the study group.

In this study, we also examined the relationship of DM duration with microalbuminuria, retinopathy and neuropathy. Incidence of retinopathy and neuropathy increased with DM duration. This was consistent with the previous data. However, such a correlation could not be find between microalbuminuria and DM duration. We attributed this to that number of patients with a high DM duration was limited in the study group.

PC and AT3 were higher in the patient group with HbA1c≥7.6 compared to the patient group with HbA1c<7.5, but the difference was not statistically significant. PS and ACA levels were similar in both group and there was no statistically significant difference. Our results suggest that hyperglycemia may cause elevated PC and AT3.

CONCLUSION

According to our results, high levels of PC and AT3 contributes to microvascular complications in DM, while PS and ACA seem to not have much effect. Increased PC and AT3 levels may be indirect indicators of preexisting vascular disease. Further studies are warranted in order to evaluate endothelial cell damage, TM secretion and PC kinetics and to investigate the effects of various therapeutic strategies regarding hemostatic abnormalities in early periods of patients with DM.

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