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CLINICAL INVESTIGATION

Evaluation of Plasma Protein C Antigen, Protein C Activity and Thrombomodulin Levels in Type 2 Diabetic Patients

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Abstract: The imbalance between coagulation and fibrinolysis leads to vascular complications in diabetics. In our study, we have investigated the effects of the protein C-thrombomodulin system as a cause of diabetic vascular complications.

Whereas PC-Ag levels were lower, thrombomodulin (TM) levels of diabetics were higher than that of the controls ($P < 0.001$, $P < 0.001$ respectively).

Furthermore, patients were divided into three groups according to their urinary albumin excretion (UAE). Group 1, 2 and 3 consisted of 27 patients with UAC ≤ 30 µg/ml, 16 patients with UAC in range 30-140 µg/ml, 3 patients with UAC >140 µg/ml, respectively. Plasma PC-Ag levels were significantly decreased in group 1 and 2 with respect to controls ($P < 0.01$, $P < 0.01$). Plasma TM concentrations were significantly increased in group 1, 2 and 3 compared with the control group ($P < 0.001$, $P < 0.01$, $P < 0.01$).

Patients were also divided into two groups according to the duration of their illness; patients whose diabetic age < 10 years (Group A) and whose diabetic age > 10 years (Group B). In both groups, PC-Ag levels were lower and TM levels higher than that of the control group ($P < 0.01$, $P < 0.01$; $P < 0.001$, $P < 0.001$ respectively). There was no significant difference between group A and B in PC-Ag levels ($P > 0.50$) but a significant difference in TM levels ($P < 0.001$).

Key Words: Protein C, Protein C activity, Thrombomodulin and Diabetes mellitus

Introduction

Patients suffering from diabetes mellitus (DM) are at increased risk of premature mortality and morbidity, mostly through the development of atherothrombotic vascular diseases. Vascular complications occur earlier and more frequently than in non-diabetic subjects. Abnormalities in the endothelium (1) and plasma coagulation system (2-5) may contribute to premature atherothrombotic diseases in DM. In this study we investigated the protein C antigen (PC-Ag), protein C activity (PCA) and thrombomodulin (TM) levels of diabetic subjects.

TM plays an important role in the protein C anticoagulant pathway which is initiated when thrombin binds to TM, an integral membrane receptor found primarily on the endothelium (6, 7). The thrombomodulin-thrombin complex activates protein C rapidly. Once activated, protein C binds to protein S and inactivates either factor Va or factor VIIIa on the surface of negatively charged phospholipids or on the membranes of activated cells. The thrombin-thrombomodulin complex is inhibited by either antithrombin or protein C inhibitor (7). Increased plasma TM levels are related to endothelial cell damage and disturbances of the organs responsible for TM clearance (6).

Materials and Methods

We studied 46 patients with type II diabetes mellitus and 24 healthy people recruited for the study (Table 1).

Patients with a history of acute ischemic heart disease, cerebrovascular and peripheral vascular disease, and liver or kidney function impairment were excluded from the study.

Table 1. Age, Glucose and HbA1c Levels of Patients and Controls.

	Age mean± SD (min-max)	Glucose mean± SD (min-max)	HbA1c mean± SD (min-max)
Patients (30 female and 16 male)	60.43± 12.02 (38-81)	173.2 ± 62.2 (72-357)	173.2 ± 62.2 (4.9-12.8)
Controls (16 female and 8 male)	59.44 ± 9.32 (45 - 84)	82.2 ± 8.4 (70-106)	5.2 ± 0.28 (4.8-5.8)
	P > 0.50	P < 0.001	P < 0.001

Activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen, TM, PC-Ag and PCA measurements were performed in citrated plasma. Hemoglobin A_{1C} (HbA_{1C}) measurement was performed on heparinized whole blood. Liver enzymes (alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase), urea, uric acid, creatinine, cholesterol and glucose levels were measured in serum on the day of blood collection. We used the urine samples which were collected over a 24-hour period for UAE detection. Urine specimens contaminated with bacteria, white blood cells or red blood cells were excluded.

Plasma TM and PC-Ag were measured by enzyme linked immunoabsorbent assay (ELISA) (Asserachrome Thrombomodulin, and Asserachrome Protein C respectively), Protein C activity measurements were made by using Staclot Prot C (Diagnostica Stago). HbA_{1C} was measured by the immunologic method (Roche Diagnostic HbA_{1C}). We determined the urinary albumin excretion (UAE) levels with the Orion Diagnostica Turbox U-Albumin assay, which was a liquid-phase immunoprecipitation assay with nephelometric end point detection.

We evaluated the statistical significance of the results and the correlation between parameters by using T-test and Pearson –Bravis Correlation coefficients, respectively.

Results

We measured PC- Ag, PCA and TM levels in both patient and control groups. PC- Ag levels of patient group were significantly lower than that of the control group (P < 0.001). PCA levels of Type II diabetic patients were lower than that of the control group but the difference between both groups was not statistically significant (P > 0.05). Finally, with respect to healthy control subjects, diabetic patients had significantly higher levels of TM concentration (P < 0.001) (Table 2).

These patients were allocated to three groups according to their albumin excretions. Group 1 consisted of 27 patients with UAE levels smaller than 30μg/ml, group 2 consisted of 16 patients with UAE levels within the range of 30- 140 μg/ml and group 3 consisted of 3 patients whose UAE levels were higher than 140 μg/ml.

Group 1, 2, 3 had lower PC levels than that of the control group. Although the differences between group 1, 2 and the control group were statistically significant (P < 0.01, P < 0.01), the difference between group 3 and the control group was not significant (P > 0.05) (Table 3).

Table 2. PC-Ag, PCA and Thrombomodulin Levels of Patients and Controls.

	PC-Ag (%) Mean± SD	PCA (%) Mean ± SD	TM (ng/ml) Mean ± SD
Patients	66.28 ± 12.98 n = 46	136.36 ± 49 n = 42	34.37 ± 11.75 n = 46
Control group	78.38 ± 15.58 n = 24	140.94 ± 32.1 n = 17	25.55 ± 8.47 n = 24
P value	< 0.001	> 0.05	< 0.001

Table 3. PC-Ag, PCA and Thrombomodulin levels of group 1, 2, 3 and control group.

	PC-Ag (%) Mean± SD	PCA (%) Mean ± SD	TM (ng/ml) Mean ± SD
Group I	66.74 ±12.77 n = 27 $P_1 < 0.01$	141.4 ± 44,24 n = 23 $P_4 > 0.05$	33.37 ± 13.43 n = 27 $P_7 < 0.001$
Group II	64.13 ± 14.18 n = 16 $P_2 < 0.01$	131.5 ± 57.22 n = 16 $P_5 > 0.05$	34.92 ± 4.64 n = 16 $P_8 < 0.01$
Group III	73.67 ± 6.66 n = 3 $P_3 > 0.05$	122.5 ± 50.65 n = 3 $P_6 > 0.05$	40.33 ± 4.64 n = 3 $P_9 < 0.01$
Control group	78.38 ± 15.588 n = 24	140.94 ± 32.1 n = 17	25.55 ± 8.47 n = 24

While PCA levels of group 1were higher, PCA levels of group 2 and 3 were lower than that of the control group, but the differences were not statistically significant (P > 0.05) (Table 3).

Plasma TM levels of group 1, 2 and 3 were higher than the control groups (P < 0.001, P < 0.01, P < 0.01). Although TM levels of the group 2 were also higher than group 1, the difference between these two groups was not statistically significant (Table 3).

The type II diabetic patients were classified into the following sub-groups according to the duration of DM: Group A consisted of 33 patients with a diabetes duration shorter than 10 years and group B consisted of 9 patients with a diabetes duration longer than 10 years. In both patient groups TM levels were significantly higher than that of the control group (P < 0.01 and P < 0.01, respectively). Moreover the difference between group A and B was also statistically significant (P < 0.01) (Table 4).

PC-Ag levels of Group A and B were significantly lower than that of the control group (P < 0.001 and P < 0.001, respectively). The differences between PC–Ag levels of Groups A and B were not significant (p_{AB}> 0.50). There were no significant differences between PCA levels of Group A, and the control group (P > 0.50) and Group B and the control group (P > 0.30). However the differences between PCA levels of Group A and Group B were significant (P_{AB}<0.001) (Table 4).

There was a positive correlation between PC-Ag and cholesterol levels in the patient group (r=+0.403, P < 0.001). We found that there was a weak negative correlation between PC-Ag and glucose and aPTT (r= -0.220 and –0.216 respectively). There were strong correlations between PC- Ag and APC levels (r=+0.521, P < 0.001). There were also a correlation between PCA and cholesterol levels of the patient group (r=+0.402, P < 0.001).

Table 4. Comparison of PC-Ag, PCA and Thrombomodulin levels of Groups A and B.

	PC-Ag (%) Mean± SD	PCA (%) Mean ± SD	TM (ng/ml) Mean ± SD
Group A (n= 33)	67.24 ± 12.64 P < 0,001	144 ± 40.03 P > 0,50	33.25 ± 11.18 P < 0,01
Group B (n= 9)	66.11 ± 15.34 P < 0,001	120.84 ± 64 P > 0,30	40.66 ± 6.83 P < 0,001
Control Group	78.38 ± 15.58 n = 24	140.94 ± 32.1 n = 17	25.55 ± 8.47 n = 24
P value (comparison of Group A and B)	P_{AB} > 0.50	P_{AB} <0.001	P_{AB} <0.01

Discussion

The non-thrombogenic properties of endothelium are very important for maintaining normal haemostatic balance. Disturbances in this balance may lead to an increase in thrombogenic tendencies or may trigger the pathophysiologic mechanism which causes atherosclerotic lesions (2). It is known that atherosclerosis and atherosclerosis-related complications are the primary cause of morbidity and premature mortality in diabetic patients (2).

In the present study we investigated the plasma levels of PC-Ag, PCA and TM in diabetic patients to evaluate the endothelial cell damage and the status of natural anticoagulants of plasma.

In our study the mean PC-Ag level was significantly lower than that of the healthy control group ($P < 0.001$). Our observation was well matched with Ceriello (5), and Koert (8). By contrast, Takahashi (9), Saito (3), and Garcia (4) found that PC-Ag levels of diabetics were higher than that of the healthy control subjects.

In Takahashi's study group, the number of diabetic patients was very small ($n = 17$), and there were significant differences between the mean age of diabetics and healthy controls (45.3 ± 14.1 years and 24.5±5 years, respectively). Dolan (10) and Tait (11) found that there were positive correlations between age and PC-Ag and PCA. As a result, differences in PC-Ag levels may depend on the age differences between both groups. Dolan (10) showed that PCA levels were higher in ages 40-45 than ages 20-25 and the differences were statistically significant. However, both investigators declared that there were not any significant age dependent differences in PC-Ag and PCA levels between 50-59 ages. On the other hand, they showed significant age dependent differences in age groups of 35-39 and 40-44.

Saito et al. (3) found that the PC-Ag levels of the diabetic patients were higher than that of the control group, but the difference between these two groups was not significant. They measured Gla-PC levels by using two monoclonal antibodies with ELISA technique. The difference between our results and Saito's would be caused by the usage of different antibodies. Garcia Frade measured PC-Ag levels using electroimmunoassay (6). The difference between our results and Garcia Frade's results may originate from the patient group containing patients suffering from acute vascular diseases.

Ceriello et al. (5) obtained lower PC-Ag and PCA levels in patient groups with respect to their control group. These investigators suggested that increased thrombin production in diabetes mellitus causes enhanced activation of protein C in the circulation and in turn enhanced clearance from the blood. They also ascertained that the low levels of PC-Ag and PCA showed negative correlation with serum glucose levels (5).

When Koert et al. (8) compared PC-Ag levels of diabetic children and adolescents with age matched controls, they observed decreased levels of protein C after a duration of 6 months. They suggested that this decrement of the PC-Ag levels was caused by accelerated turnover due to the hyperglycemia related cell damage.

We divided our patients into three further groups according to their UAE levels: Group 1 (<30 mg/ml), 2 (30 -140 mg/ml) and 3 (>140 mg/ml). The mean PC-Ag levels of Group 1, 2 and 3 were lower than that of the control group ($P < 0.01$, $P < 0.01$ and $P > 0.05$). The difference between the control group and Group 1 and Group 2 was statistically significant. But the difference between the control group and Group 3 was not statistically significant. Few numbers of patients in Group 3 would be the cause of this statistical insignificance.

UAE reflects the diabetic microangiopathy. We determined low levels of PC-Ag even in the normoalbuminuric patients (Group 1). We maintain that low levels of PC-Ag are not the result of the microangiopathy, but the cause of it.

We found that PC-Ag levels of patients whose diabetic age ≤10 years and >10 years were 67.24 ± 12.64% and 66.11 ± 15.34%, respectively. There was not a statistically significant difference between these two mean levels ($P > 0.05$). In contrast, both mean values were lower than that of the control group's mean value. As a result, we could suggest that diabetes causes decreased PC-Ag levels, but this change takes place in the early phases of diabetes mellitus.

In our study, PC-Ag and PCA levels correlated with serum cholesterol levels ($r = + 0.403$, $P < 0.01$, $n = 46$; $r = +0.402$, $P < 0.01$, $n = 42$, respectively). Saito (3), and Takahashi (12) showed that there was a correlation between plasma PC-Ag and serum cholesterol level. Cholesterol levels might affect Protein C and Protein S metabolism.

PC-Ag and PCA levels were found to be lower or higher than that of the control group in different study groups (2, 6, 13, 14). Different results obtained by different investigations may depend on either differences of the short term metabolic control of the diabetes, or different techniques used in different studies. Plasma PC-Ag levels showed a positive correlation with serum cholesterol levels and negative correlation with serum glucose. In addition, PC-Ag levels of diabetic patients having UAE levels of greater than 30 mg/ml were lower than that of the control group. As a result of these findings we may say that the PC- Ag levels are affected by the short term metabolic control of the diabetic patients.

We obtained TM levels of 33.37 ± 13.43 ng/ml, 34.92 ± 9 ng/ml, and 40.33±4.64 ng/ml in Groups 1, 2 and 3, respectively. All these levels were higher than that of the control group. Differences between the control group and patient groups were statistically significant. Mean TM levels of the Group 2 and 3 were higher than that of Group 1, but the differences were not statistically significant.

Iwassima (15) separated diabetic patients into three groups according to the UAE level in their urine. Group 1, 2 and 3 consisted of patients whose UAE levels were: ≤ 30 mg/ml, between 30-140 mg/ml, and >140 mg/ml respectively. They demonstrated that there was no statistically significant difference between the mean TM levels of group 1 and the control group. However, TM levels of Groups 2 and 3 were significantly higher than that of the control and Group1. Moreover, TM levels of Group 3 were also significantly higher than the level of Group 2.

Iwashima (15) determined that diabetic patients having UAE and proteinuria had higher TM levels than that of the control group. Endothelial injury which occurred as a result of diabetes mellitus may lead to these elevated levels of TM.

Mean TM levels of patients whose diabetic were longer than 10 years were higher than that of the patients whose diabetic were shorter than 10 years ($P < 0.001$). The mean TM levels of both groups were higher than that of the control group ($P < 0.001$, $P < 0.001$). Iwashima (15) showed a positive correlation between diabetic age and TM levels.

TM levels in diabetic patients were found to be higher than that of the healthy controls. TM levels were found increased especially in diabetic patients having UAE levels greater than 30 mg/ml (15) and microangiopathy (16). Beside renal disease, albuminuria reflects widespread vascular injury in diabetes mellitus (1). There were no correlations between TM and many metabolic products found in the serum such as glucose, insulin, cholesterol, triglyceride etc. These features make TM an ideal endothelial marker. TM show endothelial damage rather than endothelial function (16). As a result of our observations, we may suggest that plasma TM levels show the diabetes dependent microangiopathy successfully.

Diabetes mellitus is characterized by vascular complications. Coagulation abnormalties may be causally related to and/or merely secondary to vascular diseases. In our study, we show that vacular diseases and low natural anticoagulant (PC-Ag) status together may cause the thrombogenic tendencies in diabetes mellitus.

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