

Coagulation Profile in Diabetes and its Association with Diabetic Microvascular Complications

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Abstract

Objectives : To investigate the haemostatic parameters and to assess their relationship with microvascular complications in type 2 diabetes mellitus.

Materials and Methods : Coagulation and fibrinolysis parameters were measured in 60 type 2 diabetic patients (M:F 1:1) with (n=40) and without (n=20) diabetic microvascular complications and in 30 nondiabetic healthy subjects (M:F 1:1).

Results : The mean age of diabetic patients and healthy controls was 56.9±8.78 and 53.2±7.58 respectively (p=0.05). The plasma levels of PAI-1 (22.6±6.85 vs 44.8±20.8, p=0.00), serum fibrinogen (227.5±22.8 vs 252.75±40.23, p=0.002) and vWF activity (99.4±28.18 vs 144.78±36.21, p=0.00) were found to be increased in diabetics compared to healthy controls. Plasma PAI-1 levels (37.15±15.18 vs 48.65±22.29, p=0.0) and vWF activity (123.19±29.63 vs 155.57±34.61, p=0.007) were significantly increased in diabetic patients with microvascular complications than those without microvascular complications. Amongst the diabetic patients, protein S activity (63.05±16.85 vs 51.59±10.7, p=0.002) was significantly lower in patients with microvascular complications than in patients without these complications.

Diabetic retinopathy was associated with decreased protein S levels (63.05±16.85 vs 48.48±8.72, p=0.005) and vWF activity (123.19±29.63 vs 151.85±29.74, p=0.009). Diabetic nephropathy was associated with increased PAI-1 levels (39.55±13.20 vs 51.69±26.53, p=0.02) and vWF activity (134.99±32.54 vs 157.57±37.37, p=0.007). Diabetic neuropathy did not show any significant relationship with any of the haemostatic variables.

Conclusion : Hypercoagulable state as indicated by decreased fibrinolysis and increased coagulability is responsible as one of the factors for the development of microvascular complications of diabetes mellitus.

Introduction

80% of patients with diabetes mellitus die a thrombotic death. 75% of these deaths are due to cardiovascular complications and remainder due to cerebrovascular events and peripheral vascular complications.

Patients are considered to have a hypercoagulable state if they have laboratory abnormalities associated with increased risk of thrombosis. Many patients with diabetes mellitus fall into this category. Diabetics suffer from accelerated atherosclerosis too. Vascular endothelium, primary defense against thrombosis is abnormal in diabetes, which plays a role in enhanced activation of platelets and clotting factors seen in diabetes. Various mechanisms have been proposed for endothelial dysfunction eg AGEs induced activation of endothelial cells and hyperglycaemic pseudohypoxia leading to protein kinase C activation resulting in altered vascular permeability and basement membrane synthesis.

Coagulation activation markers, as indicated by many studies, such as antithrombin thrombin complexes and prothrombin activation fragment 1+2 are elevated in diabetes.^{1,2} Plasma levels of many clotting factors including fibrinogen,^{3,4,5} factor VII,⁴ factor VIII,⁷ factor XI,⁸ factor XII,⁸ kallikrein,⁸ and vWF^{5,7,9} are elevated in diabetes. Conversely, the level of anticoagulant protein C is decreased.¹⁰ The fibrinolytic system, the primary

means of removing clots, is relatively sluggish in diabetes due to abnormal clot structure that is more resistant to degradation and an increase in PAI-1 which inhibits tissue plasminogen activator.^{5,6,11,12}

This study was designed to assess changes in coagulation, fibrinolytic processes and vascular endothelial cell function parameters in patients with diabetes mellitus and to assess whether any relationship exists among changes in these parameters and development of microvascular complications.

Materials and Methods

60 type 2 diabetics were recruited in the study. This test group was divided into four subgroups – B, C, D and E on the lines of patients with diabetes with no microvascular complications (n=20), patients with diabetic retinopathy (n=14), patients with diabetic nephropathy (n=16) and patients of diabetes with neuropathy (n=10) respectively. 30 age and sex matched healthy subjects without diabetes were put in the control group i.e. group A. Retinopathy was diagnosed on the basis of fundoscopic examination and fluorescein angiography. Any patient with microalbuminuria or overt proteinuria i.e. albustix test positive in ≥2 consecutive urine samples without urinary infection, cardiac disease or prostate disease were considered to have nephropathy. Microalbuminuria is defined as protein excretion of 30-300 mg/day on at least 2 consecutive occasions. The diagnosis of neuropathy was based on presence of symptoms and signs of neuropathy i.e. any or combination of neuropathic pain, distal sensory loss, motor weakness of isolated cranial nerve palsies, and autonomic symptoms such as orthostatic hypotension, abdominal bloating, constipation, diarrhea, erectile

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Table 1 : Clinical and Biological parameters of controls and patients with diabetes

	Controls Gp A	Diabetic Pts. Gp B+C+D+E	P-value	Significance
<i>Demographics</i>				
Subjects	30	60	-	-
Age	53.2±7.58	56.9±8.78	0.05	NS
Sex(F/M)	15/15	30/30	-	-
<i>Common Coagulation Studies</i>				
Plt. Count	2.44±0.63	2.02±0.61	0.002	S
PT	13.6±0.89	14.01±0.98	0.05	NS
PTT	34.03±0.36	34.6±0.12	0.06	NS
Fibrinogen	227.5±22.8	252.75±40.23	0.002	S
<i>Special Coagulation Studies</i>				
ATIII	95.63±16.07	95.81±12.17	0.95	NS
PrC	96.94±16.4	100.02± 24.6	0.5	NS
PrS	57.97±11.54	55.41±14.06	0.39	NS
PAI-1	22.26±6.85	44.8± 20.8	0.00	S
vWF	99.4±28.18	144.78±36.21	0.00	S
Factor V	99.1±2.63	98.75±4.07	0.7	NS
Factor VIII	98.66±3.83	97.7±4.66	0.3	NS
Factor IX	100±0.0	99.28±2.45	0.07	NS

dysfunction etc. Ewing's blood pressure tests were performed to detect cardiovascular autonomic neuropathy. These included blood pressure response to standing and sustained handgrip.

Subjects with clinical/laboratory signs of liver dysfunction, malignancy, history of coagulation disorder, manifest cardiovascular disease, CVA or peripheral vascular disease, receiving medications that could affect coagulation – fibrinolytic system eg anticoagulants, antiplatelet agents, oral contraceptives, hypolipidemic drugs etc. were excluded from the study.

For coagulation and fibrinolysis, 7ml blood sample was collected in special tubes containing 3.2% sodium citrate. Platelet count, prothrombin time, activated partial thromboplastin time and serum fibrinogen were performed promptly. Aliquots and platelet poor plasma were frozen at -70°C until assays for PAI-1, vWF, protein C and S and factor V, VIII and IX were carried out. Platelet count was measured using automatic cell counter. PT and aPTT were estimated by standard methods as described by Dacie and Lewis. Serum fibrinogen levels were measured by clot method. ATIII levels were measured by chromogenic method. Protein C, S activity and PAI-1 were measured using standard kits. The normal ranges for various parameters measured were – Serum fibrinogen–150-350mg/dl, Antithrombin III–75%-125%, Protein C–72%-160%, Protein S–50%-130%, vWF–47%-197%, Factor V–50%-150%, Factor VIII–50%-150%, Factor IX–50%-150%, PAI-I–20-44 ng/mL.

Comparisons were drawn between test groups and control group and within the test groups. Statistical analysis was performed using student's t-test.

Results

In comparison between patients with and without diabetes (Table 1), platelet count was found to be decreased in patients with diabetes, but within the normal range and this difference between two groups was found to be statistically significant (2.44±0.63 vs 2.02±0.61, p=0.002). Statistically significant difference was found in serum fibrinogen levels in diabetics and controls (227.5±22.8 vs 252.75±40.23, p=0.002). PAI-1 levels were increased in diabetics as compared to controls and this difference

Table 2 : Clinical and Biological parameters of patients with diabetes without and with microvascular complications

	Without compli. Gp B	With compli. Gp C+D+E	P-value	Significance
<i>Demographics</i>				
Subjects	20	40	-	-
Age	52.45±8.21	59.12 ±8.29	0.004	S
Sex(F/M)	11/9	19/21	-	-
<i>Common Coagulation Studies</i>				
Plt. Count	1.93±0.60	2.06±0.62	0.43	NS
PT	14.1±0.91	13.97±1.02	0.64	NS
PTT	34.5±.76	34.6±1.02	0.5	NS
Fibrinogen	239.5±36.37	259.37±40.85	0.07	NS
<i>Special Coagulation Studies</i>				
ATIII	95.8±14.71	95.82±10.89	0.99	NS
PrC	101.85±16.4	99.11± 23.77	0.68	NS
PrS	63.05±16.85	51.59±10.7	0.002	S
PAI-1	37.15±15.18	48.65±22.29	0.04	S
vWF	123.19±29.63	155.57±34.61	0.007	S
Factor V	98.65±3.43	98.7±4.39	0.9	NS
Factor VIII	97.37±5.90	97.98±3.9	0.6	NS
Factor IX	98.9±3.64	99.4±1.58	0.3	NS

was found to be statistically significant (22.26±6.85 vs 44.8±20.8, p= 0.0). vWF levels were increased in diabetics as compared to controls and this difference was found to be statistically significant (99.4±28.18 vs 144.78±36.21 p=0.0).

Significant age difference was observed between diabetic patients without complications and diabetic patients with complications (52.45±8.21 vs 59.12±8.29, p=0.0004) (Table 2). This probably was a reflection of increase in complications with increasing duration of diabetes. Protein S was less in diabetic patients with complications as compared to those without complications and this difference was statistically significant (63.05±16.85 vs 51.59±10.7, p=0.002). PAI-1 and von Willebrand factor activity was raised in diabetic patients with complications compared to those without complications and again the difference was statistically significant (37.15±15.18 vs 48.65±22.29, p= 0.04 and 123.19±29.63 vs 155.57±34.61, p= 0.007 respectively).

Comparison of patients with and without diabetic retinopathy (Table 3) showed that Protein S was less in diabetic patients with retinopathy as compared to those without complications and this difference was statistically significant (63.05±16.85 vs 48.48 ±8.72, p=0.002). vWF activity was increased in patients with retinopathy compared to those without this complication (123.19±29.63 vs 151.85±29.74, p=0.009).

Statistically significant increase in PAI-1 levels (39.55±13.20 vs 51.69±26.53, p=0.02) and vWF activity (134.99±32.54 vs 157.57±37.37, p=0.007) was found in patients with diabetic nephropathy when they were compared with subjects without nephropathy (Table 4).

Discussion

Diabetes is associated with increased risk of atherosclerosis, so diabetes is a procoagulant state. To investigate the coagulation abnormalities that leads to hypercoagulability in diabetes was the purpose of this study and whether any relationship, between these hemostatic abnormalities and development of microvascular complications exists or not, was another aim of this study. Very few reports are available regarding coagulation screening tests in diabetes. Acang and Jalil reported shorter PTs

Table 3 : Clinical and Biological parameters of patients with diabetes without and with diabetic retinopathy

	Without retino. Gp B	With retino. only (Gp C)	P-value	Significance
<i>Demographics</i>				
Subjects	20	16	-	-
Age	52.45±8.21	55.14 ±8.29	0.3	NS
Sex (F/M)	11/9	8/8	-	-
<i>Common Coagulation Studies</i>				
Plt. Count	1.93±0.60	2.24±0.69	0.17	NS
PT	14.1±0.91	13.85±.94	0.45	NS
PTT	34.5±.76	34.85±1.02	0.25	NS
Fibrinogen	239.5±36.37	253.92±36.43	0.2	NS
<i>Special Coagulation Studies</i>				
ATIII	95.8±14.71	98.57±9.51	0.5	NS
PrC	101.85±16.4	101.42± 27.57	0.96	NS
PrS	63.05±16.85	48.48±8.72	0.005	NS
PAI-1	37.15±15.18	43±9.15	0.2	NS
vWF	123.19±29.63	151.85±29.74	0.009	S
Factor V	98.65±3.43	98.42±5.8	0.8	NS
Factor VIII	97.37±5.90	98.3±3.64	0.6	NS
Factor IX	98.9±3.64	99.5±1.28	0.5	NS

Table 4 : Clinical and Biological parameters of patients with diabetes with and without diabetic nephropathy

	Without nephro. Gp B+C	With nephro. Gp D + E	P-value	Significance
<i>Demographics</i>				
Subjects	34	26	-	-
Age	53.5±8.21	61.26±7.62	0.005	S
Sex(F/M)	19/15	11/15	-	-
<i>Common Coagulation Studies</i>				
Plt. Count	2.06±0.65	1.97±0.57	0.57	NS
PT	14±0.92	14.03±1.07	0.8	NS
PTT	34.64±0.88	34.57±1.02	0.77	NS
Fibrinogen	245.44±36.56	262.30±43.45	0.1	NS
<i>Special Coagulation Studies</i>				
ATIII	96.94±12.73	94.34±11.47	0.41	NS
PrC	101.67±26.6	97.86± 21.94	0.5	NS
PrS	52.05±19.52	53.26±14.06	0.3	NS
PAI-1	39.55±13.20	51.69±26.53	0.02	S
vWF	134.99±32.54	157.57±37.37	0.007	S
Factor V	98.55±4.52	98.96±3.46	0.7	NS
Factor VIII	97.75±5.05	97.80±4.20	0.9	NS
Factor IX	99.14±2.89	99.4±1.74	0.6	NS

and aPTTs in diabetics. Conversely, Collier et al found normal PTs in patients with type 2 diabetes. Erem et al reported normal PTs and aPTTs in diabetics. In our study, we did not find any difference in diabetics. Platelet counts have been found to be normal in some studies^{9,11} or decreased in others.¹³ Platelet count was found to be decreased in patients with diabetes, but within the normal range in our study. Also, platelet count did not vary significantly between patients of diabetes with and without complications.

Various researchers have reported increased serum fibrinogen levels in diabetes.^{4,5,6,9} We also observed increased fibrinogen levels in diabetics but no differences were observed among diabetics with and without complications. Clotting factor abnormalities have been less extensively studied in the past. In a study of contract activation of patients with diabetes, Patrassi et al found increase in kallikrein, factor XII and factor XI. Factor

Table 5 : Clinical and biological parameters of patients of diabetes with and without diabetic neuropathy

	Without neuro. Gp B+C+D	With neuro. Gp E	P-value	Significance
<i>Demographics</i>				
Subjects	50	10	-	-
Age	56.56±8.98	58.6±7.94	0.5	NS
Sex (F/M)	26/24	4/6	-	-
<i>Common Coagulation Studies</i>				
Plt. Count	2.03±0.59	1.95±0.75	0.69	NS
PT	14±0.96	14.1±1.10	0.7	NS
PTT	34.62±0.96	34.6±0.84	0.95	NS
Fibrinogen	253.6±41.84	248.5±32.49	0.71	NS
<i>Special Coagulation Studies</i>				
ATIII	95.82±12.62	95.8±10.19	0.99	NS
PrC	98.74±24.35	106.45± 26.18	0.37	NS
PrS	56.10±19.52	51.96±14.06	0.4	NS
PAI-1	43.3±17.17	52.4±33.9	0.2	NS
vWF	145.13±37.56	143.30±30.20	0.86	NS
Factor V	98.55±4.52	98.96±3.46	0.7	NS
Factor VIII	97.75±5.05	97.80±4.20	0.9	NS

VIII and von Willebrand factor has been studied by various groups. Factor VIII activity⁷ and von Willebrand antigen are consistently elevated.^{5,7,9} Carmassi et al demonstrated that factor VII levels are increased in poorly controlled diabetics. In our study, factor V, VIII and IX activity did not differ significantly either between diabetics and health controls or between diabetics with and without complications.

Various investigators have found normal,⁶ increased^{9,11} and decreased ATIII^{14,16} levels in diabetes. Protein C levels were found to be decreased¹⁶ or increased.¹⁷ Between controls and diabetics, levels of antithrombin III, protein C and S did not differ significantly in our study but Protein S was less in diabetic patients with complications compared to those without complications.

We found PAI-1 to be increased in diabetics compared to non-diabetics indicating decreased fibrinolysis in diabetes. This correlates well with various studies which have demonstrated high levels of PAI-1.^{5,6,11,12} PAI-1 was raised in diabetic patients with complications compared to those without complication PAI-1 has been correlated with the development of microvascular complications.¹¹

vWF activity was found to be raised in diabetics compared to controls. This is in concordance with various other studies.^{5,7,9} vWF activity was raised in diabetic patients with complications compared to those without complications. vWF has been related to development of nephropathy and neuropathy but not to development of retinopathy.¹⁸ In our study, vWF activity was raised in diabetic patients with retinopathy compared to those without retinopathy. It was also raised in patients with diabetic nephropathy compared to those without nephropathy.

Conclusion

It has been shown that diabetes is a procoagulant state. This pathophysiology of this procoagulant state is partially understood. Hypercoagulability as evidence by increased fibrinogen levels, decreased protein S levels and increased production of von Willebrand factor by endothelium and hypofibrinolysis as evidenced by increased PAI-1 levels contribute to procoagulant state observed in diabetes. This procoagulant state not only contributes to major vessel diseases

but also contributes to microvascular complications as has been observed in this study.

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