Coagulation and Fibrinolysis Parameters in Type 2 Diabetic Patients with and without Diabetic Vascular Complications

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Key Words

Type 2 diabetes · Coagulation · Fibrinolysis · Vascular complications · Lipid profile

Abstract

Objectives: To investigate the markers of endogenous coagulation/fibrinolysis and vascular endothelial cell function, and to assess the relationships between hemostatic parameters and diabetic vascular complications in type 2 diabetic patients. **Materials and Methods:** Coagulation and fibrinolysis parameters were measured in 92 type 2 diabetic patients (43 male, 49 female, mean age 50.1 ± 13.4 years) with ($n = 44$) and without ($n = 48$) vascular diabetic complications, and in 40 nondiabetic healthy subjects (20 male, 20 female, mean age 49.8 ± 15.1 years). Common lipid parameters were also measured. **Results:** The plasma levels of fibrinogen, anti-thrombin III (AT III), plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF) activity and prothrombin time were found to be significantly increased in the type 2 diabetic patients compared with the healthy subjects. Glycosylated hemoglobin 1c was inversely correlated with plasma protein S and activated prothrombin time. Protein C and S activities were positively correlated

with plasma vWF activity, and were negatively correlated with plasma t-PA levels. vWF activity was negatively correlated with plasma t-PA levels. AT III levels were positively correlated with plasma total cholesterol levels, plasma low density lipoprotein cholesterol levels, plasma triglycerides and D-dimer levels. Plasma PAI-1 levels and factor V activity in diabetic patients with microvascular complications were significantly higher than those of the diabetic patients without microvascular complications. The plasma PAI-1 and platelet count were increased in patients with diabetic retinopathy compared with the diabetic patients without retinopathy. Plasma PAI-1 levels and factor VII activity were significantly higher in the diabetic patients with nephropathy than in diabetic patients without nephropathy. Plasma concentrations of fibrinogen and PAI-1 were significantly higher in the diabetic patients with neuropathy than the diabetic patients without neuropathy. **Conclusions:** The data demonstrated that patients with type 2 diabetes mellitus had a hypercoagulable state and hypofibrinolysis, thereby indicating that activation of coagulation with a reduced fibrinolytic activity may contribute to the increased risk of vascular disease in type 2 diabetic patients.

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Introduction

Diabetes is associated with an increased risk of atherosclerosis, and coronary artery disease is a major cause of death in patients with diabetes [1, 2]. Thrombosis is the cause of death in 80% of patients with diabetes. Of these deaths, 75% are due to cardiovascular complications, while the remaining 25% are due to cerebrovascular events and peripheral vascular complications [3].

It is well known that the vascular endothelium plays an essential role in the regulation of (local) hemostatic processes [4]. The major defense mechanisms that prevent inappropriate coagulation are found at the endothelial cell surface [5]. The endothelium itself is known to produce procoagulant factors such as von Willebrand factor (vWF), and anticoagulant factors, such as heparan sulfate and thrombomodulin [6, 7]. Endothelial dysfunction has also been shown to occur in type 2 diabetes. Multiple mechanisms are most likely involved; in fact, components of the insulin resistance syndrome may be central to the development of diabetic dysfunctional endothelium [8]. Various approaches (e.g. morphological, functional, and biologic) may be used to assess endothelial integrity. The plasma levels of biological markers, such as vWF, endothelin-1, and adhesion molecules in different proatherogenic conditions [9–11], may reflect endothelial function. Increased levels of vWF, a glycoprotein synthesized by endothelial cells in subjects with type 2 diabetes, are associated with vascular injury [12].

In diabetic patients, a procoagulant state is observed, which could contribute to the risk of catastrophic cardiovascular events. The plasma levels of many clotting factors including fibrinogen, factors VII, IX, XII, kallikrein and vWF are elevated in diabetes [3, 13, 14]. This hypercoagulable state could be caused by an imbalance between hemostatic factors in plasma, and the endothelial cell surface. So far, indirect evidence for a state favoring thrombin formation has been gathered, mainly using plasma parameters like fibrinogen and antithrombin activity or endothelium-dependent parameters like vWF [6, 7].

The contribution of fibrinolytic system abnormalities to the development of diabetic micro- and macrovascular complications is still controversial [15]. In several studies activity of the fibrinolytic system in diabetics was found to be increased [16–18], decreased [19–21] or unchanged [22]. However, most studies indicate hypofibrinolysis leading to increased coagulation. Hypofibrinolysis may occur due to reduced expression of tissue plasminogen activator (t-PA) [7], which is the most important activator of plasminogen, or increased plasminogen activator in-

hibitor-1 (PAI-1) [23–25]; PAI-1 is considered an independent factor of cardiovascular risk in type 2 diabetes mellitus.

The main purpose of this study was to investigate the markers of endogenous coagulation/fibrinolysis and of vascular endothelial cell function, and to assess the relationships between hemostatic parameters and diabetic vascular complications in patients with type 2 diabetes.

Materials and Methods

Ninety-two type 2 diabetic patients were included in the study (43 male, 49 female; mean age 50.1 ± 13.4 years). The patients were divided into two subgroups: patients with ($n = 44$) and without ($n = 48$) vascular complications. The control group consisted of 40 age-, sex-, and body mass index (BMI)-matched nondiabetic healthy subjects (20 male, 20 female; mean age 49.8 ± 15.1 years) with no family history of diabetes. Patients with retinopathy, neuropathy and/or nephropathy were considered to have microangiopathy. Retinopathy was diagnosed by an ophthalmologist on the basis of fundoscopic examination. Microalbuminuria was defined as an albumin excretion rate of 3 mg/mmol creatinine in an early morning urine specimen on at least two consecutive occasions. The diagnosis of autonomic or somatic neuropathy was based on the presence of symptoms and/or signs of polyneuropathy and on the presence of at least two abnormal neurophysiological tests. The following neurophysiological tests were performed: ulnar and peroneal nerve motor conduction velocity. Moreover, to assess the presence of autonomic neuropathy the following cardiovascular autonomic tests were performed: Valsalva maneuver, deep breathing, lying to standing, orthostatic hypotension, and handgrip. Three patients were only on diet therapy, 62 on oral antidiabetic drug therapy, 22 on insulin therapy, and 5 on insulin plus oral antidiabetic combined therapy.

Subjects with clinical or laboratory signs of liver dysfunction, malignancy or a history of coagulation disorder were excluded from the study. Patients with manifest cardiovascular disease (history of myocardial infarction, angina pectoris, positive electrocardiographic changes, cerebrovascular accident and/or peripheral vascular disease) were also excluded. Another group that was excluded consisted of patients receiving medications that could affect the coagulation-fibrinolytic system and lipid metabolism, such as anticoagulants and antiplatelet agents or oral contraceptives, hormonal replacement therapy, and hypolipidemic drugs.

Blood was collected in the morning between 8.00 and 9.00 a.m. after an overnight fast. Glucose levels were determined by the glucose oxidase method in serum. Glycosylated hemoglobin (Hb A_{1c}) value (specific for Hb A_{1c}) was assayed in both diabetic and control groups with the latex immunoagglutination inhibition method (DCA 2000, Ames, Miles Inc., code 5036A). The samples for Hb A_{1c} were obtained by finger prick taking approximately 1 µl of capillary blood. Serum total cholesterol was measured using a cholesterol oxidase enzymatic method; triglycerides by a glycerol oxidase enzymatic method; high density lipoprotein cholesterol (HDL-C) by a cholesterol oxidase enzymatic method in supernatant after precipitation of other lipoproteins with phosphotungstic acid-MgCl₂. These routine analyses were carried out by Autoanalyzer (Technicon AXON). Low

Table 1. Clinical and biological parameters of controls and patients with type 2 diabetes

	Controls	Diabetic patients	p
<i>Demographics</i>			
Subjects	40	92	–
Age, years	49.8 ± 15.1	50.9 ± 13.4	NS
Gender, F/M	20/20	49/43	–
Duration of diabetes, years	–	8.8 ± 7.0	–
BMI, kg/m ²	2.4 ± 7.3	28.4 ± 6.2	NS
Systolic blood pressure, mm Hg	126.3 ± 16.8	138.7 ± 25.1	<0.05
Diastolic blood pressure, mm Hg	75.4 ± 13.5	86.5 ± 14.7	<0.05
<i>Biochemical parameters</i>			
Fasting blood glucose, mg/dl	74.5 ± 6.2	220.6 ± 81.6	<0.0001
Hb A _{1c} , %	5.4 ± 0.8	10.0 ± 2.4	<0.0001
Platelet count per µl	233.8 ± 36.7	244.0 ± 57.6	NS
Total cholesterol, mg/dl	157.5 ± 32.1	218.4 ± 69.3	<0.01
Triglycerides, mg/dl	111.2 ± 68.6	186.4 ± 130.0	NS
LDL-C, mg/dl	91.6 ± 29.9	141.5 ± 58.9	<0.01
HDL-C, mg/dl	43.4 ± 5.2	53.1 ± 10.2	NS
<i>Common coagulation studies</i>			
Prothrombin time, s	11.1 ± 0.4	12.0 ± 1.2	<0.05
aPTT	31.2 ± 3.4	30.7 ± 4.5	NS
INR	0.90 ± 0.16	1.45 ± 2.09	NS
D-dimer	280.0 ± 98.9	263.9 ± 139.4	NS
Fibrinogen, mg/dl	253.4 ± 4.1	291.2 ± 77.1	<0.05
<i>Special coagulation studies</i>			
Factor V, %	85.3 ± 16.9	85.8 ± 29.5	NS
Factor VII, %	89.6 ± 12.8	82.1 ± 16.9	NS
Factor VIII, %	123.3 ± 31.7	128.3 ± 34.8	NS
Factor X, %	92.8 ± 20.2	94.0 ± 28.8	NS
AT III Ag, mg/dl	24.5 ± 2.7	28.5 ± 4.2	<0.0001
Protein C, %	109.3 ± 29.2	115.1 ± 31.8	NS
Protein S, %	118.9 ± 32.8	112.6 ± 29.6	NS
vWF activity, %	98.7 ± 16.8	114.5 ± 27.5	<0.05
t-PA Ag, ng/ml	11.8 ± 2.9	16.2 ± 8.8	NS
PAI-1 Ag, ng/ml	21.4 ± 5.8	44.6 ± 10.6	<0.0001
INR = International normalized ratio.			

density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald's formulae.

For coagulation and fibrinolysis, a venous blood sample (9 ml) was collected into Vacutainer tubes (Becton Dickinson, Mountain View, Calif., USA) containing 0.129 mol/l trisodium citrate (1 vol). Platelet-poor plasma was obtained by centrifugation at 3,500 g at 10°C for 20 min. Platelet count, prothrombin time, activated partial thromboplastin time (aPTT), fibrinogen, antithrombin III (AT III), factors V, VII, VIII, and X measurements were performed promptly. Aliquots of plasma were transferred into plastic tubes without delay and frozen at –80°C until assays for determination of vWF, protein C, protein S, t-PA, and PAI-1. Platelet counts were measured with an automatic cell counter (Coulter Micro Diff II). Prothrombin time and aPTT were determined with coagulometer, using commercial kits of Diagnostica Stago. Fibrinogen was determined using a nephelometric assay by commercial kits for fibrinogen (Cat No. OSCA 09,

Dade Behring Marburg GmbH, Germany). Factor V, VII, VIII and X activities were measured with coagulometer (Diagnostica Stago) using commercial kits of Diagnostica Stago. AT III assay was performed with spectrophotometric method (Behring turbidimeter, Turbiquant, AT III, Dade Behring). Normal ranges are 200–400 mg/dl for fibrinogen, 50–150% for factors V, VII, VIII and X. Protein C and protein S activity assays were performed with ELISA method using commercial kits of Biopool International. The vWF activity was determined by ELISA method using commercial kits of Imtec Immundiagnostica GmbH. t-PA and PAI-1 assays were performed with ELISA using commercial kits of American Diagnostica. According to the manufacturer, normal ranges are 22–39 mg/dl for AT III, 70–150% for vWF, 72–160% for protein C activity, 60–150% for protein S activity, 1–20 ng/ml for t-PA Ag, and 20–44 ng/ml for PAI-1 Ag.

Table 2. Clinical and laboratory characteristics in diabetic patients with and without microvascular complications

	Diabetic patients		p
	with complications	without complications	
<i>Demographics</i>			
Subjects	44	48	–
Age, years	54.84±10.88	46.94±15.30	<0.01
Gender, F/M	24/20	25/23	–
Duration of diabetes, years	11.27±6.94	6.3±5.65	<0.001
BMI, kg/m ²	28.58±5.52	28.95±6.50	NS
Systolic blood pressure, mm Hg	141.13±28.66	136.11±23.95	NS
Diastolic blood pressure, mm Hg	86.13±15.30	85.74±13.28	NS
<i>Biochemical parameters</i>			
Fasting blood glucose, mg/dl	251.98±83.18	191.66±67.02	<0.001
Hb A _{1c} , %	10.47±2.17	9.26±2.29	<0.05
Total cholesterol, mg/dl	223.73±63.72	209.12±61.65	NS
Triglycerides, mg/dl	190.3±134.9	189.9±113.4	NS
LDL-C, mg/dl	152.13±57.98	121.74±42.30	<0.05
HDL-C, mg/dl	43.75±10.62	43.47±10.62	NS
<i>Common coagulation studies</i>			
Prothrombin time, s	11.87±1.20	11.92±1.20	NS
aPTT	30.56±4.45	31.15±4.84	NS
INR	1.45±0.90	0.90±0.16	NS
D-dimer	263.90±139.48	280.00±98.99	NS
Fibrinogen, mg/dl	285.38±43.64	268.80±16.61	NS
<i>Special coagulation studies</i>			
Factor V, %	90.45±27.48	73.54±20.43	<0.05
Factor VII, %	82.72±18.88	81.76±16.61	NS
Factor VIII, %	130.66±34.58	129.44±38.15	NS
Factor X, %	106.48±32.66	74.40±11.65	NS
AT III Ag, mg/dl	28.29±4.69	29.05±3.96	NS
Protein C, %	115.13±31.81	109.27±29.23	NS
Protein S, %	112.63±29.55	115.86±32.82	NS
vWF activity, %	118.90±26.76	111.36±26.34	NS
t-PA Ag, ng/ml	16.05±10.50	14.93±6.07	NS
PAI-1 Ag, ng/ml	47.55±9.45	40.82±11.50	<0.05
INR = International normalized ratio.			

Statistical analysis was performed by Student's t test, Mann-Whitney U test, and multiple regression analysis. Results are presented as the mean ± SD. The calculations were performed using SPSS for Windows version 10 (SPSS, Inc., Chicago, Ill., USA).

Results

The clinical characteristics of all subjects, including their plasma lipid profile and markers of the coagulation/fibrinolytic system, are summarized in table 1. No difference was found in age or BMI between the diabetic patients and control subjects. The levels of plasma AT-III, fibrinogen, PAI-1, and vWF activity were significantly

increased and prothrombin time was longer in the type 2 diabetic patients than the healthy subjects. Coagulation factors (V, VII, VIII and X), platelet count, aPTT, D-dimer, t-PA, protein C, and protein S did not differ between patients and control subjects. Duration of diabetes, plasma PAI-1 levels and factor V activity in type 2 diabetic patients with diabetic microvascular complications were significantly higher than those of the type 2 diabetic patients without diabetic microvascular complications (table 2). Also, the plasma PAI-1 levels and platelet count were increased in patients with diabetic retinopathy compared with the diabetic patients without retinopathy (table 3). Plasma PAI-1 levels and factor VII activity

Table 3. Clinical and laboratory characteristics in diabetic patients with and without retinopathy

	With retinopathy	Without retinopathy	p
<i>Demographics</i>			
Subjects	33	59	–
Age, years	56.94 ± 10.77	46.21 ± 13.66	<0.0001
Duration of diabetes, years	11.7 ± 7.38	6.92 ± 5.42	<0.01
BMI, kg/m ²	27.40 ± 4.33	29.74 ± 7.19	NS
Systolic blood pressure, mm Hg	153.62 ± 28.78	130.68 ± 18.79	<0.0001
Diastolic blood pressure, mm Hg	90.00 ± 16.15	85.00 ± 13.69	NS
<i>Biochemical parameters</i>			
Fasting blood glucose, mg/dl	261.67 ± 86.43	195.54 ± 66.09	<0.0001
Hb A _{1c} , %	10.64 ± 2.09	9.21 ± 2.29	<0.05
Platelet count per µl	262.90 ± 56.79	231.5 ± 56.1	<0.05
Total cholesterol, mg/dl	219.84 ± 68.13	220.16 ± 58.46	NS
Triglycerides, mg/dl	170.09 ± 116.78	207.92 ± 136.38	NS
LDL-C, mg/dl	146.96 ± 60.21	143.90 ± 45.08	NS
HDL-C, mg/dl	43.71 ± 11.57	53.95 ± 11.67	NS
<i>Common coagulation studies</i>			
Prothrombin time, s	11.79 ± 1.18	11.99 ± 1.23	NS
aPTT	30.91 ± 4.72	30.84 ± 4.68	NS
INR	1.54 ± 2.35	0.93 ± 0.19	NS
D-dimer	271.00 ± 146.01	270.00 ± 95.96	NS
Fibrinogen, mg/dl	282.53 ± 35.09	268.44 ± 15.24	NS
<i>Special coagulation studies</i>			
Factor V, %	89.22 ± 28.79	78.22 ± 21.54	NS
Factor VII, %	85.51 ± 19.42	80.24 ± 16.15	NS
Factor VIII, %	132.16 ± 37.23	130.13 ± 35.56	NS
Factor X, %	106.48 ± 32.66	74.40 ± 11.65	NS
AT III Ag, mg/dl	27.71 ± 4.64	29.60 ± 3.86	NS
Protein C, %	110.00 ± 30.81	114.84 ± 31.12	NS
Protein S, %	112.13 ± 30.11	119.04 ± 29.66	NS
vWF activity, %	114.79 ± 26.95	119.16 ± 26.88	NS
t-PA Ag, ng/ml	16.15 ± 10.33	15.28 ± 7.97	NS
PAI-1 Ag, ng/ml	48.26 ± 8.24	40.90 ± 12.05	<0.05
INR = International normalized ratio.			

were significantly higher in the diabetic patients with nephropathy than in diabetic patients without nephropathy (table 4). Plasma concentrations of fibrinogen and PAI-1 were significantly higher in the diabetic patients with neuropathy than the diabetic patients without neuropathy (table 5).

In the study group, Hb A_{1c} was negatively correlated with plasma protein S activity ($r = -0.30$, $p < 0.05$) and aPTT ($r = -0.27$, $p < 0.05$). Protein C and S activities were positively correlated with plasma vWF activity ($r = 0.65$, $p < 0.0001$; $r = 0.57$, $p < 0.0001$, respectively) and were negatively correlated with plasma t-PA levels ($r = -0.45$, $p < 0.0001$ and $r = 0.43$, $p < 0.001$, respectively). The vWF activity was negatively correlated with plasma t-PA

levels ($r = -0.29$, $p < 0.05$). AT III levels were positively correlated with plasma total cholesterol levels ($r = 0.32$, $p < 0.05$), plasma LDL-C levels ($r = 0.42$, $p < 0.05$), plasma triglycerides ($r = 0.33$, $p < 0.05$) and D-dimer levels ($r = 0.64$, $p < 0.01$).

Discussion

Enhanced activation of the clotting system has been previously reported in patients with type 1 or type 2 diabetes [26]. This activation of blood coagulation has recently been implicated as an important contributing factor for the occurrence of vascular complications in dia-

Table 4. Clinical and laboratory characteristics in diabetic patients with and without nephropathy

	With nephropathy	Without nephropathy	p
<i>Demographics</i>			
Subjects	16	76	–
Age, years	58.44 ± 8.08	48.96 ± 14.07	<0.05
Duration of diabetes, years	15.14 ± 8.34	7.13 ± 5.45	<0.0001
BMI, kg/m ²	28.19 ± 4.99	28.76 ± 6.50	NS
Systolic blood pressure, mm Hg	163.33 ± 24.69	132.69 ± 22.85	<0.0001
Diastolic blood pressure, mm Hg	91.00 ± 17.95	85.96 ± 13.14	NS
<i>Biochemical parameters</i>			
Fasting blood glucose, mg/dl	255.38 ± 67.03	209.65 ± 81.67	<0.05
Hb A _{1c} , %	10.60 ± 2.48	9.53 ± 2.22	NS
Platelet count per µl	273.56 ± 72.71	236.47 ± 53.53	NS
Total cholesterol, mg/dl	232.88 ± 76.53	215.02 ± 60.23	NS
Triglycerides, mg/dl	180.63 ± 121.21	188.70 ± 130.10	NS
LDL-C, mg/dl	173.25 ± 75.38	136.55 ± 49.50	NS
HDL-C, mg/dl	37.50 ± 5.73	45.64 ± 13.88	NS
<i>Common coagulation studies</i>			
Prothrombin time, s	11.52 ± 1.11	11.99 ± 1.23	NS
aPTT	32.05 ± 6.35	30.57 ± 4.11	NS
INR	2.16 ± 3.35	0.92 ± 0.16	NS
D-dimer	284.14 ± 165.26	261.00 ± 87.49	NS
Fibrinogen, mg/dl	272.67 ± 10.15	275.65 ± 30.42	NS
<i>Special coagulation studies</i>			
Factor V, %	90.46 ± 18.80	81.21 ± 28.00	NS
Factor VII, %	94.72 ± 21.31	78.25 ± 14.79	<0.01
Factor VIII, %	137.15 ± 34.86	127.28 ± 36.55	NS
Factor X, %	102.97 ± 28.37	89.66 ± 32.22	NS
AT III Ag, mg/dl	28.46 ± 4.98	28.80 ± 4.06	NS
Protein C, %	96.18 ± 20.38	118.61 ± 31.07	NS
Protein S, %	112.00 ± 29.24	115.58 ± 32.20	NS
vWF activity, %	113.17 ± 20.07	119.47 ± 26.16	NS
t-PA Ag, ng/ml	17.26 ± 10.98	14.24 ± 7.98	NS
PAI-1 Ag, ng/ml	51.27 ± 6.74	43.28 ± 11.15	<0.05
INR = International normalized ratio.			

betes [27, 28]. In large epidemiological studies, the pro-coagulant factors (fibrinogen and factor VII) have been described as independent predictors of cardiovascular events in diabetic and nondiabetic subjects. In the present study, the plasma levels of fibrinogen were found to be significantly higher in diabetic patients than in those of the control subjects. Equally, Asakawa et al. [29] had shown that fibrinogen level was significantly higher in diabetic patients with retinopathy or nephropathy than in patients without these complications. The detection of microalbuminuria, another well-known independent indicator of much greater risk of premature death from cardiovascular complications, has been demonstrated to correlate significantly with various hemostatic variables [3,

26, 27, 30, 31]. Accordingly, some coagulation factors did not differ between the diabetic patients and control subjects. Increased factor V activity in diabetic patients with vascular complications and increased factor VII activity in patients with diabetic nephropathy were found. Our findings for fibrinogen are in accordance with the observations of Kvasnicka et al. [14], Ostermann et al. [6], Asakawa et al. [29] and Hu et al. [32]. However, the fibrinogen levels did not differ between the diabetic patients with and without vascular complications except for the patients with diabetic neuropathy. Asakawa et al. [29] showed that the duration of diabetes was significantly longer in patients with any microangiopathy than in patients without it, similar to our data.

Table 5. Clinical and laboratory characteristics in diabetic patients with and without neuropathy

	With neuropathy	Without neuropathy	p
<i>Demographics</i>			
Subjects	59	33	–
Age, years	54.24 ± 11.68	48.35 ± 14.12	<0.05
Duration of diabetes, years	12.71 ± 6.97	6.11 ± 5.36	<0.0001
BMI, kg/m ²	28.03 ± 4.05	29.05 ± 7.17	NS
Systolic blood pressure, mm Hg	147.50 ± 27.38	134.11 ± 22.77	<0.05
Diastolic blood pressure, mm Hg	87.33 ± 16.28	86.78 ± 12.39	NS
<i>Biochemical parameters</i>			
Fasting blood glucose, mg/dl	208.02 ± 83.94	242.79 ± 75.16	<0.05
Hb A _{1c} , %	10.23 ± 2.17	9.62 ± 2.42	NS
Total cholesterol, mg/dl	220.52 ± 65.63	223.46 ± 73.27	NS
Triglycerides, mg/dl	192.94 ± 140.06	194.0 ± 118.83	NS
LDL-C, mg/dl	147.55 ± 62.02	143.61 ± 60.0	NS
HDL-C, mg/dl	44.3 ± 10.2	41.87 ± 10.35	NS
<i>Common coagulation studies</i>			
Prothrombin time, s	11.98 ± 1.21	11.83 ± 1.21	NS
aPTT	31.19 ± 4.80	30.35 ± 4.23	NS
INR	1.45 ± 2.09	0.91 ± 0.16	NS
D-dimer	271.00 ± 146.01	270.00 ± 95.92	NS
Fibrinogen, mg/dl	294.44 ± 51.23	270.80 ± 19.98	<0.05
<i>Special coagulation studies</i>			
Factor V, %	93.60 ± 30.64	79.83 ± 29.31	NS
Factor VII, %	86.94 ± 20.54	79.40 ± 15.30	NS
Factor VIII, %	139.28 ± 34.70	124.75 ± 35.82	NS
Factor X, %	106.48 ± 32.66	74.4 ± 11.65	NS
AT III Ag, mg/dl	28.02 ± 5.03	29.01 ± 3.91	NS
Protein C, %	111.84 ± 33.11	114.09 ± 29.91	NS
Protein S, %	110.84 ± 32.39	115.29 ± 29.33	NS
vWF activity, %	111.25 ± 24.12	118.03 ± 28.99	NS
t-PA Ag, ng/ml	14.37 ± 9.05	16.24 ± 8.55	NS
PAI-1 Ag, ng/ml	50.79 ± 6.66	41.39 ± 11.11	<0.001
INR = International normalized ratio.			

The pathogenetic mechanism of the clotting activation in diabetes is not completely clear. Perturbance of components of the anticoagulant system associated with hyperglycemia may play an important role as exemplified in hyperglycemia inducing depressed biological activity of the anticoagulant protein AT-III in diabetic and nondiabetic subjects [33]. Structural modification due to nonenzymatic glycation was suggested as the causative factor of this AT-III dysfunction [34]. In various studies, it was reported that plasma levels of AT-III were decreased [35] or unchanged [32]. In these studies, increased thrombin-antithrombin complex was found. In the present study we demonstrated an increased AT-III level in diabetic patients. Because AT-III is a coagulation inhibitor, this

increase could also be a compensatory reaction to a procoagulant state, as can be found in diabetes.

Abnormalities of the anticoagulant protein C pathway may also cause hypercoagulability in diabetic patients. The plasma antigen levels of protein C and of its cofactor protein S were found to be either decreased or increased in various reports depending on the type of diabetes. While reduced plasma levels of protein C antigen have been described in type 1 diabetic patients, they were found to be elevated in those with type 2 diabetes [26, 28, 36]. The activities of protein C and S did not change in our diabetic patients. Hyperglycemia has been considered to be the causative factor of the abnormalities of the protein C anticoagulant pathway. In our type 2 diabetic

patients, we found a significant negative correlation between Hb A_{1c} and protein S activity.

Endothelial dysfunction has been shown to occur in type 2 diabetes [1, 11]. The vWF, t-PA and PAI-1 are among the important endothelium-derived proteins. Although a gold standard for endothelial dysfunction is not available, high plasma vWF and/or PAI-1 have been widely considered as markers of general endothelial dysfunction in serial diseases [37–39]. Increased levels of vWF in subjects with type 2 diabetes are associated with macrovascular mortality [12]. Several studies have shown that levels of vWF are increased in both type 1 and type 2 diabetic patients [3, 32, 40] and that glycemic control normalizes vWF levels in diabetic patients [41]. In our study, we found an increased vWF activity in type 2 diabetics in comparison with the healthy controls. However, the mean values of both groups were within normal ranges of our laboratory. vWF activity did not differ between the diabetic patients with and without vascular complications.

Previous studies of the fibrinolytic system in diabetes have provided conflicting results; type 2 diabetes is often associated with profound depression of fibrinolysis asso-

ciated with high levels of PAI-1 and t-PA [23, 24, 42, 43], while Aso et al. [44] reported that both coagulation and fibrinolysis are enhanced concomitantly in patients with type 2 diabetes mellitus. Our findings of increased plasma levels of PAI-1 and decreased (but not significant) t-PA are consistent with hypofibrinolysis. We also found a significant relationships between PAI-1 and retinopathy, nephropathy and neuropathy. Correlation between AT-III and LDL-C may be interpreted as a tendency to thrombosis with cardiovascular disease for diabetic patients.

Conclusion

The results showed that type 2 diabetic patients have high fibrinogen, AT-III, PAI-1 levels and vWF activity. Our data further demonstrated that patients with type 2 diabetes mellitus had hypercoagulable state and hypofibrinolysis thereby indicating that the activation of coagulation and reduced fibrinolytic activity may contribute to the increased risk of vascular disease in type 2 diabetic patients.

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