

Coagulation pathways and diabetic retinopathy: abnormal modulation in a selected group of insulin dependent diabetic patients

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Abstract

Aims—To investigate whether diabetic retinopathy (DR), already associated with microvascular alterations, ischaemia, and endothelial dysfunction, was also characterised by abnormal modulation of coagulation pathways.

Methods—Plasma samples, collected from 67 type 1 diabetics comparable for age, duration of disease (DD), and metabolic control (MC), were processed for prothrombin degradation products (F1+2) and factor VII coagulant activity (FVII:c). 50 normal subjects served as a control group. The ETDRS-Airlie House Classification of DR was used.

Results—A significant correlation between FVII:c and F1+2 plasma concentrations was observed ($p < 0.05$). FVII:c ($p < 0.005$) and F1+2 ($p < 0.0001$) levels were higher in diabetics than in controls, especially in patients with proliferative DR (FVII:c $p < 0.0001$; F1+2 $p < 0.005$). However, cases without retinal lesions and healthy subjects did not differ significantly (FVII:c and F1+2 $p > 0.05$).

Conclusions—These findings pointed out the presence of a hypercoagulable state associated with endothelial dysfunction in patients with insulin dependent diabetes mellitus (IDDM), demonstrated both by increased FVII:c and F1+2 plasma levels. Moreover, the observation of different DR related degrees of procoagulant activity, despite comparable DD and MC, strengthens the hypothesis of multiple risk factors in the pathogenesis of DR.

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progression of retinopathy during a 9 year period in patients with insulin dependent diabetes mellitus (IDDM)¹⁷: however, a sight threatening worsening is often observed when this intensive treatment is initiated in patients with long standing poor glycaemic control, particularly if retinopathy is at or past the moderate non-proliferative stage.¹⁸ Moreover, the severity of DR is probably influenced also by familial factors.¹⁹

Experimental evidence suggests that hyperglycaemia triggers a cascade of biochemical events that lead to vascular dysfunction and early structural changes of blood vessel walls.²⁰ Several observations that vascular damage and endothelial dysfunction occur early in the course of diabetic microangiopathy have been reported in the literature: increased endothelin-1 (ET-1) levels^{21, 22}; increased plasma concentrations of tissue plasminogen activator (tPA) inhibitor,²³ von Willebrand-antihaemophilic factor A,^{24, 25} fibrinogen and activated factor VII (FVII:c)²⁶; decreased concentrations of endothelium derived relaxing factor, prostacyclin,²⁵ and tPA²³; and reduced fibrinolytic potential of vascular endothelia.^{27, 28} The net effect of all these changes is to convert the endothelium from a thromboresistant to a thrombogenic surface^{29, 30} and, consequently, impairment of coagulation and of anticoagulant pathways.^{31–33}

In the light of all these findings, the aim of the present study was to determine, in a well characterised group of patients affected by insulin dependent diabetes mellitus (IDDM), whether DR was associated with abnormally modulated haemostasis and to demonstrate whether or not a significant correlation existed between the thrombotic tendency, as measured by FVII:c and prothrombin degradation fragments (F1+2) plasma concentrations, and metabolic control.

Subjects and methods

STUDY POPULATION

Sixty seven IDDM patients (22 males, 45 females; age range 15–68 years), classified according to the National Diabetes Data Group criteria,³⁴ regularly attending the Center for the Study of Diabetes (Institute of Internal Medicine II, University of Rome “La Sapienza”) from 1989 to the end of 1996 were enrolled. Selection was made by the same diabetologist on the basis of the following inclusion criteria: type 1 IDDM, as evidenced by deficient C peptide secretion, with a

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Diabetic retinopathy (DR) is the major cause of impaired vision in the Western world and of new cases of blindness for people between the ages of 20 and 64 years.¹ However, despite the growing concern about this disease, its natural history and aetiopathogenesis are still not completely understood: several risk factors have been identified^{2–16} and much speculation surrounds the pathophysiology of diabetic microvascular lesions but, at this time, no data provide an adequate explanation for the serious and rapid involvement of the retinal microcirculation that often occur early in the disease despite a good metabolic control. The Diabetes Control and Complications Trial Research Group pointed out that intensive insulin therapy resulted in a 76% reduction in

duration of the disease longer than 5 years. Poor glycaemic control ($\text{HbA}_{1c} > 7\%$), borderline hypertension ($> 140/90$), hypertriglyceridaemia (> 1.9 mmol/l), or hypercholesterolaemia (> 5.6 mmol/l), detected on three different occasions, as well as peripheral obliterating arteriopathies or other macrovascular diseases were exclusion criteria. Fifty healthy, non-diabetic subjects, aged between 20 and 50 years, recruited from hospital staff, served as a control group.

Medical history, physical examination, electrocardiography, and Doppler analysis of extracranial and intracranial vessels were used to investigate the presence of macroangiopathy and venous or arterial thrombosis (for example, cerebral or coronary).

Nephropathy was diagnosed by the presence of microalbuminuria (30–300 mg/day: Albustix-Ames, Miles, Elkhart, IN, USA) or macroalbuminuria (> 300 mg/day: Labstix, Bayer Inc, Bridgend) in at least three samples of early morning urine collected in a 6 month period in the absence of infections, other renal diseases, or heart failure.³⁵ Blood pressure and serum creatinine concentration were determined in order to evaluate renal function.

The ophthalmological examination included: best corrected ETDRS visual acuity, applanation tonometry, and biomicroscopic evaluation of anterior segment. DR was assessed according to the ETDRS-Airlie House Classification³⁶: binocular indirect ophthalmoscopy, retinal biomicroscopy using high positive power precorneal lenses (Super Field and/or +78D Volk), and colour fundus stereophotographs (and fluorescein angiography, if necessary) were performed through dilated pupils.

All patients were treated with subcutaneous human insulin (regular and long acting) only. Both diabetics and controls were non-smokers and had not taken drugs which could affect haemostasis for at least 4 weeks (6 months in the case of oral contraceptives) before the study.

COLLECTION AND PROCESSING OF BLOOD SAMPLES

Fasting blood samples were collected using 19 gauge needles and polypropylene syringes preloaded with the appropriate solutions. All samples were drawn between 8 30 and 9 30 am, in a quiet room, following a minimum of 30 minutes of rest; blood drawing was performed without arm compression to avoid even minimal venous stasis and prevent platelet activation.

Glycosylated haemoglobin (HbA_{1c}) was determined spectrophotometrically using reagents from Bio-Rad (Richmond, CA, USA) on 3 ml of blood drawn into evacuated siliconised tubes containing EDTA. The non-diabetic range used in our laboratory is 4.0–6.0%. To determine serum glucose and serum lipids (total cholesterol, triglycerides, high density lipoprotein cholesterol), blood was drawn into evacuated siliconised tubes and measurements made by autoanalyser (Boehringer Mannheim, Germany) using enzymatic methods.

In order to determine factor VII antigen (FVII:Ag), factor VII coagulant activity (FVII:c), and prothrombin degradation fragments (F1+2), blood was collected directly into evacuated siliconised tubes containing 0.129 M buffered trisodium citrate, one part to nine parts blood, volume for volume. Samples, identified by the initials of the subjects and date of sampling only in order to leave the analysts masked to the DR diagnosis of each patient, were immediately shipped to the laboratory and processed within 1 hour of collection. Plasma was obtained by centrifugation at 2000 g at 4°C for 60 minutes, separated into small aliquots, immediately stored in plastic tubes, and frozen at -80°C until assayed.

FVII:Ag was measured with a commercial immunoenzymatic ELISA method (Diagnostica Stago, France)³⁷ and results expressed as a percentage of those given by standard plasma (% standard).

FVII:c was determined by a clotting time assay using factor VII deficient plasma as reagent (Boehringer Mannheim, Germany), according to the method described by Poggio.³⁸

The prothrombin degradation products (F1+2) assay measures the accumulation of intermediate molecules from the conversion of prothrombin to thrombin in blood. This was performed using double antibody RIA employing labelled and unlabelled F1+2 (Behring Werke AG, Marburg, Germany).³⁹

All assays were duplicate and results for each patient remained masked to the ophthalmologists until data processing was completed.

Informed consent was obtained from all subjects after full explanation of the nature of the study. This investigation was approved by the bioethics committee of the University of Rome “La Sapienza” and followed the tenets of the Declaration of Helsinki.

STATISTICAL ANALYSES

Analyses were performed by a statistician provided with a list of the patients' laboratory values but masked to the clinical diagnosis of diabetics and the identity of healthy control subjects. Upon completion of analyses, masking was broken, and assay results were matched to the clinical diagnoses. Data were expressed as mean (SD) and were normally distributed, except for age and HbA_{1c} . Statistical analyses were performed by Mann–Whitney U test for independent groups for the significance of differences. Bonferroni's correction was applied to control for the increase of type 1 error probability due to multiple comparison. A p value of less than 0.05 was considered statistically significant.

Results

CLINICAL AND METABOLIC RESULTS

Clinical, biochemical, and haemodynamic variables were comparable in diabetics and healthy controls (Table 1): no statistically significant differences were found between both groups of subjects, with the exception of HbA_{1c} and creatinine.

DR was found in 45 (67.1%) cases, associated with a higher mean age and a longer

Table 1 Clinical, biochemical, and haemostatic findings of diabetic patients and healthy controls (n = mean (SD); Ns = p value not significant)

	Diabetics (n = 67)	Controls (n = 50)	p Value
Age (years)	39.3 (15.2)	37.4 (6.6)	Ns
Sex (M, F)	22, 45	19, 31	—
Duration of diabetes (years)	16.9 (8.43)	—	—
Body mass index (kg/m ²)	24.1 (3.54)	22.9 (2.04)	Ns
Systolic blood pressure (mm Hg)	127.0 (15.4)	127.8 (11.26)	Ns
Diastolic blood pressure (mm Hg)	76.6 (8.68)	72.3 (8.28)	Ns
HbA _{1c} (%)	6.17 (0.90)	4.54 (0.64)	<0.0001
Nephropathy:			
microalbuminuria (patients)	16 (23.8%)	—	—
macroalbuminuria (patients)	9 (13.4%)	—	—
Total cholesterol (mg/dl)	188.5 (13.3)	189.6 (22.7)	Ns
HDL cholesterol (mg/dl)	49.2 (5.2)	50.4 (3.5)	Ns
Triglycerides (mg/dl)	143.1 (34.1)	144.6 (28.2)	Ns
Creatinine (mmol/l)	83.98 (32.70)	71.60 (13.26)	<0.005
FVII:Ag (% standard)	110.4 (32.1)	102.9 (29.8)	<0.05
FVII:c (U/dl)	99.2 (17.5)	88.6 (15.8)	<0.005
F1+2 (µg/l)	1.12 (0.46)	0.78 (0.24)	<0.0001

duration of diabetes compared to those without retinal lesions (p < 0.05): background (n = 21), non-proliferative (n = 15), and proliferative retinopathy (n = 9) were observed (Table 2). Other risk factors for severity of DR—for example, sex and HbA_{1c} values, were statistically insignificant in this study (p > 0.05).

Microalbuminuria was detected in 16 diabetics (23.8%), mostly associated with proliferative DR (n = 9) but also with background (n = 2) or non-proliferative retinopathy (n = 5). Macroalbuminuria, on the contrary, was observed only in those diabetics affected by proliferative DR (n = 9; 13.4%) (Tables 1 and 2).

COAGULATION STATUS

Clinical, metabolic, and haemocoagulative data of cases and healthy controls are shown in Tables 1 and 2. In diabetics, a significant correlation between FVII:c and F1+2 plasma concentrations was observed (R = 0.378, p < 0.05). FVII:Ag (p < 0.05), FVII:c (p < 0.005) and F1+2 (p < 0.0001) levels were higher in diabetics than in controls (Table 1), especially in patients with proliferative DR (FVII:Ag: p < 0.005; FVII:c: p < 0.0001; F1+2: p < 0.005). Cases without retinal lesions and healthy controls did not differ significantly (FVII:Ag, FVII:c and F1+2: p > 0.05).

Discussion

According to epidemiological studies, about 89% of IDDM patients have a clinically evident retinopathy 10 years after onset of the disease. Ten years later, this percentage increases to 99.0% and the prevalence of the proliferative form is about 53%.⁷ In our group of patients, characterised by a mean duration of the disease of about 17 years associated with a good metabolic control (HbA_{1c} = 6.17% (0.90%)) (Table 1), the overall prevalence of DR was lower (67.1%) with a percentage of proliferative retinopathy of 20%. These data strengthens the hypothesis of a multifactorial pathogenesis of diabetic vasculopathy in which the glycometabolic factor plays an important but not exclusive part.¹⁻¹⁹

In the light of the different pathogenetic hypotheses and the several risk factors that have been already reported in the literature, this study was designed to determine whether DR was associated with abnormally modulated haemostasis, as a result, and expression of endothelial damage. In fact, there are many reports that extensively document the presence of a remarkable endothelium related dysfunction of the coagulant and anticoagulant pathways in diabetics, but it is not yet clear if this condition is due to hyperglycaemia only.^{20 23-28 31-33} Abnormal coagulation is manifested by enhanced prothrombin conversion to thrombin—as demonstrated by increased F1+2 plasma concentrations⁴⁰—and down-regulation of the anticoagulant pathway, caused by reduced antithrombin III (ATIII) activity and thrombomodulin endothelial receptors.⁴¹

Increased FVII:c plasma levels have also been observed in diabetics,^{42 43} especially in association with chronic vascular complications.^{44 45} Moreover, it has been demonstrated that high FVII coagulant activity, associated with increased FXa production but low thrombin and F1+2 levels, characterises the prethrombotic condition,⁴⁶ whereas increased F1+2 and FVII:c plasma concentrations are typical features of the hypercoagulable state.^{46 47}

Table 2 Clinical, laboratory, and haemocoagulative data in diabetics (in relation to diabetic retinopathy: BDR = background DR; NPDR = non-proliferative DR; PDR = proliferative DR) and in healthy controls (n = mean(SD))

	No DR (n = 22)	BDR (n = 21)	NPDR (n = 15)	PDR (n = 9)	Controls (n = 50)
Sex (M/F)	9/13	5/15	2/5	6/12	19/31
Age range (years)	16-55	15-62	16-65	24-70	20-68
Mean age (years)	36.4 (10.5)	41.2 (17.3)	42.10 (12.3)	43.6 (13.1)	38.6 (12.2)
Duration of diabetes (years)	11.4 (6.9)	17.8 (5.7)	18.0 (1.2)	18.1 (7.2)	—
HbA _{1c} (%)	6.1 (1.0)	6.2 (1.1)	6.3 (1.2)	6.6 (1.0)	—
Microalbuminuria (patients)	—	2	5	9	—
Macroalbuminuria (patients)	—	—	—	9	—
Total cholesterol (mg/dl)	180.9 (31.0)	187.5 (46.0)	192.1 (31.1)	204.2 (49.4)	189.6 (22.7)
HDL cholesterol (mg/dl)	51.4 (9.8)	47.8 (6.7)	47.2 (5.2)	46.4 (8.4)	50.4 (3.5)
Triglycerides (mg/dl)	118.8 (52.6)	131.5 (46.8)	140.1 (31.3)	165.2 (34.5)	144.6 (28.2)
FVII:Ag (% standard)	98.6 (33.1)	108.8 (30.9)	117.6 (22.8)	131.6 (21.7)*	102.9 (29.8)
FVII:c (U/dl)	79.7 (18.4)	92.8 (16.9)	105.3 (10.4)	126.0 (34.3)**	88.6 (15.8)
F1+2 (µg/l)	0.82 (0.46)	1.08 (0.58)	1.07 (0.43)	1.41 (0.61)***	0.78 (0.24)

*PDR v No DR, PDR v BDR, PDR v controls: p < 0.005; PDR v NPDR: p < 0.05

No DR v controls: p > 0.05 (not significant).

**PDR v No DR, PDR v BDR, PDR v controls: p < 0.0001; PDR v NPDR: p < 0.05

No DR v controls: p > 0.05 (not significant).

***PDR v No DR, PDR v BDR, PDR v NPDR: p < 0.005; PDR v BDR, PDR v NPDR: p < 0.05

No DR v controls: p > 0.05 (not significant).

In this study, in order to minimise metabolic interference with our data, all diabetics were under a good metabolic control ($HbA_{1c} < 7\%$). No thrombotic events in large vessels were observed, probably due to the chronic nature of the endothelial dysfunction.

Diabetics without retinopathy had significantly lower FVII:Ag ($p < 0.005$), FVII:c ($p < 0.0001$), and F1+2 ($p < 0.005$) plasma levels than those with proliferative DR, but comparable levels with healthy controls (FVII:Ag, FVII:c, and F1+2: $p > 0.05$) (Table 2). These data suggest that in patients without retinal lesions, despite the hyperglycaemic condition, the vascular endothelium preserves its physiological thromboresistance. However, they indicate also that in cases of proliferative DR a hypercoagulable state is present: in particular, the high FVII:Ag plasma concentrations might predispose the extrinsic pathway to a hyperactivation and, consequently, the retinal microcirculation to a much more severe involvement, as a result either of endothelial dysfunction⁴⁸ or of gradually overwhelmed anticoagulant systems.⁴⁷ In conclusion, these results confirm that a stable and good metabolic control ($HbA_{1c} < 7\%$) reduces the incidence of DR^{3,4}; point out that a hypercoagulable state is present in type 1 diabetics with retinopathy; support the main part played by the endothelial dysfunction as demonstrated both by activation of the extrinsic haemostatic pathway and by increased concentrations of prothrombin degradation fragments. Particularly, the association observed between increased FVII:Ag, FVII:c, and F1+2 plasma levels supports the opinion that the endothelium in type 1 diabetics with proliferative DR, is functionally converted from a thromboresistant to a thrombogenic surface. Moreover, the findings of different DR related degrees of procoagulant activity, despite comparable duration of the disease and metabolic control, strengthens the hypothesis of multiple risk factors in the pathogenesis of DR.

Further investigations on larger diabetic populations are necessary to clarify and support this issue. If these data were confirmed by other authors, it would be possible not only to reach a better understanding of the pathogenesis of DR but also to prevent its onset and/or progression, helping primary care physicians and ophthalmologists in detecting patients at risk when they can still be treated effectively with conservative methods (for example, drugs with antithrombotic, profibrinolytic, and cytoprotective effects able to restore the endothelial function⁴⁹) and before the need for laser treatments that always destroy the retinal anatomy.

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