Von Willebrand Factor (VIII R:Ag), Fibronectin, and Insulin-like Growth Factors I and II in Diabetic Retinopathy and Nephropathy

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SUMMARY

We have measured plasma von Willebrand factor (VWF) as the factor VIII-related antigen, plasma fibronectin, and two of the serum somatomedins, insulin-like growth factor I (IGF I) and IGF II, in 51 diabetic patients and 25 nondiabetic control subjects. VWF was significantly higher in the diabetic group than in the controls (173 ± 9% SEM versus 101 ± 9%, $P < 0.001$), as has been reported by others. However, within the diabetic group there was no significant difference in VWF between those patients without retinopathy, those with background or proliferative retinopathy, or those with macular edema. There was also no difference in VWF between the diabetic subjects with and those without proteinuria. These results rule against a previously advanced hypothesis that the increase in VWF in patients with diabetes is secondary to microangiopathy. No significant difference was observed in fibronectin, IGF I, or IGF II between the diabetic and control groups, between the diabetic group without retinopathy and the retinopathic subgroups, and between the diabetic subjects with and without proteinuria. In the diabetic patients, there was no correlation between diabetic control as assessed by glycosylated hemoglobin and glycosylated serum protein, and the plasma levels of VWF, fibronectin, IGF I, or IGF II. The results of this study strongly suggest that neither plasma VWF, fibronectin, IGF I, nor IGF II plays an important primary role in the pathogenesis of diabetic microvascular disease, although one or more of these factors might play a permissive role. DIABETES 33:125–129, February 1984.

In the present study, we have measured plasma von Willebrand factor (VWF), plasma fibronectin, and serum insulin-like growth factors I and II in a group of diabetic patients, and examined the relation of these substances to diabetic retinopathy and nephropathy.

VWF is a large plasma glycoprotein, synthesized by the endothelial cells and megakaryocytes,[1] which functions in platelet agglutination[2] and mediates the adhesion of platelets to subendothelial tissue following endothelial injury.[3,4] In diabetes there is an increase in plasma VWF,[5-9] as well as an increase in the aggregatability and adhesiveness of the platelets.[5,10-14] It has been suggested that the elevation in plasma VWF plays a role in the genesis of diabetic microvascular disease by contributing to the observed abnormalities in platelet function, and thus predisposing to the occlusion of small vessels.[8,9]

Fibronectin is a high-molecular-weight plasma glycoprotein with a subunit structure similar to VWF,[15] and like VWF it is produced in part by endothelial cells[15] and is found in a secretory pool in platelets.[16] As fibronectin enhances the adhesion of erythrocytes to vascular endothelium in vitro,[17] an increase in plasma fibronectin in diabetes might promote closure of small vessels and contribute to the development of diabetic microangiopathy. There have been relatively few studies concerning plasma fibronectin in diabetic patients and animals,[18-20] and the results have been conflicting.

Plasma growth hormone is increased in patients with poorly controlled diabetes mellitus,[21] and it has been postulated that this elevation may be involved in the pathogenesis of the vascular complications of diabetes.[21,22] Since many of the metabolic actions of growth hormone are mediated by the somatomedins, which are a group of growth-hormone-dependent polypeptides,[23] it is of interest to define the relation between the concentration of the plasma somatomedins and the presence of vascular disease in patients with diabetes. Studies on the plasma somatomedins in diabetes have so far yielded conflicting results.[24-29]

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In the present study we measured plasma VWF protein, plasma fibronectin, and the serum levels of IGF I and IGF II in diabetic patients with and without evidence of diabetic retinopathy and with and without proteinuria. We also examined the relationship of these four factors to diabetic control as reflected by the levels of glycosylated hemoglobin and glycosylated serum protein.

METHODS

Control subjects and patients. Control subjects were healthy volunteers with no known history of diabetes mellitus and with normal plasma glycosylated hemoglobin levels. Most of the 51 diabetic subjects in the study were patients who had been referred to the Retina Division of the Ophthalmology Department of the Albany Medical College either for evaluation of previously diagnosed retinopathy or for screening for possible retinopathy. Subjects with a serum creatinine greater than 2.0 mg/dl were excluded from the study, to eliminate substantial renal failure as a variable. Forty-seven of the patients were studied on an outpatient basis, while four were studied during hospitalization for diabetic control. Blood samples were taken from all the diabetic patients and control subjects between 12 noon and 2 p.m., which is the time at which most of the diabetic patients were evaluated in our Diabetic Retinopathy Clinic.

In calculating the results of the study, all the 51 diabetic subjects were divided into the following subgroups: (1) no retinopathy (16 patients); (2) background retinopathy (19 patients); and (3) proliferative retinopathy (16 patients). This division was done on the basis of dilated funduscopic examination by an ophthalmologist specializing in diabetic retinopathy, and fluorescein angiography or vitreous photofluorometry was not employed in this study. Twelve of the patients with background or proliferative retinopathy also had macular edema (ME), and in one study we compared the ME group with the 23 retinopathic patients who did not have ME.

A single urine sample was obtained from 30 of the diabetic patients for determination of protein and creatinine. Patients were considered to have proteinuria if the concentration of protein in the urine exceeded 150 mg/g of creatinine.

The diabetic patients were classified as having type I diabetes if they were being treated with insulin and had onset of diabetes before age 30 yr. Table 1 summarizes some of the features of the control group and the various diabetic subgroups.

Assays. VWF protein was assayed as the factor VIII-related antigen (VIII R:Ag) by electroimmunoassay, as described previously.[30] Values are expressed as the percent of a pooled normal human plasma standard. Fibronectin was assayed in EDTA plasma by an immunoturbidimetric assay, using calibrated fibronectin standards and monospecific antibody to fibronectin supplied in kits purchased from Boehringer Mannheim Biochemicals (Indianapolis, Indiana), as published by Saba et al.[31] Plasma samples were stored at −70°C until the fibronectin assays were performed. Samples and standards were run in duplicate, and the samples from the various groups were run in random sequence in the assays. Most of the plasma samples were also assayed for fibronectin before being frozen, and there was no difference between the results obtained with the fresh and the frozen plasma samples. Serum IGF I and IGF II were determined by radioimmunoassay.[32]

Glycosylated hemoglobin (normal <9%) was measured as the total HbA_1 fraction based on a modification by Isolab, Inc. (Akron, Ohio) of the method developed by Schnek and Schroeder et al.,[33] which includes measurement of the labile fraction of HbA_1. Glycosylated protein was determined by the method of McFarland et al.,[34] with a modification involving dialysis of the serum as previously described by Kennedy et al.[35] The normal mean glycosylated protein value is 0.30 ± 0.13 nmol HMF/mg protein. Serum creatinine was measured on a SMAC Autoanalyzer (normal range 0.7–1.5 mg/dl). Urinary protein was determined by the method of Meola et al.,[36] and urinary creatinine was measured by a standard assay using an autoanalyzer (Beckman Instruments, Fullerton, California).

Statistics. All group values are expressed as the mean ± SEM. Statistical comparisons between multiple groups were made by analysis of variance (ANOVA),[37] and comparisons between two groups were made by an unpaired, two-tailed Student's *t* test. Linear correlation coefficients were calcu-

TABLE 1
Description of control and diabetic subjects

Group	Male/ female	Age range (yr)	Age (yr)	Type I/ type II	Duration diabetes (yr)	Weight (kg)	Serum creatinine (mg/dl)
Controls	13/12	25–79	52 ± 3*	—	—	70 ± 3	1.03 ± 0.03
All diabetic subjects	25/26	18–83	56 ± 2	18/33	17 ± 2	74 ± 2	1.09 ± 0.04
No retinopathy	10/6	18–83	54 ± 5	4/12	6 ± 1	73 ± 4	1.01 ± 0.06
Background retinopathy	7/12	20–75	61 ± 3	6/13	21 ± 3	75 ± 4	1.06 ± 0.06
Proliferative retinopathy	8/8	32–67	52 ± 3	8/8	22 ± 2	75 ± 5	1.21 ± 0.10
Macular edema†	3/9	47–69	64 ± 3	1/11	18 ± 2	79 ± 6	1.09 ± 0.11
Diabetic subjects without proteinuria‡	9/13	20–83	57 ± 3*	7/15	17 ± 3	70 ± 3	1.01 ± 0.05
Diabetic subjects with proteinuria‡	3/5	20–74	50 ± 6	4/4	16 ± 3	82 ± 6	1.23 ± 0.13

*Mean ± SEM.
†All of the patients with macular edema had accompanying background or proliferative retinopathy, and hence they have also been included in the background and proliferative groups described in this table.
‡Urine protein was determined in only 30 of 51 patients.

TABLE 2
Assays in the control and diabetic subjects (DM)

Group	VWF (%)	Fibronectin (μg/ml)	IGF I (ng/ml)	IGF II (ng/ml)	Glycosylated hemoglobin (%)	Glycosylated serum protein (nmol HMF/mg protein)
1(a) Controls	101 ± 9*(23)†	534 ± 23(25)	296 ± 34(23)	756 ± 46(25)		
(b) All DM	173 ± 9‡(48)	554 ± 17(51)	261 ± 17(46)	743 ± 36(51)		
2(a) DM without retinopathy	175 ± 13(15)	536 ± 33(16)	312 ± 34§(15)	811 ± 71(16)	11.1 ± 0.6(16)	0.62 ± 0.04(16)
(b) DM with background retinopathy	152 ± 12(18)	543 ± 25(19)	210 ± 22§(16)	668 ± 45(19)	11.4 ± 0.6(18)	0.54 ± 0.05(19)
(c) DM with proliferative retinopathy	195 ± 20(15)	585 ± 33(16)	264 ± 24§(15)	762 ± 74(16)	13.2 ± 0.7(15)	0.60 ± 0.07(16)
3(a) DM with retinopathy without macular edema	166 ± 14(21)	556 ± 25(23)	231 ± 22(21)	716 ± 52(23)	11.8 ± 0.6(22)	0.55 ± 0.05(23)
(b) DM with macular edema	182 ± 22(12)	577 ± 36(12)	247 ± 28(10)	703 ± 73(12)	13.0 ± 0.7(11)	0.65 ± 0.06(10)
4(a) DM without proteinuria	164 ± 12(20)	521 ± 24(22)	307 ± 29(19)	707 ± 47(22)	11.6 ± 0.6(21)	0.62 ± 0.04(22)
(b) DM with proteinuria	191 ± 20(7)	603 ± 48(8)	210 ± 47(8)	904 ± 125(8)	13.4 ± 0.9(7)	0.59 ± 0.07(8)

*Mean ± SEM.
†Number of subjects.
‡$P < 0.001$ for difference between controls and all DM.
§By analysis of variance, the P value for the difference between groups 2(a), (b), and (c) was 0.04. However, the "corrected P value" was 0.24, based on the fact that the groups were compared with regard to six different variables (see text).

lated using a linear regression model where $y = a + bx$. Significance was defined at the 0.05 level of confidence.

RESULTS

The mean plasma VWF in the diabetic subjects was 72% greater than in the nondiabetic controls ($P < 0.001$), but there was no significant difference between the diabetic and the control groups with regard to fibronectin, IGF I, or IGF II (Table 2). When the diabetic subjects were subdivided on the basis of retinopathy [subgroups 2(a), (b), and (c) in Table 2], it was found that there were no significant differences between them with regard to VWF, fibronectin, IGF II, glycosylated hemoglobin, or glycosylated protein, as determined by ANOVA. The difference between the mean IGF I levels in the three subgroups was significant at a P level of 0.04 by ANOVA. However, the subgroups were compared by ANOVA with regard to a total of six variables and the possibility of observing a difference at a P level of 0.04 for one of six variables by chance alone is 0.24. We therefore conclude that the subgroups did not differ significantly with regard to IGF I in this study. Furthermore, when the type I and type II diabetic groups were studied separately, and each was divided into subgroups on the basis of retinopathy, there was no significant difference between the three subgroups (based on "corrected P" by ANOVA) with regard to any of the six variables studied (data not shown).

The 12 patients with ME did not differ substantially from the other 23 patients with retinopathy who did not have ME, with regard to any of the six variables studied [subgroups 3(a) and (b), Table 2].

There was no significant difference between the diabetic patients with and those without proteinuria, with regard to the plasma and serum factors studied [subgroups 4(a) and (b), Table 2]. In interpreting this observation, it should be noted that the number of proteinuric patients was relatively small, and that we studied only one urine sample from each patient.

Several statistically significant correlations were noted between age or weight, and VWF or fibronectin, and these are summarized in Table 3. It was calculated from the linear

TABLE 3
Correlation of VWF and fibronectin with age and weight

Correlation	Group	P	r	Relation
VWF and weight	Control	NS		
	Diabetic	<0.01	0.47	VWF = y = 64 ± 0.7 × weight in pounds
VWF and age	Control	<0.02	0.49	VWF = y = 28 + 1.5 × age in years
	Diabetic	NS		
Fibronectin (FN) and weight	Control	NS		
	Diabetic	<0.002	0.43	FN = y = 333 + 1.3 × weight in pounds
Fibronectin and age	Control	NS		
	Diabetic	NS		

regression equations presented in Table 3 that the small differences in weight and age between the controls and the various diabetic groups (Table 1) did not substantially affect the results obtained. There was no significant correlation between IGF I or II and weight or age. In the diabetic subjects, neither VWF, fibronectin, IGF I, nor IGF II correlated with the duration of diabetes or level of glycosylated hemoglobin or glycosylated serum protein, and there was no difference between the type I and type II groups with regard to any of these four variables.

DISCUSSION

In the present study, we found that VWF, measured as VIII R:Ag, was significantly elevated in the diabetic group as a whole (Table 2), as has been found by others.[5-9] There was no significant difference in mean VWF between the diabetic patients without and those with retinopathy. Similar findings were obtained by Coller et al. using an assay for VIII R:Ag.[6] Coller et al. also measured VWF by a functional assay, based on the capacity of VWF to cause platelet clumping in the presence of ristocetin (VIII R:VWF), and found a significant increase in VIII R:VWF in their diabetic subjects. They also observed that the ratio of mean VIII R:VWF in their diabetic patients to that in matched control subjects was almost identical in the diabetic subjects with and without retinopathy. Data from a group of patients with proliferative retinopathy were interpreted as indicating that VIII R:VWF was higher in these patients than in the nonretinopathic diabetic subjects, but the difference between the two groups was not statistically significant.[6]

Bensoussan et al.[5] also found increased levels of VIII R:VWF in three of six diabetic patients without retinopathy, consistent with our own study of VIII R:Ag. However, in contrast to our findings, VIII R:VWF was significantly higher in a group of six patients with proliferative retinopathy than in the six diabetic patients without retinopathy. The reason for the discrepancy between their results and ours is unclear, but it is of note that their patient groups were relatively small.

The fact that mean VWF was very similar in our nonretinopathic and retinopathic diabetic groups argues against the previously advanced hypothesis that the increase in VWF in diabetes is secondary to diabetic microangiopathy.[5] Our conclusion is supported by the fact that in the present study VWF in proteinuric diabetic subjects was not significantly higher than in those without proteinuria. Our observations also suggest that the increase in VWF in diabetes is not a major causal factor in the genesis of diabetic retinopathy and nephropathy, although they do not exclude the possibility that VWF plays a permissive role.

We did not find a significant difference in the plasma fibronectin level between our diabetic patients as a whole and our control group, which suggests that plasma fibronectin does not play a primary role in the genesis of diabetic angiopathy, but this does not exclude the possibility that there is an abnormality in the tissue component of fibronectin in diabetes.[38] Alexander et al.[18] and Schwarz and Schernthaner[19] have reported that well-controlled diabetic patients have a normal level of plasma fibronectin, but Alexander et al. noted a marked decrease in plasma fibronectin in untreated or ketoacidotic diabetic subjects, whereas Schwarz and Schernthaner observed a significant increase in plasma fibronectin in poorly controlled diabetic subjects. We cannot comment on the relevance of these studies to our own work, as both have been published only in abstract form and details concerning the patient population and methods used for measuring fibronectin are not available. Cornell reported a 22% decrease in plasma fibronectin in rats made diabetic by the administration of streptozotocin (STZ) 14 days previously, as compared with rats not given STZ.[20] However, no fibronectin measurements were made on STZ-treated rats treated with insulin. Furthermore, the body weight of the diabetic rats was markedly reduced, and it has been found that food deprivation causes a fall in plasma fibronectin.[39] Therefore, the decrease in fibronectin may have been due to impaired nutrition rather than to diabetes per se.

In the present study we measured IGF I (somatomedin C) and IGF II by radioimmunoassay, and we did not find any significant difference in the concentration of these factors between diabetic and nondiabetic subjects, between diabetic subjects with and without retinopathy, or between diabetic subjects with and without proteinuria. Other investigators who have measured IGF I in the serum of diabetic patients by immunoassay have also found it to be normal,[27,28] but we are not aware of any previous studies on immunoreactive IGF II in diabetic subjects. Our finding of normal serum levels of IGF I and IGF II in diabetic subjects suggests that diabetic microangiopathy is not secondary to an absolute increase in the concentration of these factors. However, these factors are bound to plasma proteins,[27] and we cannot exclude the possibility that the fraction of these factors that is bioavailable might be abnormal in diabetic patients. Plasma somatomedin activity, as measured by bioassay techniques, has been reported to be either reduced[24,25] or increased[26] in diabetic patients, but these bioassay studies are difficult to interpret, since serum may contain factors other than somatomedins that affect the bioassay systems.[40] In studies in which plasma somatomedin has been measured in diabetic subjects by radioreceptor assay, it has been found to be normal,[29] consistent with our radioimmunoassay findings.

Overall, the results of the present study suggest that neither plasma VWF, fibronectin, IGF I, nor IGF II plays a primary role in the pathogenesis of diabetic microvascular disease. However, our results do not exclude the possibility that one or more of these factors might play a permissive role.

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