Elevation of Factor VIII Coagulant Activity Over Factor VIII Coagulant Antigen in Diabetic Children Without Vascular Disease

A Sign of Activation of the Factor VIII Coagulant Moiety During Poor Diabetes Control

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SUMMARY

To investigate whether the elevation of factor VIII coagulant activity observed in children with poor control of diabetes is due to increased levels of the factor VIII coagulant moiety of the factor VIII complex or reflects activation of the factor VIII coagulant moiety, factor VIII coagulant activity (VIII C), factor VIII coagulant antigen (VIII C:Ag), and factor VIII-related antigen (VIII R:Ag) were determined in 75 insulin-dependent children. All children were without signs of vascular disease based on negative funduscopy, negative fluorescein angiography, normal serum creatinine levels, and absence of proteinuria. Children with poor actual control of diabetes had significantly higher VIII C values than did children with good actual control of diabetes based on HbA_1 values, but VIII C:Ag values did not differ in children with good or poor actual control of diabetes. A significant elevation of VIII C over VIII C:Ag values was observed in children with poor actual control of diabetes, but no elevation of VIII C over VIII C:Ag was found in children with good actual control. VIII R:Ag values were higher in children with poor actual control. VIII C, VIII C:Ag, and VIII R:Ag did not differ significantly in children with short or long duration of clinical diabetes.

Our observation of significantly higher VIII C values than VIII C:Ag levels strongly suggests intravascular activation of the factor VIII coagulant moiety during poor diabetes control. The process leading to activation of the coagulant moiety seems to be different from the process leading to the elevation of the other moiety of the factor VIII complex, the factor VIII-related antigen, in diabetic subjects. DIABETES 1985; 34:140–44.

The factor VIII complex is formed by two functionally and immunologically different moieties. A high-molecular-weight moiety exerts its activities in primary hemostasis by supporting platelet adhesion and platelet aggregation. When measured by means of heterologous antisera, the high-molecular-weight moiety is termed factor VIII-related antigen (VIII R:Ag). A low-molecular-weight moiety displays factor VIII coagulant activity (VIII C). This low-molecular-weight coagulant moiety of the factor VIII complex is usually measured by determining its clotting activity by means of factor VIII coagulant activity-deficient plasmas. Recently, immunologic methods for the determination of the factor VIII coagulant moiety have been described that use antibodies derived from patients with acquired inhibitors against the coagulant moiety of the factor VIII complex. When measured by these methods, the coagulant moiety is termed factor VIII coagulant antigen (VIII C:Ag).[1–5]

In previous work, we have shown that alterations of the factor VIII complex can be observed in children without signs of diabetic vascular disease and even in children with a short duration of clinical diabetes.[6] In that study, we found an elevation of the factor VIII-related antigen only in children with a relatively long duration of clinical diabetes, while in children with a short duration but poor control of diabetes no elevation of the VIII R:Ag was observed. A similar elevation of VIII R:Ag has been observed in patients with vascular disease[7–13] and most likely represents a sign of endothelial damage, since VIII R:Ag is synthesized in the vascular endothelium. More interesting was our previous observation of a significant elevation of VIII C in patients with poor actual control of diabetes. This elevation of VIII C was correlated with urinary sugar excretion and HbA_1 levels, but not with duration of diabetes.[6]

Two mechanisms might lead to high factor VIII C during a phase of poor diabetes control: factor VIII coagulant moiety might be released in greater amounts from its synthesis or storage sites, or its degradation might be decreased. Alternatively, the measurement of an increased factor VIII coagulant activity might be due to a slow-grade intravascular activation of the coagulant moiety by thrombin or some other protease.

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The present study was performed to further clarify the elevation of VIII C during poor diabetes control. To investigate whether the elevation of VIII C in the plasma of these patients is due to increased levels of the coagulant moiety or reflects activation of the coagulant moiety, factor VIII coagulant activity (VIII C) and factor VIII coagulant antigen (VIII C:Ag) were determined in parallel. For the first time, we report a significant difference in VIII C and VIII C:Ag levels, suggesting activation of the factor VIII coagulant moiety in subjects with poor control of diabetes mellitus.

MATERIALS AND METHODS

Patients. Seventy-five children, 40 female and 35 male, regularly attending the diabetes unit of the Department of Pediatrics were included in the study. Informed consent was obtained from the parents of each patient. Age ranged from 4 to 20 yr. Duration of clinical diabetes ranged from 0.7 to 13.8 yr with a median duration of 5.2 yr. All of the patients were insulin-dependent and free of any other medication at the time of the study. All subjects included in the study were nonsmokers and of normal weight; their blood pressures were in the normal range. Only subjects without signs of microangiopathic disease were included in the study: no retinal lesions were demonstrable by funduscopy and by fluorescein angiography, plasma creatinine levels were <1.0 mg/dl, and no proteinuria (Combur test, Boehringer) was demonstrable. No clinical signs of neuropathy were present. Blood samples were taken before breakfast and insulin by a clean venipuncture of an antecubital vein without venicompression. Nine parts of blood were collected into plastic syringes containing 1 part of 0.1 M sodium citrate.

Procedures. Urinary sugar excretion was determined by means of a glucose-oxidase method. The HbA_1 determinations were done by means of a commercially available microcolumn procedure (Isolab Lab., Akron, Ohio).

Plasma factor VIII coagulant activity (VIII C) was assayed by one-stage assay using a commercially available test kit from Behring Company. Plasma factor VIII coagulant antigen (VIII C:Ag) was determined by immunoradiometric assay as described by Peake and Bloom.[3,5] The antibody used in the assay was purified from the plasma of a hemophilic patient with an inhibitor against VIII C. The antibody did not cross-react with VIII R:Ag. The assay detected VIII C:Ag even after complete inactivation of VIII C by thrombin; VIII C:Ag values decreased during inactivation of VIII C by thrombin (to about 60% after complete inactivation of VIII C as compared with 100% before inactivation).

Plasma factor VIII-related antigen (VIII R:Ag) was assayed by quantitative immunoelectrophoresis using a specific rabbit antiserum commercially available from Behring Company.[2]

Calibration curves for all measurements were done with pooled plasma from 20 healthy adults.

Statistical methods. After a thoroughly descriptive examination, the data were checked by analysis of variance (ANOVA) and correlation analysis. The validity of the ANOVA was checked by Levene's test for equal variances and confirmed by robust test statistics. Each significant F-value obtained by the ANOVA was further examined by a sequential multiple-test procedure proposed by Holm—a method that tests the difference among several groups using a sequential correction of the level of significance to avoid unnecessary conservative decisions.[14-16]

RESULTS

The correlations between the various variables are shown in Table 1. For further analysis, the patients included in the study were grouped according to the following criteria:

HbA_1 levels. Patients were grouped as follows: Group 1: HbA_1 levels <9%, group 2: HbA_1 levels 9–11%, and group 3: HbA_1 levels >11%. The results are summarized in Table 2. The mean values for VIII C were lowest in the group of children with HbA_1 levels <9% and highest in the group with HbA_1 levels >11%. The elevation of VIII C in the group with high HbA_1 levels was statistically significant. VIII C:Ag values were highest in the group with HbA_1 levels >11%, but the differences in the groups were not statistically significant.

A statistically significant elevation of VIII C values over VIII C:Ag values was observed in the groups with HbA_1 levels of 9–11% and >11%, but no significant difference in VIII C and VIII C:Ag values was observed in the group with HbA_1 levels <9% (Table 3).

VIII R:Ag levels were significantly lower in the group with low HbA_1 levels (Table 2).

Urinary sugar excretion. Patients were kept on a diet individually planned for their daily schedules. Daily intake of carbohydrates was not only calculated on the basis of the patients' weight, but also according to their personal needs. Therefore, urinary sugar excretion was calculated as percent of the carbohydrates (glucose, starch, and fiber) ingested on the day before the day of the investigation: group 1, urinary sugar excretion <5% of the ingested carbohydrates; group 2; urinary sugar excretion 5–10% of the ingested carbohydrates and group 3; urinary sugar excretion >10% of the ingested carbohydrates. The results are summarized in Table 2.

The mean value of VIII C was lowest in the group of children with the smallest sugar excretion and highest in the group

TABLE 1
Correlation between variables

	Glucosuria	HbA_1	Duration	VIII C	VIII C:Ag	VIII R:Ag
Glucosuria	1.00					
HbA_1	0.50	1.00				
Duration	0.28	0.22	1.00			
VIII C	0.26	0.37	0.16	1.00		
VIII C:Ag	0.01	0.24	0.02	0.64	1.00	
VIII R:Ag	0.06	0.29	0.28	0.71	0.057	1.00

TABLE 2
ANOVA results: mean ± SD, P-value, and significant multiple tests of variables VIII C, VIII C:Ag, and VIII R:Ag divided into groups based on values of HbA_1, glucosuria, and duration of clinical diabetes

	HbA_1				
	Low: <9% (25)	Mid: 9–11% (28)	High: >11% (22)	All (75)	P-value
VIII C	159.60 ± 74.62	175.79 ± 60.64	222.27 ± 68.32	184.03 ± 71.65	0.007 < 0.01
VIII C:Ag	146.72 ± 81.35	135.14 ± 60.53	176.27 ± 70.39	151.07 ± 71.97	0.125 NS
VIII R:Ag	136.44 ± 42.50	167.36 ± 55.56	174.46 ± 50.23	159.13 ± 51.06	0.02 < 0.05
	Glucosuria of the ingested carbohydrates				
	Low: <5% (36)	Mid: 5–10% (19)	High: >10% (20)	All (75)	P-value
VIII C	164.61 ± 65.54	196.21 ± 71.28	207.00 ± 76.36	184.03 ± 71.65	0.07 NS
VIII C:Ag	147.42 ± 71.05	161.79 ± 83.87	147.45 ± 63.71	151.07 ± 71.97	0.76 NS
VIII R:Ag	157.67 ± 52.92	154.00 ± 47.56	166.65 ± 52.56	159.13 ± 51.06	0.73 NS
	Duration of clinical diabetes (yr)				
	Short: <3 (25)	Mid: 3–7 (29)	Long: >7 (21)	All (75)	P-value
VIII C	174.88 ± 73.21	177.17 ± 56.59	204.36 ± 86.44	184.03 ± 71.65	0.31 NS
VIII C:Ag	150.12 ± 85.46	150.62 ± 73.71	152.81 ± 52.84	151.07 ± 71.79	0.99 NS
VIII R:Ag	143.12 ± 45.53	159.38 ± 40.27	177.86 ± 64.83	159.13 ± 51.06	0.07 NS

with the highest urinary sugar excretion, but the differences were not significant. VIII C:Ag values did not differ significantly in the groups. Also, VIII R:Ag values did not differ significantly in the groups with low or high urinary sugar excretion.

Duration of clinical diabetes. Patients were grouped as follows: group 1, duration <3 yr; group 2, duration 3–7 yr; and group 3, duration >7 yr.

VIII C values did not differ significantly in the groups; also, VIII C:Ag levels did not differ significantly in the groups.

VIII R:Ag levels were highest in the group with the longest duration of clinical diabetes, but the elevation did not reach significance (Table 2).

The interpretation of the above results relies on the assumption of HbA_1 being the "hard" parameter in regard to actual control of diabetes, while glucosuria depends more on the patient's most recent diabetes control. This assumption is confirmed by the frequency table (Table 4).

The frequency table shows a rather good correspondence of the low levels of the HbA_1, duration, and glucosuria variables, while a mid-level HbA_1 value does not necessarily correspond to a mid-level glucosuria. Similar results are found for the relationship between duration and HbA_1 and also for duration and glucosuria.

A significant elevation of VIII C values over VIII C:Ag levels was observed only in the groups with mid- and high-HbA_1 levels; the significant differences in the group with low sugar excretion depend obviously on the unstable character of the glucosuria evaluation.

DISCUSSION

Alterations of the factor VIII complex have been reported mostly in adult diabetic subjects and in patients with overt signs of diabetic vascular disease, the most frequent reported finding being an elevation of VIII R:Ag that correlates with the degree of diabetic vascular disease.[7,13,17] In previous work, we have shown that, in children without signs of vascular disease and even in children with a short duration of clinical diabetes, alterations of the factor VIII complex can also be observed.[6] In the study mentioned, we found that alterations of the two moieties of the factor VIII complex showed a different pattern when correlated with duration and

TABLE 3
P-values of difference between levels of VIII C and VIII C:Ag

	Glucosuria	HbA_1	Duration
All	0.001	0.001	0.001
Low (short)	0.031	0.25	0.022
Mid	0.047	0.001	0.041
High (long)	0.001	0.006	0.001

TABLE 4
Frequency tables

		Glucosuria			
		Low	Mid	High	Total
HbA_1	Low	21	3	1	25
	Mid	13	7	8	28
	High	2	9	11	22
	Total	36	19	20	75
		Glucosuria			
		Low	Mid	High	Total
Duration	Low	17	6	2	25
	Mid	10	11	8	29
	High	9	2	10	21
	Total	36	19	20	75
		HbA_1			
		Low	Mid	High	Total
Duration	Low	14	6	5	25
	Mid	7	12	10	29
	High	4	10	7	21
	Total	36	19	20	75

quality of actual control of diabetes: the elevation of VIII R:Ag correlated with the duration of clinical diabetes, while elevation of VIII C without concomitant elevation of VIII R:Ag correlated with the quality of actual diabetes control.[6] In the present study, we were able to confirm the results of our previous study in another cohort of patients: VIII C was significantly elevated in the groups with poor actual control of diabetes as compared with the group with good control as shown by low HbA_1 levels in the latter group.

In addition, we show that a significant difference in VIII C and VIII C:Ag levels can be found in patients with poor diabetes control. There was also no significant difference in VIII C:Ag levels in the groups with good or poor diabetes control despite a significant elevation of VIII C in the groups with poor control (based on HbA_1 levels) as compared with the group with good control. These findings strongly suggest that, during poor diabetes control, there is no increased synthesis or release from possible storage sites and no decreased degradation of the coagulant moiety of the factor VIII complex, since no elevation of antigenic material was observed despite the significant elevation of VIII C by functional testing.

In plasma of normal persons, a good correlation exists between VIII C and VIII C:Ag levels. When the factor VIII complex is activated by thrombin in vitro, a rapid increase in VIII C occurs that is followed by a time-dependent inactivation of VIII C.[18] Hoyer et al.[19] and Fulcher et al.[20] have shown that, during thrombin inactivation, the factor VIII coagulant moiety is cleaved to smaller fragments. VIII C:Ag values decrease during inactivation of the coagulant moiety by thrombin to values of about 60% after complete inactivation as compared with 100% before the addition of thrombin.[3,5] Consequently, in vitro, at an early stage of activation of the factor VIII coagulant moiety, VIII C values are found to be higher than VIII C:Ag values. This phase of activation is followed by inactivation after which VIII C:Ag values are lower than before inactivation, but VIII C is no longer detectable.

The pattern of significantly higher VIII C values than VIII C:Ag values observed in our patients with poor actual control of diabetes is compatible with the in vitro findings of slight activation of the factor VIII coagulant moiety, therefore suggesting that, during poor diabetes control, an intravascular activation of the coagulant moiety of the factor VIII complex occurs.

The mechanism for this possible intravascular activation of the factor VIII coagulant moiety is not clear at this point. Our findings of higher activity than antigenic material levels of the factor VIII coagulant moiety may be a sign of activation of the coagulant moiety alone, or more likely represent a sign of a more general activation of the coagulation system. Other laboratory signs suggestive of an activation of the hemostatic system in diabetes have been reported;[21-27] even signs of disseminated intravascular coagulation have been observed at autopsy in patients who died from diabetic coma.[28] Trace amounts of thrombin that lead to activation of the factor VIII coagulant moiety may be generated by alterations of the primary hemostatic system, resulting in increased platelet aggregation, or may be a sign of direct activation of the coagulation system during poor control of diabetes.

Again, most of the signs of activation of the hemostatic system have been observed in patients with overt vascular disease. In contrast, in this study, we found strong evidence for the activation of the factor VIII coagulant moiety during poor diabetes control in children without any signs of vascular disease. The mechanism of this activation and the significance of this activation of the coagulation system, or at least the factor VIII coagulant moiety, remain to be elucidated. Mechanisms other than intravascular activation of the coagulant moiety cannot be ruled out as the reason for the observed elevation of VIII C over VIII C:Ag. Catecholamines are found elevated in diabetic subjects and can increase VIII C.[29] Phospholipids alter the factor VIII complex[30] and cause a disparity of VIII C and VIII C:Ag values.[31] Phospholipids might be elevated during poor control of diabetes, but are unlikely to be responsible for the alterations observed in our study, since phospholipids decrease measurable VIII C:Ag by interference with the VIII C:Ag assay, and VIII C:Ag levels did not differ in the groups. Our studies, however, strongly suggest that the two moieties of the factor VIII complex play a completely different role in diabetic patients: the high-molecular-weight moiety is found elevated with long duration of diabetes and most likely reflects a sign of endothelial damage. The low-molecular-weight moiety that exerts factor VIII coagulant activity seems to be altered during phases of poor diabetes control. This process seems not necessarily to be correlated with endothelial damage, since the factor VIII coagulant moiety is not synthesized in the vascular endothelium.[32,33]

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