Elevated Factor VIII Activity and Factor VIII-related Antigen in Diabetic Children Without Vascular Disease

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SUMMARY

Factor VIII coagulant activity (VIII C) and factor VIII-related antigen (VIII R:Ag) were studied in 86 insulin-dependent diabetic children. All children were without signs of vascular disease based on a negative funduscopy, negative fluorescein angiography, normal serum creatinine levels, and absence of proteinuria. Age ranged from 4 to 17 yr; duration of clinical diabetes ranged from 1 to 12 yr. The children were grouped according to their urinary sugar excretion, the HbA_1 levels, and the duration of clinical diabetes. The group with high urinary sugar excretion and the group with high HbA_1 levels had a significantly higher VIII C than the group with low urinary sugar excretion and the group with low HbA_1 levels. VIII C levels did not differ significantly in the groups with a different duration of clinical diabetes, but VIII R:Ag was significantly higher in the group with the longest duration of diabetes as compared with the group with the shortest duration. VIII R:Ag levels did not differ significantly in the groups with different degrees of urinary sugar excretion or different HbA_1 levels.

The results show that in children without vascular disease, and even in children with a short duration of diabetes, alterations of the factor VIII complex can be demonstrated. DIABETES *31*:1006–1009, November 1982.

Diabetes mellitus is associated with a high prevalence of vascular disease. The pathogenesis of diabetic vascular disease is not completely clear at this point. Changes in the hemostatic system may play a role in pathogenesis and have been investigated in numerous studies. A number of abnormalities have been reported: increased platelet adhesiveness, hypersensitivity to platelet aggregating agents,[1–8] an increase of plasma β-thrombo-globulin,[9–11] changes in prostaglandin synthesis,[12–14] and abnormalities in the factor VIII complex.[15–22]

Most of the studies cited above were done in adults with type I or type II diabetes with a long duration of diabetic symptoms and with diabetic vascular disease. Only a few reports deal with diabetic children.[23] In children with insulin-dependent diabetes mellitus (IDDM) and a short duration of diabetic symptoms, there are usually no diabetic angiopathies demonstrable, but these children presumably are at the onset of diabetic vascular disease. In addition, most children do not have other risk factors for vascular disease like nicotine abuse, hypertension, and obesity. Therefore, we studied the factor VIII complex in children with IDDM in an attempt to find signs of an early beginning of vascular disease in diabetes mellitus.

PATIENTS AND METHODS

Eighty-six diabetic children, 46 girls and 40 boys, 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 17 yr. Duration of clinical diabetes ranged from 1 to 12 yr with a median duration of 4.9 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. All were without signs of microangiopathic disease: no retinal lesions were demonstrable by funduscopy and by fluorescein angiography, plasma creatinine levels were lower than 1.0 mg/dl, and no proteinuria was demonstrable. No signs of neuropathy were present. Blood samples were taken in the fasting condition between 7:00 and 8:30 a.m. by a clean venipuncture of an antecubital vein without venicompression. 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, which uses undialyzed blood samples (Biorad Lab., Richmond, California).

Plasma factor VIII-related antigen (VIII R:Ag) was assayed by the quantitative immunoelectrophoretic technique

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TABLE 1
Urinary sugar excretion as percent of the carbohydrates ingested on the day before the day of the investigation

Group	N	VIII C (U/ml)		VIII R:Ag (U/ml)		VIII C/VIII R:Ag	
1	47	1.855 ± 0.122	$P < 0.05$	1.854 ± 0.117	NS	1.016 ± 0.890	$P < 0.05$
2	16	2.144 ± 0.357		1.986 ± 0.219		1.237 ± 0.170	
3	23	2.242 ± 0.350		1.682 ± 0.176		1.399 ± 0.230	

$\bar{x}$ ± SEM of factor VIII activity (VIII C)(U/ml), factor VIII-related antigen (VIII R:Ag)(U/ml), and the ratio VIII C/VIII R:Ag. Group 1: < 5%; group 2: <10%; and group 3: >10%.

of Laurell using factor VIII antibodies commercially available from Behring Company.[24,25] The two-dimensional immunoelectrophoresis was done using the same antiserum: Barbital buffer (pH 8.6), 1% agarose; first dimension 6–7 V/cm for 4 h; second dimension 2–3 V/cm for 12–14 h.

Plasma factor VIII coagulant activity (VIII C) was assayed by a one-stage method using a commercially available test kit from Behring.

Calibration curves for all measurements were done with pooled plasma from 20 healthy adults. Statistical analyses were done by means of the Student's *t* test.

RESULTS

VIII C was $\bar{x}$ 1.904 U/ml ± 0.141 SEM. VIII R:Ag was $\bar{x}$ 1.840 U/ml ± 0.088 SEM. The ratio of VIII C/VIII R:Ag was $\bar{x}$ 1.2 ± 0.01 SEM. The results of the studies were grouped according to the following criteria.

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 ingested on the day before the day of the investigation. The following values were noted for each group: group 1: urinary sugar excretion < 5% of the ingested carbohydrates; group 2: urinary sugar excretion < 10% of the ingested carbohydrates; and group 3: urinary sugar excretion > 10% of the ingested carbohydrates. The results are summarized in Table 1.

The mean value of VIII C was lowest in the group of patients with the smallest urinary sugar excretion and highest in the patients with the highest urinary sugar excretion. The difference was statistically significant ($P < 0.05$). The VIII R:Ag was not significantly different in the groups with low or high urinary sugar excretion. The median of the ratio of VIII C/VIII R:Ag was lowest in the group with the smallest urinary sugar excretion and highest in the group with the highest urinary sugar excretion. The difference was statistically significant ($P < 0.05$).

HbA_1 levels. The HbA_1 levels for each group were as follows: group 1: HbA_1 levels < 10%; group 2: HbA_1 levels < 12%; and group 3: HbA_1 levels > 12%. The results are summarized in Table 2.

The mean values of VIII C were lowest in the group with HbA_1 < 10% and highest in the group with HbA_1 > 12%; the difference was statistically significant ($P < 0.05$). VIII R:Ag was not different in the group with low or high HbA_1 values. The ratio of VIII C/VIII R:Ag was significantly different in the group with HbA_1 < 10% and in the group with HbA_1 > 12% ($P < 0.05$).

Duration of clinical diabetes mellitus. The range of duration for each group was as follows: group 1: < 4 yr; group 2: < 8 yr; and group 3: < 12 yr. The results are summarized in Table 3.

Although the mean value of VIII C was lowest in the patients with the shortest duration of diabetes and highest in the patients with the longest duration of diabetes, the difference was not statistically significant. VIII R:Ag, however, was lowest in the group of patients with the shortest duration of diabetes and highest in the group of patients with the longest duration; the difference was statistically significant ($P < 0.05$). The ratio of VIII C/VIII R:Ag did not significantly differ in the three groups.

No statistically significant differences were found between male and female patients in all groups investigated.

The two-dimensional immunoelectrophoresis of VIII R:Ag of the diabetic children showed no differences in the form of the precipitate and no differences in regard to the distance of migration from the application point as compared with normal plasma.

DISCUSSION

Factor VIII procoagulant activity (VIII C) is exerted by a low-molecular-weight moiety of the factor VIII molecule that can be separated from a high-molecular-weight moiety by high-ionic-strength buffers.[26,27] The two moieties of the factor VIII molecular complex also have different antigenic sites. The high-molecular-weight moiety precipitates with heterologous antisera and can be measured by means of such antisera, then termed factor VIII-related antigen (VIII R:Ag).[25] This part of the factor VIII molecule or molecule complex supports ristocetin-induced thrombocyte aggregation and

TABLE 2
HbA_1 levels

Group	N	VIII C (U/ml)		VIII R:Ag (U/ml)		VIII C/VIII R:Ag	
1	26	1.550 ± 0.139	$P < 0.05$	1.912 ± 0.181	NS	0.964 ± 0.151	$P < 0.05$
2	37	1.966 ± 0.221		1.914 ± 0.139		1.093 ± 0.090	
3	23	2.210 ± 0.317		1.596 ± 0.135		1.500 ± 0.242	

$\bar{x}$ ± SEM of factor VIII activity (VIII C)(U/ml), factor VIII-related antigen (VIII R:Ag)(U/ml), and the ratio VIII C/VIII R:Ag. Group 1: HbA_1 levels < 10%; group 2: HbA_1 levels < 12%; and group 3: HbA_1 levels > 12%.

TABLE 3
Duration of clinical diabetes mellitus

Group	N	VIII C (U/ml)		VIII R:Ag (U/ml)		VIII C/VIII R:Ag	
1	33	1.733 ± 0.235	NS	1.642 ± 0.118	$P < 0.05$	1.215 ± 0.184	NS
2	30	1.855 ± 0.206		1.935 ± 0.148		1.045 ± 0.103	
3	23	2.156 ± 0.260		2.011 ± 0.206		1.254 ± 0.139	

$\bar{x}$ ± SEM of factor VIII activity (VIII C)(U/ml), factor VIII-related antigen (VIII R:Ag)(U/ml), and the ratio VIII C/VIII R:Ag. Group 1: <4 yr; group 2: <8 yr; and group 3: <12 yr.

thrombocyte adhesion.[28] The high-molecular-weight factor VIII moiety is synthesized in the vascular endothelium.[29,30] The synthesis site of the low-molecular-weight factor VIII moiety that exerts coagulant activity is not known.

Plasma factor VIII-related antigen (VIII R:Ag) levels have been found to be elevated in adults with IDDM and with NIDDM, mostly in patients with overt signs of vascular disease.[5,15–22] Elevated levels of VIII R:Ag in six diabetics with background retinopathy, but normal levels in seven diabetics without retinopathy, were reported by Porta and co-workers.[22] Normal levels of VIII R:Ag in a group of 30 diabetic children were found by Masperi et al.[23] We studied a fairly homogeneous group of 86 insulin-dependent diabetic children without any evidence of vascular disease. The levels of VIII R:Ag were found to be significantly higher in children with a long duration of diabetic symptoms than in children with a short duration (Table 3).

At this point it is not clear whether the elevation of VIII R:Ag is one of the causes for the angiopathy or only the consequence of endothelial damage. VIII R:Ag levels have been found to be elevated in a number of vascular diseases like atherosclerosis or in connective tissue disease.[8] It seems likely that release of VIII R:Ag from the damaged endothelial cells would also account for the elevated levels of VIII R:Ag in diabetes.[29,30] On the other hand, VIII R:Ag is involved in platelet adhesion to the subendothelium.[30] Evidence for the involvement of VIII R:Ag in the pathogenesis of vascular disease has been provided by animal experiments. Pigs with von Willebrand's disease and, therefore, a lack of VIII R:Ag did not develop atherosclerosis or coronary heart disease on a diet rich in cholesterol, whereas normal pigs did.[31] Therefore, the increase of plasma factor VIII-related antigen might be a risk factor for thrombogenesis and for the propagation of vascular disease.

Another possible explanation for the elevated values of VIII R:Ag in diabetes is that in diabetes an altered VIII R:Ag that migrates faster in the immunoelectrophoresis might be present. In our study, no differences between the factor VIII R:Ag of normal and diabetic children were found in the two-dimensional immunoelectrophoresis.

Factor VIII coagulant activity (VIII C) has been found to be normal in diabetic adults and in diabetic children, but elevated VIII C together with elevated VIII R:Ag levels have also been reported.[5,16,21,23] Interestingly, elevation of VIII C in our studies was correlated with the quality of control of diabetes and was not associated with an elevation of VIII R:Ag (Tables 1 and 2). No statistically significant elevation of VIII C was found in patients with long duration of diabetes as compared with patients with short duration of diabetes (Table 3).

Elevated levels of plasma VIII C might indicate a hypercoagulable state.[5] In patients with diabetic ketoacidosis, a striking rise of VIII C has been found; even the occurrence of disseminated intravascular coagulation has been reported.[21,32] The reason for these findings is not known. It was suggested that it represents a nonspecific response to acute stress in diabetic ketoacidosis.[33]

At this time, the implications of the described alterations of the factor VIII complex in diabetes are not completely clear. However, the results reported in this study show that similar alterations of the factor VIII complex, as described in adults with vascular disease, can be found in children with diabetes mellitus without overt signs of vascular disease and even in children with a short duration of clinical diabetes.

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