Endothelial Perturbation in Children and Adolescents With Type 1 Diabetes

Association with markers of the inflammatory reaction

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OBJECTIVE — The progression of diabetic angiopathy is, in most cases, unpredictable. The aim of this study was to investigate early events that could influence the development of diabetic angiopathy.

RESEARCH DESIGN AND METHODS — Circulating levels of von Willebrand factor (vWF) and tissue-plasminogen activator (tPA), defining endothelial perturbation, were measured in 40 young patients with type 1 diabetes. Patients were divided into two groups according to the duration of diabetes (group A, <1 year; group B, >1 year) and compared with a control group of age- and sex-matched healthy individuals. Prothrombin fragment 1 and 2 (F1+2), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) levels were also determined as markers of a prothrombotic state and inflammatory response. A total of 16 of the 20 children in group A were re-examined after 12 months.

RESULTS — Compared with either normal subjects or patients in group B, children in group A showed increased levels of vWF, tPA, F1+2, TNF-α, and CRP. Significant direct correlations between TNF-α or CRP and either vWF, tPA, or F1+2 were observed. Endothelial perturbation was shown in 70% of group A and 20% of group B. After 1 year, 16 of the 20 patients in group A showed a significant reduction in vWF, tPA, F1+2, TNF-α, and CRP levels, whereas endothelial perturbation was reversed in 5 of these patients.

CONCLUSIONS — Endothelial perturbation represents an early and, in some cases, reversible event in the chronology of type 1 diabetes in children. A correlation might exist between the initial inflammatory reaction and the appearance of endothelial perturbation.

Diabetes Care 24:1674–1678, 2001

Type 1 diabetes is associated with the development of vascular complications involving both the micro- and macrocirculation. Nephropathy, retinopathy, and atherosclerosis are frequently observed in patients with type 1 diabetes, and the progression of these conditions has a considerable impact on the prognosis of this disease (1).

Vascular endothelium is a primary target of the unbalanced glycemic metabolism in type 1 diabetes. Evidence indicates that high glucose levels may alter the anti-atherogenic, anti-thrombotic properties of vascular endothelium, thus favoring the development of diabetic angiopathy (2). On the other hand, inflammatory reactions taking place in the early phase of diabetes determine an increase in serum levels of a wide array of cytokines, including TNF-α, interleukin-1β (IL-1β), and interleukin-6 (IL-6), which may impair endothelial cell functions (3).

The initial phase of vascular endothelial damage is defined by the term “endothelial perturbation,” which refers to a reversible dysfunction of the endothelial cell. In vivo, endothelial perturbation is shown by a simultaneous increase in circulating vWF and tPA to levels 2 SD higher than the control values (4,5). Endothelial perturbation is associated with an ongoing prothrombotic state and an increased risk of thrombotic events (6), and its persistence could represent a negative prognostic index.

Because the risk of developing vascular disease is not distributed homogeneously among patients with type 1 diabetes and the pathogenesis of vascular complications is not completely defined, we examined the presence of endothelial perturbation in two populations of type 1 diabetic children and adolescents at different stages of the disease. We also investigated the possible relationship between inflammatory reaction and endothelial dysfunction. In this report, we show that endothelial perturbation represents an early and, in some cases, reversible event in children with type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 40 young patients with diabetes (18 girls and 22 boys, 12.5 ± 4.6 years of age) attending the Diabetes Clinic at the Division of Pediatrics, University of Chieti, Italy, were examined several times between December 1996 and November 1998. All patients had type 1 diabetes, as defined in accordance with the criteria of the American Diabetes Association (7). None of the
children had clinical evidence of retinopathy, as assessed by routine ophthalmologic examinations and stereoscopic fundus photography, or nephropathy (absence of microalbuminuria or macroalbuminuria). Patients were subdivided into two groups according to the duration of diabetes (defined as time since first injection of insulin): Group A (n = 20), duration of diabetes <1 year (±2.3 ± 3.8 months); and group B (n = 20), duration of diabetes >1 year (±58 ± 35 months). All patients were being treated with insulin (0.8–1.2 units kg\(^{-1}\) day\(^{-1}\)). A total of 10 age- and sex-matched healthy children were also studied. Of the 20 children in group A, 16 were re-examined after 12 months. Informed consent was obtained from each subject (or from his/her parent) participating in the study. The protocol was approved by the local ethical committee. The baseline characteristics of patients and control subjects are detailed in Table 1.

Venous blood samples were collected in tubes containing 3.8% sodium citrate. Plasma was prepared by centrifugation (2000g for 20 min), aliquoted, and stored at \(\sim\)20°C. vWF and tPA antigens were determined by enzyme-linked immunosorbent assay (ELISA) (American Diagnostic, Greenwich, CT). The intra- and interassay variation coefficients were 9 and 13% and 7 and 9%, respectively. Patients were considered to have endothelial perturbation when both vWF:Ag and tPA:Ag were more than 2 SD higher than control values (endothelial perturbation). In contrast, only 20% of patients in group B had signs of endothelial perturbation. In the remaining patients, vWF:Ag and tPA:Ag were highly correlated (0.81). The mean plasma tPA:Ag levels were significantly higher in patients in group A compared with age- and sex-matched healthy children or patients in group B.

### Table 1—Clinical characteristics of patients with diabetes and healthy subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy subjects</th>
<th>Patients with type 1 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>4/6</td>
<td>9/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.6 ± 2.6</td>
<td>10.6 ± 3.4</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months</td>
<td>—</td>
<td>4.2 ± 3.8</td>
</tr>
<tr>
<td>Range</td>
<td>—</td>
<td>7–10</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>ND*</td>
<td>174 ± 88</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.5 ± 0.7</td>
<td>8.9 ± 2.5</td>
</tr>
</tbody>
</table>

Data are n or (mean ± SD). *Not determined.

### Results

**Circulating levels of vWF:Ag** were significantly higher in patients in group A compared with age-matched healthy subjects or patients in group B (1.536 ± 438 vs. 671 ± 146 units/l, \(P = 0.0001\), and vs. 979 ± 252 units/l, \(P = 0.0008\), respectively) (Fig. 1A). Patients in group A also had increased plasma tPA:Ag levels in comparison with healthy subjects or patients in group B (11.2 ± 4.4 vs. 5.3 ± 1.3 ng/ml, \(P = 0.0036\), and vs. 7.4 ± 3.0 ng/ml, \(P = 0.0087\), respectively) (Fig. 1B). Of the 20 patients in group A, 14 (70%) had an increase in both vWF and tPA >2 SD higher than control values (endothelial perturbation). In contrast, only 20% of patients in group B had signs of endothelial perturbation.

**Higher F\(_{1+2}\) levels** compared with normal subjects or patients in group B (1.05 ± 0.42 vs. 0.419 ± 0.144 nmol/l, \(P = 0.0016\), and vs. 0.498 ± 0.295 nmol/l, \(P = 0.002\), respectively) were found in patients in group A (Fig. 1C). Direct correlations were found between F\(_{1+2}\) and either vWF (\(r = 0.809\), \(P = 0.0001\)) or tPA levels (\(r = 0.81\), \(P = 0.0001\)) (results not shown).

Patients in group A also had elevated circulating TNF-\(\alpha\) levels compared with normal subjects or patients in group B (21.9 [6.5–44.4] vs. 4.4 [1.5–8.2] pg/ml,
P = 0.0003, and vs. 2.45 [1.4–4.1] pg/ml, P = 0.0001, respectively) (Fig. 2A). This was paralleled by an increase in CRP levels (1.67 ± 0.99 vs. 0.38 ± 0.1 mg/l, P = 0.0051, and vs. 0.77 ± 0.5 mg/l, P = 0.004) (Fig. 2B). Interestingly, patients with endothelial perturbation displayed the highest levels of F1+2 (1.13 ± 0.37 vs. 0.45 ± 0.19 mmol/l), TNF-α (20.8 [2.6–44.4] vs. 6.9 [1.5–36.4] pg/ml), and CRP (2.31 ± 1.1 vs. 1.1 ± 0.65 mg/l). In addition, statistically significant correlations were found between TNF-α and CRP (r = 0.71, P = 0.0001); between TNF-α and vWF (r = 0.685, P = 0.0001); tPA (r = 0.469, P = 0.0034), or F1+2 levels (r = 0.61, P = 0.0001); and between CRP and vWF (r = 0.838, P = 0.0001), tPA (r = 0.694, P = 0.0001), or F1+2 (r = 0.718, P = 0.0001). In contrast, neither blood/urinary glucose, nor HbA1c, influenced vWF, tPA, and F1+2 levels (results not shown).

A total of 16 patients in group A were reviewed after 1 year. The results of the follow-up are summarized in Table 2. Endothelial perturbation was still appreciable in 6 of the 11 patients in whom it was initially displayed, whereas in five cases, it was reversed. Interestingly, when patients were divided into groups according to changes in endothelial perturbation, their TNF-α levels showed significant differences. In fact, the patients who still had endothelial perturbation after 1 year were those who had the highest TNF-α levels at the beginning of the study, whereas patients in whom endothelial perturbation was reversed after 1 year were those who had significantly lower initial TNF-α levels. Patients who did not have endothelial perturbation from the beginning were those with the lowest initial TNF-α values (Fig. 3). A similar pattern was observed with CRP (results not shown). In these patients, statistically significant correlations were observed between ICA and TNF-α (r = 0.44, P = 0.014), vWF (r = 0.54, P = 0.0025), tPA (r = 0.45, P = 0.0012), and F1+2 levels (r = 0.56, P = 0.0018) levels.

**CONCLUSIONS** — This study shows for the first time that the occurrence of endothelial perturbation represents an early and, in some cases, reversible event in the chronology of type 1 diabetes in children and adolescents. We observed that patients with duration of diabetes shorter than 1 year had a significant increase in both vWF and tPA levels and that endothelial perturbation was reversed in ~45% of these patients after 1 year (Fig. 1). Therefore, it seems that signs of a vascular involvement can be appreciated as early as during the first decade of life of patients with diabetes.
Considering the central role played by the endothelium in the regulation of vascular homeostasis (10), the question arises regarding the pathophysiological relevance of an early and, in most cases, transient endothelial perturbation in children and adolescents with diabetes. Should it be regarded as a transient epiphenomenon of the initial inflammatory autoimmune events taking place in diabetes? Or should it be considered an indicator of the risk for developing diabetic angiopathy in children and adolescents with type 1 diabetes? Long-term studies will hopefully answer this question. Consistent with previous observations (11), the present results also indicate that measurements of vWF and tPA may be useful to detect initial endothelial involvement in type 1 diabetes. In this regard, it must be noted that vWF is considered the best available noninvasive marker for endothelial dysfunction (12,13). Indeed, an elevation in vWF levels may be observed in diabetes (14) (in which it can precede the occurrence of microalbuminuria by ~3 years [2]), as well as in hypertension (15), smoking (16), hypercholesterolemia (17), and ischemic heart disease (18). However, because vWF may also originate from platelets (19), the combined determination of vWF and tPA, which is also secreted by endothelial cells (20), may be a more specific and sensitive index of endothelial cell alteration.

In our patients, endothelial perturbation was associated with an increment in F1+2 levels (Fig. 1). This finding, previously unappreciated in children with diabetes, indicates that at the beginning of the disease, these patients may have a subclinical prothrombotic disorder. In fact, F1+2 generates thrombin and is formed when active tissue factor and activated coagulation factor X accumulate (21). Increased F1+2 levels can be observed during coagulative disorders and are associated with a prothrombotic condition (4). Therefore, an increase in F1+2 levels is both a marker of an unbalanced tissue factor/tissue factor inhibitor pathway and an indicator of augmented thrombin generation. However, in approximately half of our children with type 1 diabetes, endothelial perturbation was a transient event; therefore, it would be difficult to draw a conclusion on the validity of endothelial perturbation and F1+2 determinations as predictive markers of diabetic angiopathy. However, it is striking that signs of endothelial perturbation and coagulative disorders can be already detected during childhood. Future studies will determine whether children showing persistent endothelial perturbation are at higher risk for developing vascular complications.

How is endothelial perturbation established in children with type 1 diabetes? Although previous studies have shown that poor glycemic control is related to the occurrence of vascular complications in juvenile diabetes (22,23), we were unable to observe a correlation between endothelial perturbation and HbA1c levels. On the other hand, an inflammatory response is frequently observed in the initial phase of type 1 diabetes. Therefore, taking into account that among our patients, those with the shorter duration of disease had the higher incidence of endothelial perturbation, we hypothesized that a relationship might exist between the appearance of immune-inflammatory markers and the occurrence of endothelial perturbation. Indeed, we observed a strong increment in the levels of the inflammatory cytokine TNF-α and of the inflammation marker CRP, which correlated with the levels of endothelial perturbation indexes (Figs. 2 and 3). Moreover, ICA, which appeared at a very early stage in type 1 diabetes, positively correlated with TNF-α as well as with vWF, tPA, and F1+2 levels. Considered together, these findings seem to be consistent with the possibility that the early immune-inflammatory reaction may influence the appearance of endothelial perturbation in children with type 1 diabetes. These results are consistent with a recent observation showing a significant correlation between CRP and markers of endothelial dysfunction in patients with type 1 diabetes (24) and suggest that TNF-α may represent at least one of the agents that link the inflammatory reaction to the appearance of endothelial perturbation in children with type 1 diabetes. Indeed, TNF-α induces vWF release and transient and reversible endothelial dys-function in humans (25,26). However, this does not exclude the possibility that some other factors may intervene in this process to determine whether endothelial perturbation becomes persistent. In this regard, the observation that patients in group A with the highest initial TNF-α levels (Fig. 3) corresponded to those with persistent endothelial perturbation after 1 year is intriguing. It might indicate that the degree of the initial inflammatory reaction could influence the chance of developing permanent endothelial dys-function. However, due to the relatively small number of patients observed, this hypothesis must be investigated further.

In conclusion, in this study, we have shown that endothelial perturbation is an early event that correlates with the appearance of inflammatory markers in children and adolescents with type 1 diabetes. Although the persistence of endothelial perturbation could represent a potential index of the risk for developing vascular complications, the relationship between the occurrence of endothelial perturbation and the development of diabetic angiopathy in young patients still remains to be established by future prospective studies.

Acknowledgments — This work was supported, in part (60%), by a grant from the Italian Ministry of University. Financial support was also provided by the local Juvenile Diabetes Foundation (AGDA).

We thank Antonella Bascelli for secretarial help and the patients and their families for their participation in this study.

References

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Endothelial perturbation in diabetic children


