Fibrinogen and von Willebrand factor in type II diabetes mellitus

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Abstract. A hypercoagulable state may contribute to the formation of early vascular lesions in diabetes. The von Willebrand factor is required for the attachment of platelets to the subendothelium; fibrinogen is required for platelet aggregation. This study was designed to assess in type II diabetic patients plasma levels of fibrinogen and von Willebrand factor to see if these variables are associated with platelet aggregation responses to adenosine diphosphate (ADP). Fibrinogen and the von Willebrand factor were significantly increased in diabetics but only fibrinogen was significantly related to platelet aggregation for ADP. Strict metabolic control does not reduce the increased concentrations of these two proteins. Hyperfibrinogenaemia was related to the presence of macrovascular disease. Therefore measurements of plasma fibrinogen could be added to the cardiovascular risk factor profile of diabetic patients. Intervention studies are also needed to reduce the increased incidence of thrombotic diseases in patients with diabetes mellitus.

Key words: Diabetes mellitus – Fibrinogen – von Willebrand factor – Platelet aggregation

Introduction

Diabetes mellitus is associated with early vascular complications. It has been suggested that a hypercoagulable state associated with metabolic disturbances [1] may contribute to vascular lesions. Blood platelets might initiate the formation of atheroma by attaching to denuded subendothelial structures. The von Willebrand factor (vWF) is required for the attachment of platelets to the subendothelium [2] and also has a key role in platelet aggregation at high shear stress. Because vWF-deficient pigs show a remarkable resistance to atherosclerosis, the possible role of vWF as a risk factor in cardiovascular disease in man has been investigated [3].

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Fibrinogen is a major blood glycoprotein that plays an essential role in haemostasis and maintenance of blood viscosity. Fibrinogen is important for effective in vitro and in vivo platelet function as supported by studies on patients with Glanzmann’s thrombasthenia, which might help in explaining the thrombogenic potential of fibrinogen. Moreover, several epidemiological studies have indicated an independent association of cardiovascular mortality with fibrinogen levels [4–6].

This cross-sectional study was designed to assess plasma levels of fibrinogen and vWF related antigen (vWF Ag) in diabetic patients in order to investigate whether these variables are associated with platelet aggregation response to adenosine diphosphate (ADP). In fact, diabetic patients show increased binding of fibrinogen to platelets and effective inhibition of binding to diabetic platelets corrects the hyperaggregable state [7, 8].

Subjects and methods

One hundred and twenty-five patients with type II diabetes mellitus (69 women and 56 men; mean age 65.1 ± 12.3 years), 40 young healthy volunteers (20 women and 20 men; mean age 29.5 ± 15.0 years) and 40 healthy subjects of equivalent age (18 women and 22 men; mean age 62.2 ± 13.4 years) were studied between June 1987 and October 1991 (Table 1). Type II diabetes was defined in accordance with the criteria of the American Diabetes Association [9]. All had non-insulin-dependent diabetes with a mean duration of 16.2 years (range: 7–26 years).

Forty-nine patients had a history of and/or a clinical examination showing evidence of macrovascular complications. Twenty-seven patients had stable angina pectoris or previous myocardial infarction, 16 had peripheral vascular disease and 6 had a history of previous episodes of stroke. Patients with coronary heart disease were in a stable phase, as judged on the basis of clinical symptoms, electrocardiographic monitoring during exercise, Holter monitoring, and echocardiographic observations. Patients with peripheral vascular disease were in Fontaine’s stage II (intermittent claudication, ankle-arm pressure index < 0.85, and no resting pain), with a constant level of pain while walking. Their disorder had been diagnosed on the basis of clinical symptoms, ability to walk long distances, cycle ergometry, and Doppler echographic study of the lower limbs. Patients with cerebrovascular disease had a clinical history of and positive results on carotid Doppler testing and Doppler echography.
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Table 1. Summary of demographic data on subjects studied

<table>
<thead>
<tr>
<th></th>
<th>Young controls</th>
<th>Controls of equivalent age</th>
<th>Diabetes (all)</th>
<th>Diabetes without vascular disease</th>
<th>Diabetes with vascular disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>40</td>
<td>40</td>
<td>125</td>
<td>76</td>
<td>49</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>19/21</td>
<td>18/22</td>
<td>56/69</td>
<td>32/44</td>
<td>25/24</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.5 ± 15.0</td>
<td>62.2 ± 13.4</td>
<td>65.1 ± 12.3</td>
<td>60.3 ± 12.5</td>
<td>72.0 ± 7.8</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>-</td>
<td>-</td>
<td>16.2 ± 9.5</td>
<td>12.2 ± 8.7</td>
<td>21.2 ± 9.4</td>
</tr>
</tbody>
</table>

Values are given as mean ± 1 SD

They had relatively stable disease, with one or two episodes of transient ischaemia per month before they were admitted to hospital. In none of the patients had vascular disease progressed significantly during the previous 12 months, as established at outpatient visits. Moreover, all had mild-to-moderate symptoms compatible with a virtually normal lifestyle. All patients continued cardiovascular drug therapy (beta-blockers, calcium-channel blockers, diuretics) during the study period and were also asked to abstain from aspirin-like drugs. At the time of the study, all patients were being treated with three daily injections of insulin (intermediate-acting and regular insulin).

Study design

A cross-sectional comparison of plasma levels of fibrinogen and vWF was performed between patients and controls. Patients were admitted to hospital and fasted overnight. Before they received insulin, blood was drawn for measurement of plasma levels of fibrinogen and vWF. Paired measurements of these plasma proteins and ADP-induced platelet aggregation were carried out in 62 diabetic patients while they were on standard therapy.

Biochemical analyses

Blood was drawn between 8 and 9 a.m. and collected in 3.8% sodium citrate (1 ml per 9 ml blood). Platelet-rich and platelet-poor plasma was prepared as previously described [10]. Platelet aggregation was measured in an Elvi 840 (Logos) aggregometer by Born's method [11]. Percent aggregation was determined assuming that platelet-poor plasma blank represented 100% aggregation and platelet-rich plasma blank represented 0% aggregation. Threshold aggregating concentration with ADP was defined as the lowest concentration of the agent that caused at least a 50% increase in light transmittance within 3 min.

Plasma fibrinogen was measured with a commercially available nephelometric assay (Behringwerke, Marburg, FRG). The normal range was 200–450 mg/dl. Plasma vWF Ag was measured by ELISA (Boehringer Mannheim, Mannheim, FRG). The normal range was 60–150%. The measurements were done without prior knowledge of the clinical diagnosis [12, 13].

Blood glucose levels were measured by a glucose oxidase method. Measurement of the extent of glycation of serum proteins, as determined by the reduction of alkaline nitro blue tetrazolium salts (fructosamine test), was used as an integrated glycaemic index, reflecting glycaemic control during the 2 weeks preceding blood analysis [14].

Statistical analysis

The data were analysed by non-parametric methods to avoid assumptions on the distribution of the measured variables [15]. An analysis of variance was performed by the Kruskall-Wallis method. Subsequent pairwise comparisons were made by the Mann-Whitney U test. All values are reported as mean ± 1 SD. Statistical significance was defined as $P < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Young controls</th>
<th>Controls of equivalent age</th>
<th>All diabetics (n=)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma fibrinogen (mg/dl)</td>
<td>287 ± 51</td>
<td>301 ± 38*</td>
<td>430 ± 122 (125)</td>
</tr>
<tr>
<td>Plasma von Willebrand factor antigen (%)</td>
<td>98 ± 15</td>
<td>103 ± 19*</td>
<td>138 ± 47 (62)</td>
</tr>
<tr>
<td>AC$_{50}$ for ADP (μM)</td>
<td>1.56 ± 0.28</td>
<td>1.45 ± 0.36**</td>
<td>0.76 ± 0.27 (0.62)</td>
</tr>
</tbody>
</table>

Values are given as mean ± 1 SD
* $P<0.001$, ** $P<0.005$ versus controls of equivalent age

Fibrinogen and vWF Ag were significantly increased in diabetic patients, with or without clinically evident vascular disease (Table 2).

Moreover, platelets from the patients with type II diabetes mellitus required significantly less ADP to aggregate. Among the patients in whom paired measurements were obtained ($n=62$), a strong and significant direct correlation ($r=0.62; P<0.001$) was found between ADP-induced platelet aggregation and fibrinogen levels. The highest concentrations of fibrinogen and vWF Ag were found in the diabetic patients with evidence of macrovascular complications (Table 3).