Platelet Activation in Patients with Diabetic Retinopathy

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To clarify the diabetic complications mediated by increased platelet activity, we undertook a study of the mean platelet component (MPC), as determined by an automated hematologic analyzer (ADVIA 120®, Bayer). Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen were also measured to investigate blood viscosity abnormalities. MPC was determined in 100 healthy controls and in 100 diabetic patients, the latter of which were subdivided into no diabetic retinopathy (DR) (n = 25), nonproliferative DR (n = 30), and proliferative DR (n = 45) groups. The mean MPC level was 26.9 g/dl in the control group and 22.5 g/dl in the diabetic patients (p < 0.05). PT and aPTT were similar for the diabetic patients and the controls; however, their corresponding fibrinogen levels were significantly different between the two groups (3.26 ± 1.14 g/L vs. 4.21 ± 2.35 g/L, p < 0.05). Our results suggest that platelet hyperfunction in diabetic patients may be implicated in the pathogenesis of diabetic retinopathy.

Key words: diabetic retinopathy, mean platelet component, platelet hyperfunction

INTRODUCTION

After 20-25 years of suffering from diabetes, more than 80% of patients experience some type of retinal lesion, and these patients are 20 times more likely to become blind than other members of the general population.1,2 Sustained hyperglycemia leads to a series of interrelated alterations that can cause evident endothelial dysfunction. Such alterations have been suggested to cause endothelial dysfunction, and vascular lesions in the retinas of patients with diabetes and retinopathy.3,4 Possible mechanisms by which elevated glucose induces vascular abnormalities, include, the non-enzymatic glycation theory5 and the sorbitol-myoinositol theory.6 Abnormalities of most aspects of the hemostatic system have been described in diabetes, and a hypercoagulable state is said to exist.

The recent development of improved assays has allowed the detection of the in vivo activation of the coagulation system. Platelet hyperactivity has been reported in diabetic men and animals, both in vivo and in vitro.7 Although the mechanisms underlying this hyperactivity remain unknown, some evidence suggests that platelet hyper-aggregation may participate in the pathogenesis of diabetic complications.8

In the present study, our aim was to determine if these platelets and the coagulation system are activated in diabetes, and, if so, whether activation could be a consequence of existing vascular disease. In this study, we examined platelet activation, pro-
thrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and glycosylated hemoglobin A1c (HbA1c), in both patients with diabetes mellitus with different types of retinopathy, and normal subjects. We used a modified version of the 2-angle, laser light scattering, flow-cytometric method used by Bayer hematology analyzers for red blood cell (RBC) analysis as further developed in the ADVIA 120® Hematology System (Bayer Corporation, Tarrytown, NY) for the measurement of platelet parameters. The platelet refractive index is related linearly to platelet density, which is a measure of the overall concentration of components within a platelet, and, which is indirectly an index of platelet activation status.9,10

**MATERIALS AND METHODS**

**Patients**

The participants in this study were 100 healthy subjects and 100 diabetic patients. We excluded patients with nephropathy, cardiovascular disease, and clotting disorder. All patients and normal subjects underwent a complete ophthalmologic examination, which included an evaluation of best-corrected visual acuity, intraocular pressure, lens, and fundus examination, and fundus photography.

On the basis of the fundus photography the participants were divided into four groups. Group 1 (n = 100) was comprised of normal individuals, group 2 (n = 25) of patients with diabetes mellitus (DM) with no signs of diabetic retinopathy (DR), group 3 (n = 30) of patients with DM and non-proliferative DR (NPDR), and group 4 (n = 45) of patients with diabetes and proliferative DR (PDR). The presence of HbA1c was confirmed from patients’ clinical records (Table 1).

**Laboratory procedures**

The parameters PT and aPTT, measured in the conventional manner, are screening tests for hypercoagulability. Fibrinogen was measured using STA-Compact® (Diagnostica stago, France). Venous blood samples were collected with EDTA as anticoagulant and tested within 1 hour of collection to minimize variations due to sample age. Samples were maintained at room temperature.

The ADVIA 120® used 2-dimensional platelet analysis, based on an extension of the optical method for counting RBCs. This method involves the simultaneous measurement of laser light scattered at 2 different angles; both of which were converted into volume and refractive index values. A cytogram was produced, where platelets were identified in the region corresponding to a volume of 1 to 60 fl and of refractive index 1.35 to 1.40. Volume and refractive index were then converted, by using a mathematical algorithm, into platelet components, from which the mean platelet component (MPC) was obtained. Platelet activation was defined as an MPC of < 26.7 g/dl.

**RESULTS**

A significant difference was found in blood sugar regulation between patients with and without DR, as determined by glycosylated hemoglobin (Table 1). The mean MPC level was 26.9 g/dl in healthy sub-

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**Table 1. Demographic findings of normal & diabetic patients**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetics</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Number (DM)</td>
<td>100</td>
<td>25</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>No</td>
<td>No</td>
<td>NPDR</td>
<td>PDR</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45 ± 2.1</td>
<td>62.3 ± 3.6</td>
<td>59.7 ± 3.1</td>
<td>62.4 ± 1.3</td>
</tr>
<tr>
<td>Gender (F:M)</td>
<td>1:4.5</td>
<td>1:1.2</td>
<td>1:1.3</td>
<td>1:1.1</td>
</tr>
<tr>
<td>Hb A1c (%)</td>
<td>3.8</td>
<td>5.7</td>
<td>7.63</td>
<td>7.73</td>
</tr>
</tbody>
</table>

Table 2. Mean MPC and coagulation profiles of the four groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean MPC (g/dl)</td>
<td>26.90</td>
<td>25.96</td>
<td>23.22</td>
<td>20.16</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.26 ± 1.14</td>
<td>4.21 ± 1.21</td>
<td>4.32 ± 1.31</td>
<td>4.15 ± 2.1</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Platelet count (× 10^3/µL)</td>
<td>298 ± 50</td>
<td>278 ± 74</td>
<td>283 ± 88</td>
<td>277 ± 43</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>PT (%)</td>
<td>98.4 ± 13.2</td>
<td>96.1 ± 15.8</td>
<td>94.2 ± 11.2</td>
<td>97.5 ± 21.2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>35.1 ± 2.5</td>
<td>36.0 ± 3.7</td>
<td>34.2 ± 4.4</td>
<td>35.7 ± 3.1</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Group 1: normal, group 2: diabetes without retinopathy, group 3: diabetes with NPDR, group 4: diabetes with PDR, MPC: mean platelet component, PT: prothrombin time, aPTT: activated partial thromboplastin time

jects and 22.5 g/dl in diabetic patients. The patients with nonproliferative and proliferative DR showed similar decreases (p < 0.05) in MPC level, while those without DR occupied an intermediate position between the normal controls and the patients with DR.

The mean MPCs of groups 2, 3, and 4 were 25.96 g/dl, 23.22 g/dl, and 20.16 g/dl, respectively (Table 2). The MPC level was negatively correlated with HbA1c, but did not differ significantly in diabetes patients. However, diabetic patients did have significantly higher plasma fibrinogen levels than normal control subjects. (4.21 ± 2.35 g/L vs. 3.26 ± 1.14 g/L, p <0.05) (Table 2), although no significant difference was found between groups 2, 3, and 4. PT and aPTT did not show significant abnormality in any patients group (Table 2).

**DISCUSSION**

A direct relation between platelet dysfunction and the development of diabetic complications has yet to be established. Some studies have suggested that the enhanced activation of circulating platelets is particularly apparent in diabetics with microvascular disease.11 Platelets are small, anucleate, discoid cells that circulate in the bloodstream and participate in hemostasis.12 Their main function is to plug holes in blood vessel walls. Platelets do this by changing shape, adhering to subendothelial surfaces, secreting the contents of intracellular organelles, and aggregating to form a thrombus in response to stimuli generated by the endothelium of damaged blood vessels. These pro-aggregatory stimuli include thrombin, collagen, and epinephrine and agents such as ADP, which is secreted from platelet storage granules, and thromboxane A2, which is synthesized by activated platelets.13 During aggregation, platelets secrete components of the blood coagulation pathway and the growth factors necessary for wound healing. Activation of platelets also results in changes in the levels of expression of surface glycoproteins.14 Fibrinogen levels may also be elevated in diabetes, and this would contribute to fibrin clot formation and platelet aggregation.15,16 Endothelial injury or plaque rupture with platelet adhesion and aggregation at the site of injury may be a critical event leading to morbidity of the retina.17 Platelets may, therefore, assume an important role in the signaling of atherosclerosis in diabetes.

In the present study, platelet activation was measured by flow-cytometry, immunofluorescence, and ELISA. Rauch and colleagues evaluated platelet activation by the flow cytometric detection of specific platelet surface markers, such as P-selectin, thrombospondin or the active complex of glycoprotein GP IIb/IIIa, in diabetic patients with and without microangiopathy.18 They found reduced expression of these markers in subjects with microangiopathy, but no differences between controls and diabetic patients without complications. However, these decreased expressions of the markers of platelet activation may reflect the increased consumption of activated platelets.19 Recently, increases in specific markers of platelets reactivity, like thrombomodulin and platelet factor 4, have been observed, but not all authors have confirmed their role in the prediction of disease progression.20, 21 However, these methods are time-consuming and expensive. The ADVIA120® hematology analyzer allows fast and accurate determination of platelet
count and various parameters, both traditional, such as mean platelet volume, and platelet volume distribution width, and new, such as MPC and mean platelet mass. This newly developed approach for platelet analysis also offers the precise determination of platelet parameters, in particular of MPC, which can be used in platelet function assessment.

In agreement with some recent studies, we found platelet activation in diabetics. In addition, we have shown enhanced platelet activation in patients with DR.

Our findings suggest that these platelet hyperactivations may play a role in the development of DR, and in the detection of platelet activation. These concentration changes of MPC can be used by the sensitive ADVIA 120® hematology analyzer to indicate the level of platelet activation.

REFERENCES


