Factor VIII, von Willebrand Factor and Platelet Adhesiveness in Diabetic Retinopathy

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Thirteen non-diabetic controls, 40 diabetics without retinopathy and 19 diabetics with retinopathy, approximately matched for age and sex, were studied for plasma levels of F VIII:C, vWF:Ag and R Cof, and platelet adhesiveness. F VIII:C was elevated in both diabetic groups compared with normal controls, but no differences were found between the diabetic groups. vWF:Ag was significantly higher in both diabetic groups than in normal controls, and it was also elevated in diabetics with retinopathy compared with those without. R Cof was higher in diabetics with retinopathy than in normal controls or in diabetics without retinopathy, but there were no differences between normal controls and diabetics without retinopathy. We could not find any differences in platelet adhesiveness between the groups. The results in the present study suggested that F VIII / vWF might play an important role in the pathogenesis of diabetic retinopathy.

(Key Words: F VIII : C, vWF : Ag, R Cof, Platelet Adhesiveness, Diabetic Retinopathy)

INTRODUCTION

A complex of factor VIII (F VIII) and von Willebrand factor (vWF) is essential in hemostasis and blood coagulation. The two proteins are controlled under distinct genes and have special physiologic functions. F VIII accelerates blood coagulation via its cofactor role in the enzymatic activation of factor X by factor IXa. The site of F VIII synthesis and the cell type responsible for it are not known. Patients with hemophilia A have a low concentration of F VIII and show a prolonged clotting time, as well as hemorrhages and muscular hematomas clinically (6).

vWF is a glycoprotein synthesized both by endothelial cells and by megakaryocytes. This protein is essential for platelets to adhere to subendothelial cells in blood vessels. Patients with von Willebrand's disease with low concentrations of vWF exhibit a prolonged bleeding time and bleeding complications such as epistaxis, gingival bleeding and menorrhagia.

On the other hand, high plasma levels of F VIII and vWF may result in thrombus formation in vessels. There have been several reports demonstrating between relationships of F VIII, vWF or platelet adhesiveness and the development of diabetic microangiopathy. However, definite conclusions have not yet been obtained. To clarify the role of F VIII / vWF in the pathogenesis of diabetic retinopathy, we measured plasma levels of F VIII procoagulant activity (F VIII : C), vWF antigen (vWF : Ag) and vWF ristocetin cofactor (R Cof), and platelet adhesiveness in diabetics with or without retinopathy.

PATIENTS AND METHODS

Forty diabetics without retinopathy (20 males and 20 females), 19 diabetics with retinopathy (12 males and seven females) and 13 healthy non-diabetic controls (eight males and five females) were concurrently investigated. The classification of retinopathy was performed on the basis of dilated funduscopic examination by ophthalmologists. Details of subjects studied are given in Table 1.

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Table 1 Clinical data of the subjects studied

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic controls</th>
<th>Diabetics without retinopathy</th>
<th>Diabetics with retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>13</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>8/5</td>
<td>20/20</td>
<td>12/7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52 ± 13</td>
<td>54 ± 12</td>
<td>59 ± 10</td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>—</td>
<td>5 ± 5</td>
<td>9 ± 6</td>
</tr>
<tr>
<td>Treatment Diet (no.)</td>
<td>—</td>
<td>26</td>
<td>7</td>
</tr>
<tr>
<td>HA (no.)</td>
<td>—</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Insulin (no.)</td>
<td>—</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>No. of IDDM</td>
<td>—</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Values given as mean ± SD
HA = hypoglycemic agent
IDDM = insulin dependent diabetes mellitus

Fasting blood was drawn from the antecubital vein using a 21 gauge vein needle and transferred immediately into a siliconized glass cylinder containing 3.2% trisodium citrate (9 vol:1 vol). F VIII : C was assayed by the one-stage method based on the PTT. vWF : Ag was tested by immunoelectrophoresis as described by Laurell (8). R Cof was measured according to the method of MacFarlane et al. (10) using washed formalin-fixed platelets.

Platelet adhesiveness was examined with a glass bead column (Igakushoin Kikai Co., Japan) by the modified method of Salzman (12). The results were calculated by the following formula:

\[
\text{Platelet adhesiveness (\%)} = \left(\frac{A - B}{A}\right) \times 100 (\%)
\]

A : Number of platelets which passed through the control column
B : Number of platelets which passed through the glass bead column

Statistical analysis was carried out by Student's t-test. The results were expressed as mean ± standard deviation.

RESULTS

Table 2 summarizes the results for plasma levels of F VIII : C, vWF : Ag and R Cof, and platelet adhesiveness. First, F VIII : C was elevated in diabetics with or without retinopathy compared with normal controls, but there was no difference between the diabetic groups. vWF : Ag was significantly higher in diabetics with or without retinopathy than in normal controls, and it was more elevated in diabetics with retinopathy than in those without. The R Cof was higher in diabetics with retinopathy than in normal controls or in diabetics without retinopathy, but no differences were found between normal controls and diabetics without retinopathy. We could not determine any differences in platelet adhesiveness between the groups although high platelet adhesiveness (> mean + 2SD) was observed in two cases of non-retinopathic diabetics with myocardial infarction.

DISCUSSION

Controversy remains with respect to the plasma level of F VIII : C in diabetics. Colwell et al. (4) reported that there was no change in the concentration of plasma F VIII : C between diabetics and normal controls. On the other hand, Pandolfi et al. (11) and Gensini et al. (5) demonstrated high levels in diabetics. Few researchers have recognized differences in plasma levels of F VIII : C between diabetic groups.

Most investigators have found higher levels of plasma vWF : Ag in diabetics than in normal controls. Additionally, Pandolfi et al. showed higher levels in diabetics with retinopathy than in those without. Lamberton et al. (7) and Borsey et al. (2) did not find any differences between diabetic groups. Coller et al. (3) reported higher vWF : Ag only in diabetics with retinopathy.

Regarding R Cof, Colwell et al. and Gensini
et al. found higher levels in all diabetic groups, while Coller et al. showed high R Cof only in diabetics with retinopathy. Bensoussan et al. (1) demonstrated higher R Cof in diabetics with retinopathy than in those without.

As mentioned above, definite conclusions concerning F VIII and vWF in diabetics have not been obtained. The present study confirmed that F VIII : C was higher in diabetics than in normal controls but that there were no differences between the diabetic groups. A small amount of vWF is complexed with F VIII, and the concentrations of these two proteins are closely correlated in normal plasma (6). However, we have found a discrepancy between these plasma levels in diabetics. This might be partially attributed to the different origins of the proteins and the different modes of cleavage in plasma (9). A discrepancy between plasma levels of vWF : Ag and R Cof was also revealed in diabetics in the present study. Cleavage of vWF by proteases in vivo may result in a high level of vWF : Ag, but not R Cof. High production of this protein may lead to high levels of both vWF : Ag and R Cof. Our study suggested that complete vWF might be produced at high levels in diabetics with retinopathy, but not in those without. This indicated that thrombus formation might occur most easily in diabetics with retinopathy.

Finally, we could not find any clear differences in platelet adhesiveness between the groups, although two cases of diabetics with myocardial infarction had high platelet adhesiveness. The method used in this study did not appear to reflect true platelet adhesiveness in diabetics with microangiopathy.

**ACKNOWLEDGMENTS**

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**REFERENCES**


### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic controls</th>
<th>Diabetics without retinopathy</th>
<th>Diabetics with retinopathy</th>
<th>Control v diabetic without retinopathy</th>
<th>Control v diabetic with retinopathy</th>
<th>Diabetics with retinopathy v those without</th>
</tr>
</thead>
<tbody>
<tr>
<td>F VIII: C %</td>
<td>104 ± 23</td>
<td>154 ± 44</td>
<td>135 ± 45</td>
<td>+</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>vWF: Ag %</td>
<td>105 ± 24</td>
<td>159 ± 58</td>
<td>190 ± 81</td>
<td>+</td>
<td>#</td>
<td>**</td>
</tr>
<tr>
<td>R Cof %</td>
<td>106 ± 28</td>
<td>116 ± 24</td>
<td>166 ± 63</td>
<td>**</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Platelet adhesiveness %</td>
<td>32 ± 12</td>
<td>32 ± 13</td>
<td>31 ± 9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values given as mean ± SD
NS = not significant, *P < 0.05, **P < 0.02, + P < 0.01, ²P < 0.001