The Tissue Thromboplastin Inhibition Test in Diabetics without Cerebro-Cardiovascular Diseases

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In 45 patients with diabetes mellitus (DM) without cerebro-cardiovascular diseases (CCVD) the modified method of the tissue thromboplastin inhibition test (TTIT) was studied. TTIT is the method of detection of the lupus anticoagulant (LA). LA, first recognized in patients with systemic lupus erythematosus, is presented by a prolonged activated partial thromboplastin time (APTT), a slightly to moderately prolonged prothrombin time (PT), and high incidence of biological false-positive seroreactions for syphilis (BFP). In patients with LA, thrombotic events have been reported. Six of the 45 diabetic patients were TTIT-positive (13.3%). All control subjects were TTIT-negative. In the TTIT-positive diabetics APTT and PT were normal. BFP also were not observed. The difference between LA and these results in TTIT-positive diabetics remains unclear. Clinical profiles except for duration of DM between the TTIT-negative and TTIT-positive diabetics did not differ. Follow-up studies may resolve an association between the results of TTIT and DM.

Key words: Lupus anticoagulant, Activated partial thromboplastin time, Prothrombin time, Diabetes mellitus

The lupus anticoagulant (LA) was first reported in 1952 (1). It was first recognized in patients with systemic lupus erythematosus (SLE), but has been subsequently observed in other clinical conditions (2, 3). LA is a spontaneously acquired inhibitor of blood coagulation that interferes with the activation of prothrombin via the prothrombin activator complex (4). In patients with LA, activated partial thromboplastin time (APTT) is prolonged and prothrombin time (PT) is slightly to moderately prolonged (4). Occasionally, biological false-positive seroreactions for syphilis (BFP) are observed in patients with LA (5). Patients with LA usually remains free of bleeding manifestations, despite the coagulation abnormalities. Paradoxically, thrombotic events have been reported (6, 7).

The occurrence of cerebro-cardiovascular diseases (CCVD) in patients with diabetes mellitus (DM) is well documented (8, 9). Blood clotting disorders that may be responsible for diabetes’ contribution to the risk of CCVD have been reported in patients with DM (10, 11). Among patients treated for DM in the Okura National Hospital, a case of a middle-aged man who presented with widespread thrombotic diseases in spite of good control of DM was examined. In that patient LA was observed and other blood clotting disorders were not found. From the results of that patient, in patients with DM it was suggested that LA might be related to blood clotting disorders. The tissue thromboplastin inhibition test (TTIT) is one of the methods of detection of LA (2). An association between DM and TTIT has not yet been reported. Therefore, the modified method of TTIT was studied in 45 diabetics without CCVD. The results of the modified method of TTIT and clinical profiles in DM are discussed.

SUBJECTS AND METHODS

Subjects: Forty-five patients with DM, 20 males and 25 females, aged between 26 and 76 yr old, were
studied. Eighteen of these patients were on insulin, 11 on sulfonylurea, and 16 on diet alone therapy [6 insulin-dependent DM (IDDM), 39 non-insulin-dependent DM (NIDDM)].

Control subjects (9 males, 12 females) were 21 outpatients. Of the 21 patients, eleven had hypertension, three hyperthyroidism, two hyperlipidemia, and one each of gastric ulcer, bronchial asthma, iron deficiency anemia, subacute thyroiditis, and bronchiecstasis. None of the control subjects had DM. None of the patients with DM nor the control subjects had clinical evidence of CCVD.

Methods: Blood was drawn after an overnight fast. APTT and PT were measured using Amelung KC-10 (Heinrich Amelung GmbH, Lemgo, Germany). Serologic tests for syphilis were performed. Plasma glucose was measured by glucose oxidase methods. Total cholesterol and triglyceride were measured by enzymatic methods. High-density lipoprotein cholesterol was measured with dextran sulfate-Mg²⁺ precipitation procedures. HbA₁c was measured by HPLC methods. Body mass index was calculated as the ratio of body wt (kg) to ht (m)². The modified method of TTIT was measured according to the following method:

1) Plasma was obtained from nine parts of blood, mixed with one part of 3.8% sodium citrate and spun at 3,000 g for 15 min; 0.1 ml of this plasma was added to 0.2 ml of Simplastin (Organon Teknika Co., Durham, North Carolina, USA) and the formation of a clot was measured using Coag-A-Mate-X2 (Organon Teknika Co.). This time denominated PT1.

2) Simplastin was diluted 1:50 with calcium chloride solution (0.025 M). Simplastin, (0.2 ml of the 1:50 dilution) was added to 0.1ml of patient plasma, and the clotting time was measured using Coag-A-Mate-X2. This time denominated PT2.

3) The ratio of the clotting time (PT2/PT1) was calculated.

Normal values: Less than 3.0 was considered a normal ratio (TTIT-negative); a ratio of 3.0 or greater was abnormal (TTIT-positive) (personal communication from Special Reference Laboratories Co., Tokyo, Japan).

The results were expressed as the mean ± standard deviation and statistical analysis was performed using the Student’s t test or χ² test. Only values with p<0.05 were regarded as significant.

RESULTS

1) Age, body mass index and duration of DM

The mean age was 57.6 ± 13.9 yr for the DM group and 56.4 ± 12.6 yr for the control group. The mean body mass index was 21.7 ± 2.6 for the DM group and 23.8 ± 3.2 for the control group. The mean duration of DM was 8.9 ± 6.0 yr for the DM group.

2) Incidence of TTIT-positive patients with DM

None of the control subjects were TTIT-positive. In 45 patients with DM, 6 (13.3%) patients were TTIT positive. One of the 6 patients was IDDM (16.7%). Of the 39 TTIT-negative patients with DM, 5 patients were IDDM (12.8%).

3) APTT and PT in control subjects and in patients with DM

All control subjects, TTIT-negative and TTIT-positive patients with DM did not have a prolonged APTT or PT (Table 1).

4) Clinical profiles of TTIT-negative and TTIT-positive patients with DM (Table 2)

Age and body mass index between the TTIT-negative and TTIT-positive patients with DM were not significantly different. Duration of DM in TTIT-negative patients with DM was significantly longer than that in TTIT-positive patients with DM (p <0.02).

Fasting plasma glucose, HbA₁c, total cholesterol level, triglyceride level and high-density lipoprotein cholesterol level were not significantly different

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>APTT (s)</th>
<th>PT (s)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21</td>
<td>30 ± 3*</td>
<td>10.4 ± 0.2</td>
<td>109 ± 12</td>
</tr>
<tr>
<td>TTIT</td>
<td>(-)</td>
<td>39</td>
<td>29 ± 4</td>
<td>10.5 ± 0.6</td>
</tr>
<tr>
<td>(+)</td>
<td>6</td>
<td>29 ± 1</td>
<td>10.5 ± 0.4</td>
<td>113 ± 16</td>
</tr>
</tbody>
</table>

APTT, activated partial thromboplastin time; PT, prothrombin time; TTIT, tissue thromboplastin inhibition test
*Values are mean ± SD
Table 2. Clinical profile of TTIT-negative and TTIT-positive patients with diabetes mellitus.

<table>
<thead>
<tr>
<th>TTIT</th>
<th>(-)</th>
<th>(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>39</td>
<td>6</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>57.9 ± 13.2*</td>
<td>56.2 ± 19.0</td>
</tr>
<tr>
<td>BMI</td>
<td>21.6 ± 2.1</td>
<td>22.8 ± 4.9</td>
</tr>
<tr>
<td>Duration of DM (yr)</td>
<td>9.6 ± 6.2**</td>
<td>4.7 ± 2.8**</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>145 ± 53</td>
<td>151 ± 93</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.6 ± 1.4</td>
<td>7.6 ± 2.4</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>208 ± 28</td>
<td>206 ± 46</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>109 ± 52</td>
<td>107 ± 49</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>53 ± 17</td>
<td>50 ± 12</td>
</tr>
<tr>
<td>BFP</td>
<td>0/39</td>
<td>0/6</td>
</tr>
</tbody>
</table>

TTIT, tissue thromboplastin inhibition test; BMI, body mass index; DM, diabetes mellitus; FPG, fasting plasma glucose; TC, total cholesterol level; TG, triglyceride level; HDL, high-density lipoprotein cholesterol level; BFP, biological false-positive seroreactions for syphilis

**p<0.02, *Values are mean ± SD

between the TTIT-negative and TTIT-positive patients with DM. BFP were not found in either TTIT-negative or in TTIT-positive patients with DM.

**DISCUSSION**

It has been reported that incidence of CCVD in diabetics is higher than in non-diabetics (8, 9) and many factors in DM contribute to that result. Blood disorders in diabetics have been recognized as one of the many factors. Fibrinolytic activity has been found to be diminished in patients with DM (10). Increased platelet aggregation and adhesiveness have been noted in patients with DM. Raised levels of fibrinogen, factor VII, and factor VIII have been reported (10, 11).

LA, first described by Conley and Hartmann in 1952 (1), is a spontaneously acquired inhibitor of blood coagulation that interferes with the activation of prothrombin via the prothrombin activator complex (factor Xa, V, calcium, and phospholipid) (4). The presence of LA is recognized by the prolongation of APTT observed in patients and a prolonged PT observed in some patients (4). Patients with LA also have a strikingly high incidence of BFP (5). The paradoxical association of an apparent coagulation defect with a thrombotic disorder, rather than a bleeding diathesis, is a well recognized feature of patients with LA (6, 7).

Among patients treated for DM in the Okura National Hospital, a 56-year-old man who had suffered from myocardial infarction, transient cerebral ischemic attack, and frequent thromboses retinae centralis for 4 yr was examined. He was not obese and was a non-smoker. He had mild hypertension. In the present patient diabetic control was fairly good. Hyperlipidemia was not present. Different blood clotting examinations were performed. In the present patient the presence of LA was demonstrated and other blood clotting disorders were not found. From the results of the present patient, it was considered that LA might be related to blood clotting disorders in patients with DM. The present patient was excluded from the present study because he had clinical evidence of CCVD.

In the present study using the modified method of TTIT of detection of LA, 6 of 45 diabetics with no clinical evidence of CCVD were revealed to be TTIT positive (13.3%). This incidence is similar to the incidence of SLE with LA reported by many authors (4, 12). All control subjects were TTIT negative. Control of DM between TTIT-negative and TTIT-positive patients was not significantly different. Incidence of IDDM in TTIT-negative patients was as high as in TTIT-positive patients. The state of DM control or type of DM, that is, IDDM or NIDDM, might not influence the result of whether diabetics are TTIT negative or TTIT positive. Duration of DM in TTIT-negative patients was longer than that in TTIT-positive patients. And it is possible that the duration of DM does not affect the result of the modified method of TTIT in DM. These results may suggest that some of the patients with DM possess TTIT-positive diathesis.

In the TTIT-positive patients with DM, APTT and PT were normal, moreover BFP were not observed which is the distinction from SLE with LA. It has been reported that increasing the concentration of phospholipid, especially phosphatidyl serine, in an APTT test system markedly reduces the sensitivity to LA (13). TTIT-positive patients with DM could best be detected by a screening method using an APTT test with a reagent of low phosphatidyl serine content. An approach using different types of the reagent might facilitate detection of TTIT-positive patients with DM.
Lupus Anticoagulant and Diabetes Mellitus

It has been reported that LA is an antibody to negatively charged phospholipids, that is, phosphatidylerine, phosphatidylinositol, and phosphatidic acid (14). In particular, LA has been regarded as an anticardiolipin antibody (15). Cardiolipin is part of the antigen used in serologic tests for syphilis. Therefore, BFP may be observed in patients with LA. In TTIT-positive patients with DM the presence of antibodies to phospholipids different from cardiolipin might be suggested because TTIT-positive diabetics with BFP were not found. Follow-up studies have confirmed the significance of TTIT in DM.

REFERENCES