DIAGNOSTIC VALUE OF D-DIMER IN PREDICTING MYOCARDIAL INFARCTION AMONG DIABETIC MAKKAH PILGRIMS

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ABSTRACT

Introduction Diabetes mellitus is a leading cause of vascular morbidity. It is often associated with cardiovascular risk factors such as hypercholesterolemia, hypertension, obesity, and increased markers of coagulation and inflammation. It has been recently confirmed that diabetes mellitus is associated with disturbances in haemostasis that result in an increased incidence of thrombotic complications and cardiovascular disease. Thus, the main aim of the present work was to study the state of D-dimer and activated partial thromboplastin time (APTT) and their association with cases of myocardial infarction in pilgrims with type 2 diabetes mellitus. Methods: Patients and Control The study group comprised 101 diabetic pilgrims with type 2 diabetes mellitus. They were 60 men and 41 women. Of those patients 40 cases were diagnosed as having myocardial infarction (MI). The control group consisted of 42 healthy volunteers of matching age and sex. Laboratory Methods Fasting and 2-hours postprandial venous samples from the whole groups were measured. To monitor coagulation these samples were assayed for D-dimer. The activated partial thromboplastin time (APTT) and prothrombin time (PT) were also measured. Results Using the \( \chi^2 \) test, significant high plasma D-dimer levels were observed in patients with myocardial infarction, compared with patients without myocardial infarction and the control group (445 ± 352 \( \mu \)g/L, 340 ± 249 \( \mu \)g/L, and 156 ± 107 \( \mu \)g/L, respectively). However, plasma activated partial thromboplastin time (APTT) levels of patients with myocardial infarction were slightly lower than those of patients without myocardial infarction and of the control group, but the difference was not statistically significant. Myocardial infarction and INR levels were identified as major determinants of plasma D-dimer levels. A plasma D-dimer level of >394 \( \mu \)g/L predicted the presence of myocardial infarction with 57.5% sensitivity and 98.4% specificity. Conclusions Current data suggest that increased plasma D-dimer levels can be clinically helpful in predicting the presence of myocardial infarction among diabetic pilgrims.

Keywords: Fibrin Formation and Degradation - Thrombosis - D-dimer - Vascular Complications – Diabetes Mellitus - Myocardial Infarction - Activated Partial Thromboplastin Time (APTT) - Prothrombin Time (PT)

INTRODUCTION

Diabetes mellitus is a leading cause of vascular morbidity. It is often associated with cardiovascular risk factors such as hypercholesterolemia, hypertension, obesity, and increased markers of coagulation\(^1\) and inflammation\(^2\). It has been recently confirmed that diabetes mellitus is associated with disturbances in haemostasis that result in an increased incidence of thrombotic complications and cardiovascular disease\(^3\). It is also reported that 80% of patients with diabetes mellitus die a thrombotic death. Seventy five percent of these deaths are due to cardiovascular complications\(^4\).
The acute coronary syndrome, including myocardial infarction, is characterized by a ruptured, vulnerable plaque and subsequent intraluminal thrombus formation. Besides local hypercoagulability, a profound systemic imbalance of the hemostatic system with a shift to increased procoagulation have been shown. Various hemostatic proteins like fibrinogen, plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) have been found to be associated with coronary events in apparently healthy subjects and in patients with manifest atherosclerosis.

On the other hand, fibrin D-dimer, the degradation product of cross-linked fibrin, has gained increasing interest for several reasons. First, it can be considered as a global marker of the turnover of cross-linked fibrin and of activation of the hemostatic system. Second, in contrast to several other markers of hemostasis, D-dimer assays are more stable and more practical to measure and therefore may be more suitable for routine clinical use.

It is well recognized that heavy physical exertion sometimes immediately precedes, and indeed appears to trigger, the onset of acute myocardial infarction. Descriptive studies have established that in approximately 5% of patients with myocardial infarction, such exertion immediately precedes the onset of symptoms. At the same time, it is well known that performing Hajj rituals involves a lot of physical effort and long periods of walking which represent a high risk factor for acute myocardial infarction.

If increased plasma D-dimer levels could be clinically helpful in predicting the presence of myocardial infarction, it would be possible to predict cases of myocardial infarction among diabetic pilgrims and thus decrease the high costs of hospitalization and treatment of overt cases by taking preventive medical measures as early as possible. Thus, the main aim of the present work was to study the state of D-dimer and activated partial thromboplastin time (APTT) and prothrombin time (PT). Blood sampling for patients group was carried out after admission to hospital and before the initiation of drug therapy. For determination of D-dimer levels, the D-Dimer PLUS kit (Dade Behring Marburg GmbH) was used. D-Dimer PLUS is a latex-enhanced, immunoturbidimetric test for the quantitative determination of cross-linked fibrin decomposition products (D-dimers) in human plasma. In this method polystyrene particles covalently linked to a monoclonal antibody (DD5) to the cross-linkage region of cross-linked fibrin are agglutinated when mixed with samples containing D-Dimer. The agglutination reaction is then detected turbidimetrically via the increase in turbidity.

METHODS

Patients and Control
The study group comprised 101 diabetic pilgrims with type 2 diabetes mellitus. They were 60 men and 41 women. They were admitted to the Department of Internal Medicine and Cardiology at King Abdul Aziz Hospital in Holy Makkah during the Hajj period from 22nd December 2006 to 19th January 2007. Of those patients 40 cases were diagnosed as having myocardial infarction (MI). The diagnosis of MI was confirmed according to the World Health Organization criteria for MI, which includes symptoms such as elevations of cardiac enzyme levels and diagnostic changes on the ECG.

The control group consisted of 42 healthy volunteers of matching age and sex from workers in King Abdul Aziz Hospital in Holy Makkah. They were 26 men and 16 women. All were in sinus rhythm with no drug treatment. The control subjects were exposed to the same investigations as the patient group. Demographic characteristics were assessed for all participants by a standardized questionnaire including medical history and history of smoking.

Informed consent
All patients admitted to the Department of Internal Medicine and Cardiology at King Abdul Aziz Hospital in Holy Makkah during the Hajj period from 22nd December 2006 to 19th January 2007 were fully informed of the purposes of the study, and all the participating patients gave their written consent. Moreover, all patients were informed of the results of their tests. Healthy controls, who participated in this study, also gave informed, written consent.

Laboratory Methods
Fasting and 2-hours postprandial venous blood samples were drawn from all participants. Blood collection kits including potassium citrate Vacutainer tubes were used for blood drawing. Blood was centrifuged immediately at 3000g for 10 minutes and the plasma was divided into aliquots and stored at -80°C until analysis. To monitor coagulation these samples were assayed for D-dimer, the activated partial thromboplastin time (APTT), and prothrombin time (PT). Blood sampling for patients group was carried out after admission to hospital and before the initiation of drug therapy. For determination of D-dimer levels, the D-Dimer PLUS kit (Dade Behring Marburg GmbH) was used. D-Dimer PLUS is a latex-enhanced, immunoturbidimetric test for the quantitative determination of cross-linked fibrin degradation products (D-dimers) in human plasma. In this method polystyrene particles covalently linked to a monoclonal antibody (DD5) to the cross-linkage region of cross-linked fibrin are agglutinated when mixed with samples containing D-Dimer. The agglutination reaction is then detected turbidimetrically via the increase in turbidity.
For determination of activated partial thromboplastin time (APTT), Pathromtin SL kit (Dade Behring Marburg GmbH) was used. For determination of the prothrombin time (PT) and international normalized ratio (INR), Thromborel S kit (Dade Behring Marburg GmbH) was used.

Other tests performed on the samples included total and HDL cholesterol, triglycerides, fasting plasma glucose (FPG), post prandial glucose (PPG), and glycated heamoglobin (HBA1C). These tests were determined by routine enzymatic methods.

Statistical Analysis
All data are expressed as mean ± standard deviation. Differences between the different groups for discrete variables were analyzed by the χ² test. For continuous variables, groups were compared with the unpaired Student’s t-test or Mann-Whitney U test, where appropriate. Correlation between different variables was analyzed with Pearson linear regression analysis or Spearman rank correlation. Analysis of variance was used for making comparisons between multiple groups. A multiple regression analysis was performed for identifying independent factors for myocardial infarction occurrence in patients with type 2 diabetes mellitus. Multiple regression analyses were performed for identifying factors that determine the D-dimer and APTT levels. Receiver operating characteristics (ROC) curves were generated using Analyse-it statistical software, which also provides a calculated area under the ROC curve. The larger the area under the curve (AUC), the more accurate the test. A P value of <0.05 was accepted as significant in all analyses. All statistical analyses were performed by using Analyse-it statistical program (Analyse-it Software).

RESULTS

Study Group:
Table 1 shows the general laboratory characteristics of the study group. There are small differences in the lipid profile between groups. HDL cholesterol shows higher levels in controls compared with disease groups.

Prothrombin time (PT) and INR levels:
Prothrombin time (PT) and INR levels of diabetic patients with and without myocardial infarction are presented in Table 2. The PT levels were significantly lower in patients with myocardial infarction, compared with the patients without myocardial infarction and with control group (P = 0.004). The INR levels were significantly lower in patients with myocardial infarction, compared with the patients without myocardial infarction and with control group (P = 0.003).

Both univariate regression analysis and multivariate regression analysis were used to assess the effects upon myocardial infarction occurrence of age, sex, PT, and INR. Multivariate regression analysis identified INR levels as a significant independent factor for myocardial infarction occurrence (r = -0.28. P = 0.05). The other factors were not significantly related to myocardial infarction occurrence, either in univariate or multivariate analyses. The combined factors regression analysis, age/sex, age/PT, age/INR, sex/PT, sex/INR, INR/PT, were not significantly related to each other. The distribution of age values in the different studied groups in this work was normal.

D-Dimer and APTT Levels:
The mean ± SD and median D-dimer and APTT levels of the control group and patients with and without myocardial infarction are presented in Table 3. Patients with myocardial infarction seemed to have relatively lower APTT levels than did the control group and the patients without myocardial infarction. However, analysis of variance did not reveal a significant difference between the APTT levels of the 3 studied groups (P = 0.8). There was a considerable amount of overlap between the APTT levels of the 3 groups.

D-dimer levels of patients with myocardial infarction were significantly higher than levels in the patients without myocardial infarction and the control group (P = 0.002 and P = 0.001. respectively). Patients without myocardial infarction also had significantly higher D-dimer levels, when compared with the control group (P = 0.004). A cutoff D-dimer value of 394 µg/L was determined for discriminating between the patients with and without myocardial infarction. In patients with myocardial infarction, 23 out of 40 (57.5%) patients had D-dimer levels of >394 µg/L. On the other hand, of the diabetic patients with no myocardial infarction, only 1 out of 61 (0.02%) patients had D-dimer levels of >394 µg/L. A plasma D-dimer level of >394 µg/L predicted the presence of myocardial infarction with 57.5% sensitivity and 98.4% specificity. (Figure 1) (Table 4).

The whole study group (control group and all patients with and without myocardial infarction) was also evaluated with multiple regression analysis to identify major independent factors affecting APTT and D-dimer levels. None of the factors, including the presence of myocardial infarction, was identified...
The presence of myocardial infarction was identified as the most significant independent factor affecting the D-dimer levels in multiple regression analysis ($P = 0.001$, $r = 0.30$). Having myocardial infarction and low INR levels were also identified as significant independent determinants of D-dimer levels ($P = 0.005$, $r = 0.33$; and $P = 0.03$, $r = -0.22$ respectively). Age and sex were not significantly related to D-dimer levels ($P = 0.093$, $P = 0.66$ respectively).

**DISCUSSION**

The laboratory measurement of D-dimer detects cross-linked fibrin degradation products and has been demonstrated to be a useful marker of activation of endogenous coagulation. The plasma D-dimer level also reflects fibrin turnover, as well as the efficacy of fibrinolysis, without being affected by fibrinogen or non-cross-linked fibrin. High plasma D-dimer levels have been detected in patients with acute ischemic stroke and peripheral vascular disease and have proved to be useful in the diagnosis of deep vein thrombosis.

This current study demonstrates increased plasma D-dimer levels in pilgrims with type 2 diabetes mellitus, when compared with control subjects. This probably is a consequence of increased fibrin turnover and continuous fibrinolysis in association with the diabetic state. The increase in plasma D-dimer levels was observed to be most prominent in patients with myocardial infarction, who probably experience markedly increased fibrin turnover, fibrinolysis, and production of fibrin degradation products. This is expected to be clinically useful and may have a predictive value in identifying those patients with myocardial infarction. High levels of plasma D-dimer ($>394 \mu g/L$) predicted the presence of myocardial infarction with a high specificity and moderate sensitivity. The reason for the relatively lower sensitivity may be that D-dimer levels were influenced by the presence of diabetes mellitus, as well as by the level of coagulation activity. It has been shown that INR levels in patients with type 2 diabetes mellitus are an important predictor of D-dimer levels.

On the other hand, age as a predictor factor was not significantly related to myocardial infarction occurrence. In this study age values were normally distributed. Although this was not the case in the study of Simioni et al. who found that age values were not distributed normally and expressed age values as median and range, the results of our study are in line with the work of Burke et al. who found age values normally distributed and expressed it as mean and standard deviation. This is explained by the fact that Makkah pilgrimage, although it is a very tough and strenuous physical activity, is not only attended by young people, but is also attended by other age groups and in large numbers.

Although the plasma APTT activity of patients with myocardial infarction was lower than it was in both the control subjects and the patients without myocardial infarction, the presence of myocardial infarction was not identified as an independent determinant of plasma APTT levels. In addition, there was a substantial overlap between the APTT levels of the 3 groups, which makes it difficult to use APTT levels as a differentiating parameter. Therefore, these results suggest that APTT levels cannot be used as a clinical marker for identifying patients with myocardial infarction.

It is recommended that future studies with larger groups of patients should confirm these data and may help to identify better cutoff values for plasma D-dimer levels, in order to discern patients with myocardial infarction.

**CONCLUSION**

In conclusion, the current data suggest that plasma D-dimer levels may be used as an early marker of myocardial infarction with a moderate sensitivity and relatively high specificity in diabetic pilgrims. Increased plasma D-dimer levels are not by themselves diagnostic of myocardial infarction but may alert the clinician to refer the patient for more detailed examination. Normal plasma D-dimer levels highly predict the absence of myocardial infarction. Current data also suggest that plasma APTT levels are not of diagnostic value in predicting myocardial infarction.

**ACKNOWLEDGEMENTS**

The study was funded by The Institute of the Custodian of the Two Holy Mosques for Hajj Research, Holy Makkah, Saudi Arabia.
REFERENCES


**Table 1.** The General and Laboratory Characteristics of the Study Groups

<table>
<thead>
<tr>
<th>Diabetics with MI (n=40)</th>
<th>Diabetics only (n=61)</th>
<th>Controls (n=42)</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (years)*</td>
<td>60 ± 10</td>
<td>59 ± 11</td>
<td>53 ± 6</td>
</tr>
<tr>
<td>Male, %</td>
<td>66</td>
<td>54</td>
<td>62</td>
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<tr>
<td>Duration of smoking, (years) *</td>
<td>9.8 ± 11.8</td>
<td>8.9 ± 12.9</td>
<td>6.3 ± 8.3</td>
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<tr>
<td>Duration of diabetes, (years) *</td>
<td>13.2 ± 7.4</td>
<td>10.6 ± 7.6</td>
<td>11.9 ± 6.4</td>
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<tr>
<td>Total cholesterol, mg/dL*</td>
<td>225 ± 38</td>
<td>202 ± 40</td>
<td>161 ± 41</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL*</td>
<td>37 ± 5</td>
<td>42 ± 7</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>176 ± 81</td>
<td>127 ± 97</td>
<td>113 ± 46</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dL*</td>
<td>192 ± 46</td>
<td>201 ± 54</td>
<td>89 ± 21</td>
</tr>
<tr>
<td>Postprandial blood glucose, mg/dL*</td>
<td>301 ± 65</td>
<td>293 ± 72</td>
<td>118 ± 29</td>
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<tr>
<td>Glycated hemoglobin (%)</td>
<td>9.6 ± 1.8</td>
<td>9.1 ± 1.5</td>
<td>4.6 ± 1.2</td>
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</table>

*Values are mean ± SD.

**Table 2.** Prothrombin Times (PT), and International Normalized Ratios (INR) in the Study Groups

<table>
<thead>
<tr>
<th>Diabetics with MI (n=40)</th>
<th>Diabetics only (n=61)</th>
<th>Controls (n=42)</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (seconds)</td>
<td>10.2 ± 1.2</td>
<td>12.6 ± 4</td>
<td>13.2 ± 1.4</td>
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<tr>
<td>INR</td>
<td>1.1 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>2 ± 0.2</td>
</tr>
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Table 3. D-Dimer and APTT Levels of the Control Group and Patients with and without Myocardial Infarction

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Diabetics with MI(n=40)</th>
<th>Diabetics only(n=61)</th>
<th>Controls (n=42)</th>
<th>Median (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Dimer(µg/L)</td>
<td>445 ± 352 µg/L</td>
<td>340 ± 249 µg/L</td>
<td>156 ± 107 µg/L</td>
<td>340</td>
</tr>
<tr>
<td>APTT (seconds)</td>
<td>34.2 ± 14.4</td>
<td>35.6 ± 14.2</td>
<td>36.5 ± 13.1</td>
<td>36.5</td>
</tr>
</tbody>
</table>

(M) Median

Table 4. Characters of ROC curve for D-Dimer levels in patients with and without Myocardial Infarction.

<table>
<thead>
<tr>
<th>Infarcted cases having higher values than the No discrimination line</th>
<th>95% CI of Area Under the Curve</th>
<th>P</th>
<th>SE</th>
<th>Area Under the Curve</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>0.810 to 0.948</td>
<td>&lt;0.0001</td>
<td>0.0353</td>
<td>0.879</td>
</tr>
</tbody>
</table>

CI: Confidence Interval
P: Probability
SE: Standard Error
**Fig. 1** ROC curve for D-Dimer levels in patients with and without myocardial infarction