Originals

Background. Some studies have shown that diabetic patients had hypercoagulability.

Objective. To compare the haemostasis pattern between overweight diabetic patients and control subjects.

Design. Twenty three overweight patients of our Diabetes Unit chosen at random (16 males/7 females) with type 2 diabetes mellitus were enrolled. The clinical characteristics of the patients were: age 81.3 ± 13.2 years e body mass index (BMI) 27.6 ± 3.1 kg/m² and duration of diabetes 8.4 ± 6.7 years. A group of twenty three voluntary controls chosen at random (15 males/8 females) without diabetes were studied. The clinical characteristics of this group were: age 62 ± 13 years and BMI 27.8 ± 3.1 kg/m². All patients (diabetics and controls) underwent the following examinations: plasma TAT, activated TPA, factor, D dimer (DD), plasmin-antiplasmin (PAP), and prothrombin activation fragment F1+2 (F12).

Results. Overweight diabetic patients showed an increment in procoagulant parameters (F12 1.38 ± 0.6 vs 1.21 ± 0.25 mmol/l; p < 0.05; VWFα 223 ± 67 vs 181.7 ± 28 (MU/ml); p < 0.05), and a decrease in fibrinolytic parameters (TAT 27.4 ± 14.3 ng/ml; p < 0.05), and antiocoagulant parameters (Thrombomodulin 27.4 ± 11.7 vs 45.1 ± 21.7 ng/ml; p < 0.05), with a increase in D dimer (DD 22.3 ± 28.9 vs 9.7 ± 5.4 µg/l; p < 0.05) and (F1+2 1.21 ± 0.6 vs 7.4 ± 3.1 ng/ml; p < 0.05). In diabetic patients, there was no difference according the absence or presence of microangiopathy or macroangiopathy. In a correlation analysis between BMI and Hba1c in controls, no correlations were found (r = 0.32; p < 0.05), respectively). A correlation analysis was also performed between BMI and haemostasis parameters and no correlations were found between haemostasis parameters with BMI and HbA1c in controls.

Conclusion. Hypercoagulable state is present in diabetic patients which with present knowledge, can be viewed as a risk factor for chronic complications.

Key words: Type 2 diabetes mellitus. Haemostasis. Overweight.

High risk haemostasis patterns in overweight patients with type 2 diabetes mellitus

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PATRÓN DE HEMOSTASIA DE ALTO RIESGO EN PACIENTES CON SOBREPESO Y DIABETES TIPO 2

Introducción. Algunos estudios han demostrado un estado de hipercoagulabilidad en los pacientes diabéticos.

Objetivo. Comparar el patrón de hemostasia entre pacientes diabéticos con sobrepeso y controles.

Diseño. Un total de 23 pacientes con diabetes mellitus tipo 2 y sobrepeso atendidos en nuestra unidad fueron incluidos en el estudio (16 varones/7 mujeres). Las características clínicas de los pacientes fueron: 61,3 ± 12,3 años, índice de masa corporal (IMC) 27,2 ± 3,9 kg/m² y duración de la diabetes 8,4 ± 6,7 años. Un grupo de 23 voluntarios sanos fue elegido al azar entre donantes de sangre sin diabetes mellitus (15 varones/8 mujeres). Las características clínicas de estos pacientes fueron: 62 ± 13 años e IMC 27,6 ± 3,1 kg/m². A todos los sujetos, casos y controles, se les realizaron las siguientes pruebas: inhibidor del activador del plasminógeno: tipo 1 (PAI-1), complejo trombina/antitrombina III (TAT), activador titular del plasminógeno (t-PA), antígeno Von Willebrand (vW), proteína C (PC), proteína S (PS), trombomodulina (TH), factor VII activado, dimero D (DD), plasmina-antiplasmina (PAP) y fragmento activado protrombina F1+2 (F12). Estos parámetros fueron comparados en ambos grupos, y dentro de los diabéticos en los grupos con y sin macroangiopatía. En ambos grupos, se realizó un análisis de correlación entre los parámetros clínicos y los hemostásicos.

Resultados. Los pacientes diabéticos con sobrepeso evidenciaron un incremento en los factores procoagulantes (F12 1.38 ± 0.4 frente a 1.21 ± 0.25 mmol/l; p < 0.05; VWFα 223 ± 67 frente a 181.7 ± 28 (MU/ml); p < 0.05), y un descenso en los parámetros fibrinolíticos (TAT 27.4 ± 14.3 frente a 94.6 ± 45.1 ± 21.7 ng/ml; p < 0.05), así como en los parámetros antiocoagulantes (Thrombomodulin 27.4 ± 11.7 frente a 45.1 ± 21.7 ng/ml; p < 0.05), con un incremento en los niveles de dimero D (DD 22.3 ± 28.9 frente a 9.7 ± 5.4 µg/l; p < 0.05) y (F1+2 1.21 ± 0.6 frente a 7.4 ± 3.1 ng/ml; p < 0.05). En los pacientes diabéticos no hubo diferencia en función de la ausencia o presencia de micro o macroangiopatía. La proteína C y tPA mostraron una correlación negativa (r = 0.34; p < 0.01; y r = 0.32; p < 0.05, respectivamente) con la hemoglobina glucosilada (HbA1c). FvW se correlacionó de una manera positiva con el IMC (r = 0.32; p < 0.05). No se encontraron correlaciones entre los parámetros de hemostasia, con el IMC y HbA1c, en los sujetos control.

Conclusión. En los pacientes con diabetes tipo 2, hay un estado de hipercoagulabilidad que puede influir en las complicaciones crónicas de esta población.

INTRODUCTION

Diabetes mellitus is an independent risk factor for the development of atherosclerosis. The possible mechanisms are unclear. It is postulated that chronic inflammation may contribute to increase the risk of coronary heart disease in different ways: increasing serum concentrations of acute phase reactants (such as fibrinogen or C reactive protein) or modifying the serum lipid pattern (such as decrease of HDL cholesterol and increase of triglycerides). Another factor involved in the atherogenesis of diabetic patients is the promotion of the oxidation of LDL-cholesterol since oxidation enhances the atherogenic capacity of these molecules. More recently, some authors have shown that diabetic patients had a hypercoagulable state.

The aim of our study was to compare the haemostasis pattern between overweight patients with diabetes mellitus type 2 and a control group.

MATERIALS AND METHODS

Population

Twenty-three overweight patients of our Diabetes Unit (16 males/7 females) and twenty three controls (blood donors) (15 males/8 females) without diabetes were studied. Both groups were chosen at random and their characteristics are shown in table 1. Patients and controls did not take either anti-hypertensive or hypolipidemic drugs. Diabetic patients took sulphonylureas as anti-hyperglycemic agents. The study was approved by the local ethical committee and each patient gave informed consent to participate in the study.

Design

All patients (diabetic and controls) underwent the following examinations: plasma/serum activator inhibitor type I (PAI-1), throm-binoantithrombin III complex (TAT), tissue plasminogen activator (t-PA), von Willebrand antigen (vW), protein C (PC), protein S (PS), thrombomodulin (TH), activated VII factor, D dimer (DD), plasminogen/antiplasmin (PAP) and prothrombin activation fragment F1+2 (F12). Blood samples for coagulation testing were collected into 3.8% trisodium citrate solution between 07:00 and 09:00 am, supine position (Mac PC Electrocardiograph, Marquette Electronics, Inc., Overlandpark, KS, USA). Factor VIIa (normal range 5-85 µIU/ml) was determined by coagulometry, (normal range 70-150% for both). Thrombo- modulin (normal range 14-55 ng/ml), and prothrombin activation fragment F1+2 (F12) were determined by enzyme immunoassay (Enzygnost TAT micro, Marburg, Germany). Factor VIIa (normal range 5-85 µIU/ml) was determined by coagulometry, (normal range 70-150% for both). Thrombomodulin (normal range 14-55 ng/ml), and prothrombin activation fragment F1+2 (F12) were determined by enzyme immunoassay (Enzygnost TAT micro, Marburg, Germany).


Haemostasis assessment

Plasminogen activator inhibitor type I (PAI-1) (normal range < 10 U/ml) and tissue plasminogen activator (t-PA) (normal range 1-12 ng/ml) were determined by enzyme immunoassay, (TintElize PAI UNica, Sweden). Thrombomodulin/antithrombin III complex (TAT) (normal range 1.0-4.1 ng/l) was determined by enzyme immunoassay with a commerical kit (Staclot, Asnieres-herlands). Factor VIIa (normal range 5-85 µIU/ml) was determined by coagulometry, (normal range 70-150% for both). Thrombomodulin (normal range 14-55 ng/ml), and prothrombin activation fragment F1+2 (F12) were determined by enzyme immunoassay (Enzygnost TAT micro, Marburg, Germany). Factor VIIa (normal range 5-85 µIU/ml) was determined by coagulometry, (normal range 70-150% for both). Thrombomodulin (normal range 14-55 ng/ml), and prothrombin activation fragment F1+2 (F12) were determined by enzyme immunoassay (Enzygnost TAT micro, Marburg, Germany).

Chronic diabetic complications assessment

All diabetic patients were checked in the Clinic for chronic complications. Ischaemic heart disease was assessed by anamnesis and in addition, a 12-lead resting electrocardiogram was recorded in supine position (Mac PC Electrocardiograph, Marquette Electro nics) and evaluated by a cardiologist. The presence of any of the following findings was considered suggestive of coronary heart di-

<table>
<thead>
<tr>
<th>TABLE 1. Population characteristics of overweight diabetic patients and controls</th>
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<tr>
<td><strong>Diabetic patients</strong></td>
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<tr>
<td><strong>Age (years)</strong></td>
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<tr>
<td><strong>Sex (male/female)</strong></td>
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<td><strong>BMI (kg/m²)</strong></td>
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<td><strong>Diabetes duration (years)</strong></td>
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<td><strong>HbA1c (%)</strong></td>
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<td><strong>Macroglobulinemia (%)</strong></td>
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<td><strong>Microangiopathy (%)</strong></td>
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**TABLE 2. Differences in hemostasis parameters in diabetic and control patients**

| **Diabetic patients** | **Control subjects** | **p** |
| --- |
| **PAI (U/ml)** | 25.3 ± 24.9 | 37.3 ± 51.7 | NS |
| **TAT (ng/ml)** | 3.52 ± 2.3 | 5.83 ± 4.5 | NS |
| **Prothrombin activation fragment F1+2 (F12) (nmol/L)** | 1.38 ± 0.4 | 2.12 ± 0.25 | < 0.05 |
| **Plasmin-antiplasmin (PAP) (ng/l)** | 262.9 ± 107.5 | 348.5 ± 143 < 0.05 |
| **t-PA (ng/ml)** | 12.6 ± 5.1 | 7.4 ± 3.1 | < 0.05 |
| **Factor VIIa (%)** | 106 ± 32.1 | 120.7 ± 23 | NS |
| **DD (µg/ml)** | 22.3 ± 26.8 | 9.7 ± 5.4 | < 0.05 |
| **Protein C (%)** | 106 ± 32.1 | 120.7 ± 23 | NS |
| **Protein S (%)** | 97.7 ± 24 | 98 ± 25 | NS |
| **Thrombomodulin (ng/ml)** | 27.4 ± 11.7 | 45.1 ± 21.7 | < 0.05 |

**Statistical analysis**

The results were expressed as mean ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student’s t test. Non-parametric variables were analyzed with the U-Mann-Whitney test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher’s test. Pearson and Spearman tests were used in correlation analysis. A p-value under 0.05 was considered statistically significant.

**RESULTS**

Twenty three overweight diabetic patients and 23 control overweight diabetic patients were enrolled in the study. The mean age and BMI were similar in both groups (table 1). The diabetes duration was 8.9 ± 2.4 years, macroglobulinemia was present in 48.8% and microangiopathy in 12.8% of diabetic patients. Table 2 shows differences between both groups with an increase in procoagulant parameters in diabetic patients, with
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... a significant increase in prothrombin activation fragment FI+2 and factor VIII(a). Values of PAI and TAT did not have statistical differences. Fibrinolytic parameters showed significant differences in TP and D dimer (increased) and PAP (decreased), without differences in FvW. A decrease in anti-coagulant parameters was observed in diabetic patients (thrombomodulin), without differences in protein S and C.

All haemostasis parameters were compared in diabetic patients in the group with c8.9% and without microangiopathy (51.1%), but no differences were found. In a correlation analysis between BMI and haemostasis parameters, only protein C and TP showed significant inverse correlations (r = -0.34; p < 0.01 and r = -0.32; p < 0.05, respectively). Another correlation analysis was performed between BMI and haemostasis parameters, only FvW was correlated with BMI (r = 0.32; p < 0.05). No correlations were found between haemostasis parameters with BMI and HbA1c.

No correlations were found among diabetes evolution, age or microalbuminuria levels with haemostasis parameters in diabetic patients.

DISCUSSION

Patients with type 2 diabetes mellitus have a variety of coagulation dysfunctions, which could contribute to microvascular and macrovascular complications. The hypercoagulable state has been demonstrated in a group of overweight diabetic patients under strict metabolic control who had an increase in TAT levels. In our study no significant differences in TAT levels were detected between overweight diabetic and control subjects, but FI+2 and activated factor VII were higher in diabetic patients, showing a hypercoagulable state. In diabetic patients, it has been shown that fibrinolytic parameters, such as PAI-1 and tPA antigen, were strongly related to insulin resistance, whereas the link with factor VII and other procoagulant parameters remained weak. These alterations might contribute to increased cardiovascular mortality in diabetes. For example, Motoshi et al showed significantly higher levels of TAT, fibrinogen and PAI-1 in 22 diabetic patients with coronary heart disease than 51 patients without diabetic microangiopathy.

Another haemostasis alteration in overweight diabetic patients is a decrease in the anticoagulant system. Patients with diabetes have activated protein C resistance, suggesting that final steps of the protein C/S inhibiting system could be abnormal. These abnormalities of anticoagulant system might constitute a potential trigger for haemostatic activation. Gabarra et al demonstrated alterations in overweight diabetic patients in the plasma levels of fibrinogen, FVIIa, fibrin monomer, protein C antigen, total protein S antigen, and thrombomodulin. Patients with microalbuminuria showed low plasma levels of activated protein C-protein C inhibitor complex and significant low values of the anticoagulant response to exogenous thrombomodulin, indicating a poor plasma reactivity to the anticoagulant effect of thrombomodulin. Our study showed a decrease in thrombomodulin, but no differences between diabetic patients with micro or macroangiopathy were found.

Previous studies have showed alterations in fibrin/fibrinogen system in overweight diabetic patients, such as a significant increase in D dimer levels. Increased levels of plasminogen activator-1 (PAI-1) might be involved in the pathogenesis of the vascular complications of diabetes mellitus. However, Mansfield et al showed low PAI-1 levels in subjects with retinopathy, without a clear explanation. The lack of relation between glycemic control and haemostasis parameters in our study, could be due to a intrinsic altered state in diabetic patients. This haemostasis alteration with other risk factors such as hyperglycaemia or hyperlipidaemia could start micro- and macro-angiopathy, and haemostasis could act in a second step, so that there was a lack of relationship between diabetic complications and haemostasis parameters. For example, Alhutus et al in diabetic patients achieving good control after 3 months of therapy, observed a significant reduction in protein S and cdf-binding protein, however, no differences could be observed in other parameters and HbA1c did not show any correlation with plasma antigenic levels or functional activities of coagulation inhibitors either at baseline or at 3 months of good glycaemic control. Our study only showed correlation between protein S and tPA with HbA1c. Previous data have indicated that even mild postprandial hyperglycaemia in diabetic subjects, who are concerned to be in good control, activates haemostasis. In this study, the postprandial levels of glucose, triglycerides, fibrinogen, FI+2, TAT and D dimer were lower after glibenclamide administration compared to placebo, while the concentrations of insulin and C-peptide were higher. These data showed a continuous alteration in coagulation in diabetic patients, another interesting detail is the prethrombosis state demonstrated in relatives of type 2 diabetic patients, who in a case-control study exhibited levels of prothrombin FI+2 and D dimer than control subjects.

An additional point of interest is the relationship between some haemostasis parameters and BMI. One possibility is that changes in these parameters are related to adipose tissue derived cytokines.

In conclusion, hypercoagulable state is present in diabetic patients which with present knowledge can be viewed as a risk factor for chronic complications. The role of adipose tissue as a possible cause of chronic inflammatory activity in diabetic patients requires further investigation.
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