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: el inhibidor del activador del plasmineño: tipo 1 (PAI-I), complejo trombina/antitrombina III (TAT), activador titular del plasmineño (t-PA), antígeno Von Willebrand (vW), proteína C (PC), proteína S (PS), fibrinogen, factor VII (FVII) a 94,6 ± 48 frente a 81,7 ± 28 (MU/ml); p < 0,05), y un descenso en los parámetros fibrinolíticos (t-PA 12,6 ± 5,1 vs 7,4 ± 3,1 ng/ml; p < 0,05) y un incremento en los niveles de dímero D (DD 22,3 ± 26,8 vs 9,7 ± 5,4 ng/l; 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.

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

Palabras clave: Diabetes mellitus tipo 2. Hipercoagulabilidad. Sobrepeso.


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INTRODUCTION
Diabetes mellitus is an independent risk factor for the de-
velopment of atherosclerosis. The possible mechanisms are un-
clear. It is postulated that chronic inflammation may con-
tribute to increase the risk of coronary heart disease in dif-
ferent ways; increasing serum concentrations of acute phase re-
actants (such as fibrinogen or C reactive protein) or mod-
difying the serum lipid pattern (such as decrease of HDL-
cholesterol and increase of triglycerides). Another factor in-
volved in the atherogenesis of diabetic patients is the pro-
motion of the oxidation of LDL-cholesterol since oxidation enhances the atherogenic capacity of those mole-
ecules. More recently, some authors have shown that diabetic pa-
ients 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 ma-
les/7 females) and twenty-three controls (blood donors) (15 ma-
les/8 females) without diabetes were studied. Both groups were
chosen at random and their characteristics are shown in table 1. Pa-
ients and controls did not take either anti-hypertensive or hypoli-
pemic agents. Diabetic patients took sulphonylureas as antihy-
pglycemic 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 exa-
minations: plasminogen activator inhibitor type (t-PA), tissue plasminogen activator (t-PA), von Willebrand antigen (vW), protein C (PC), protein S (PS), thrombomodulin (TH), activated Vii factor, D dimer (DD), (normal ranges for both). Thrombomodulin activation fragment F1+2 (F12). Blood samples for coagulation testing were collected into 3.8% sodium citrate solution between 07:00 and 09:00 am, after the subjects had fasted for 12 h. Samples were centrifugated for 15 min at 3000 g at room temperature.

Haemostasis assessment
Plasminogen activator inhibitor type (t-PA) (normal range < 10 U/ml) and tissue plasminogen activator (t-PA) (normal range 1-12 ng/ml) were determined by enzyme immunoassay, (TintElize Pat Umea, Sweden). Thrombomodulin (normal range 1.0-4.1 ng/ml) was determined by enzyme immunoas-
say with a commercial kit (Flaunyost TAT inaco, Marburg, Ger-
many). Von Willebrand antigen (vW) (normal range 50-160%) was determined by enzyme immunoas-

sessment

Chronic diabetic complications assessment
All diabetic patients were checked in the Clinic for chronic com-
plications. Ischemic heart disease was assessed by anamnestic and in addition, a 12-lead resting electrocardiograph was recorded in supine position (Mac PC Electrocardiograph, Marquette Electro-

TABLE 1. Clinical characteristics of overweight diabetic patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients (n = 23)</th>
<th>Control subjects (n = 23)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.3 ± 12.3</td>
<td>62.1 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>16/7</td>
<td>15/8</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 ± 3.9</td>
<td>27.6 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>8.4 ± 6.7</td>
<td>8.9 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Macroangiopathy (%)</td>
<td>12.8%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Microangiopathy (%)</td>
<td>46.9%</td>
<td>0</td>
<td></td>
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</tbody>
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results: T wave inversion, ST segment depression and Q waves. Pa-

tients with signs or symptoms of cerebrovascular disease were eva-

uated with a CNS computerized tomography. The final diagnosis was reviewed by a neurologist.

Peripheral vascular disease was clinically defined by the presen-
tice of intermittent claudication, absent or weakened peripheral pul-
ses, or both. Retinopathy was documented by fundus eye exami-
nation and diagnosed on the presence of microaneurysms, venous dilatation, cotton-wool spots, neovascularization or he-

morrhages. Clinical neuropathy was defined by an abnormal neu-

ologic examination, consistennt with the presence of peripheral sensorymotor neuropathy. Nephropathy was defined by the presen-

cence of urinary albumin excretion of 30 mg or more per 24 hours.

Statistical analysis
The results were expressed as mean ± standard deviation. The distri-
bution 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

RESULTS
Twenty three overweight diabetic patients and 23 over-

weight no diabetic patients were enrolled in the study. The mean age and BMI were similar in both groups (table 1). The diabetes duration was of 8.9±2.4 years, microangio-

pathy was present in 48.9% and macroangiopathy in 12.8% of diabetic patients.

Table 2 shows differences between both groups with an in-

crease in pro-

coagulant parameters in diabetic patients, with

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a significant increase in prothrombin activation fragment F1+2 and factor VIII(a). Values of PAI and TAT did not have statistical differences. Fibrinolytic parameters showed significant differences in TPA and D dimer (increased) and FAP (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 c4b%9 and without microangiopathy (51.1%), but no differences were found. In a correlation analysis between HbA1c and haemostatic parameters, only protein C and TAT showed significant inverse correlations (r = –0.35; p = 0.001 and r = –0.32; p < 0.05, respectively). Another correlation analysis was performed between BMI and haemostasis parameters, only FV was correlated with BMI (r = 0.32; p < 0.05). No correlations were found between haemostasis parameters with BMI and Hba1c in non diabetic subjects.

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 F1+2 and activated factor VII were increased in diabetic patients, showing a hypercoagulable state. In diabetic patients, it has been shown that fibrinolytic parameters, such as PAI-1 and t-PA antigen, were strongly related to insulin resistance, whereas the link with factor VII and other procoagulant parameters remained weak. These alterations might contribute to increase cardiovascular mortality in diabetes. For example, Motishita et al. showed significantly higher levels of TAT, fibrinogen and PAI-1 in diabetic patients with coronary heart disease than 51 patients without diabetic macroangiopathy.

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 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, F1+2, 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 fibrinolysis 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 glycaemic control and haemostasis parameters in our study, could be due to an 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 Altunbas et al. in diabetic patients achieving good control after 3 months of therapy intensification, observed a significant reduction in protein S and cP-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 PAI 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, F1+2, TAT and D dimer were lower after glimepiride 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 prothrombin state demonstrated in relatives of type 2 diabetic patients, who in a case-control study exhibited levels of prothrombin F1+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.

REFERENCES

De Luis DA, et al. High risk haemostasis patterns in overweight patients with type 2 diabetes mellitus


