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

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PATRON 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 plaquetoplasminógeno: tipo I (PAI-1), complejo trombina/antitrombina III (TAT), activador titular del plaquetoplasminógeno (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).

Haemostasis parameters were compared in both groups and within the diabetic subjects in the subgroups with and without micro and macroangiopathy. In both groups, correlation analysis was performed between haemostasis and clinical parameters.

Results. Overweight diabetic patients showed an increment in procoagulant parameters (F12 1.38 ± 0.4 vs 1.21 ± 0.25 nmol/l; p < 0.05; VWF% 81.7 ± 28 (MU/ml); p < 0.05), and a decrease in fibrinolytic parameters (F1+2 107.5 ± 248.5 μg/l; p < 0.05), and a correlation analysis between haemostasis and clinical parameters. In diabetic patients, there was no difference according to the absence or presence of microangiopathy or macroangiopathy. In a correlation analysis between HbA1c and haemostasis parameters, only protein C and TPA showed significant negative correlations (r = –0.34; p = 0.01 and r = –0.32; p = 0.05, respectively). A correlation analysis between diabetics and controls was also performed between BMI and haemostasis parameters and, FvW was correlated with BMI (r = 0.32; p < 0.05). No correlations were found between haemostasis parameters with BMI and HbA1c in controls. Conclusion. Hipercoagulable 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.

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Palabras clave: Diabetes mellitus tipo 2. Hipercoagulabilidad. Sobrepeso.
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 those 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 (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 antihyperglycemic 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: plasminogen activator inhibitor type (t-PA); von Willebrand antigen (vW); protein C (PC); protein S (PS); thrombomodulin (TH), activated VII factor, D dimer (DD); fibrinogen; tissue plasminogen activator (t-PA) and plasmin-antiplasmin (PAP), prothrombin 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 2500 × 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 (MabtechDia Diagnosis, Sweden). Thrombomodulin and von Willebrand factor (normal range 0.4-1.1 nmol/L) were determined by enzyme immunoassay (Staclot, Asnieres-sur-Seine, France) (Boehringer Mannheim, Almere, The Netherlands). Factor VIIa (normal range 5-85 µM/l) was determined by enzyme immunoassay with a commercial kit (Staclot, Asnieres-sur-Seine, France). Von Willebrand antigen (vW) (normal range 50-160%) was determined by enzyme immunoassay (George King Biomedical, Netherlands). Protein C (%) and protein S (%) were determined by enzyme immunoassay (George King Biomedical, Netherlands). Thrombin/antithrombin III complex (TAT) and tissue plasminogen activator (t-PA) (normal range < 10 U/ml) were determined by enzyme immunoassay (MabtechDia Diagnosis, Sweden). Thrombomodulin (normal range 14.5-55 ng/ml), D dimer (normal range 2.5-5.27 µg/L), plasmin-antiplasmin (PAP) (normal range 99-368 ng/L), and prothrombin activation fragment F1+2 (F12) (normal range 1.4-16.1 nmol/L) were determined by enzyme immunoassay (Staclot, Asnieres-sur-Seine, France).

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 Electrocardio-

results

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 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, microangiopathy was present in 48.9% and macroangiopathy in 12.8% of diabetic patients. Table 2 shows differences between both groups with an increase in procoagulant parameters in diabetic patients, with
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 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 HbA1c and haemostasis parameters, only protein C and TPA 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 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 VIIChere were increased in diabetic patients, showing a hypercoagulable state. In diabetic patients, it has been shown that fibrino-lytic parameters, such as PAI-1 and t-PA antithrombin were strongly related to insulin resistance, whereas the link with factor VII and other procoagulant parameters remained weak. These alterations might contribute to an increase in cardiovascular mortality in diabetes. For example, Mortellet 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 anti-coagulant 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 anti-coagulant 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 and protein C inhibitor complex and significant low values of the anti-coagulant response to exogenous thrombomodulin, indicating a poor plasma reactivity to the anti-coagulant 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 fibrinolytic system in overweight diabetic patients, such as a significant increase in D dimer levels. Increased levels of plasmino-gen activator (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 a intrinsic altered state in diabetic patients. This haemostasis alteration with other risk factors such as hyperglycaemia or hyperlipidaemia could start macro- 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, Alhunna et al. in diabetic patients achieving good control after 3 months of therapy intensification, observed a significant reduction in protein S, and C-prothrombin. 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 patients, 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 gibletanamide 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 pre-thrombotic 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.

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