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 these patients were: age 61.3 ± 12.3 years, body mass index (BMI) 27.2 ± 3.9 kg/m² and duration of diabetes 8.4 ± 8.7 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 27.6 ± 3.1 kg/m². All patients (diabetic and controls) underwent the following examinations: plasma; inactivator inhibitor type 1 (PAI-1), thrombomodulinantithrombin III complex (TAT), tissue plasminogen activator (tPA), von Willebrand antigen (vWF), protein C (PC), protein S (PS), thrombomodulin (TH), activated VII factor, D dimer (DD), plasmin-antiplasmin (PAP), and plasminogen activation fragment 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 BMI and HbA1c in controls.

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; VWFα 503.7 ± 28 (MU/ml); p < 0.05), and a decrease in fibrinolytic parameters (TAT 107.5 ± 24.5 µg/l; p < 0.05) and anticoagulant 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 ± 26.8 vs 9.7 ± 5.4 µg/l; p < 0.05) and (t-PA 1.38 ± 0.4 frente a 1.21 ± 0.25 nmol/l; p < 0.05; VII(a) 94.6 ± 48 frente a 81.7 ± 28 (MU/ml); p < 0.05). A correlation analysis between haemostasis parameters and clinical parameters.

Conclusion. High risk haemostasis patterns with type 2 diabetes mellitus

D.A. DE LUIS, R. ALLER, L. CUÉLLAR, J.J. TORTOSA, E. ROMERO, D. BELLIDO Y C. TERROBA.


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 ± 8,7 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: tipo1 (PAI-1), complejo trombina/antitrombina III (TAT), activador titular del plasminógeno (t-PA), antígeno Von Willebrand (vWF), proteína C (PC), proteína S (PS), plasminógeno: tipo1 (PAI-1), plasmin-antiplasmin (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 micro y 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 nmol/l; p < 0,05; VWFα 503,7 ± 28 (MU/ml); p < 0,05), y un descenso en los parámetros fibrinolíticos (TAT 107,5 ± 24,5 µg/l; p < 0,05) y anticoagulantes (Thrombomodulin 27,4 ± 11,7 frente a 45,1 ± 21,7 ng/ml; p < 0,05), con un incremento en los niveles de dímero D (DD) 22,3 ± 26,8 frente a 9,7 ± 5,4 µg/ml; p < 0,05) y (t-PA 1,38 ± 0,4 frente a 1,21 ± 0,25 nmol/l; p < 0,05; VII(a) 94,6 ± 48 frente a 81,7 ± 28 (MU/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.

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

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

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Correspondence: Dr. D.A. de Luis.

Associated Professor of Endocrinology and Nutrition.

Executive Director of Institute of Endocrinology and Nutrition.

Medical School. Valladolid University.

Cuarto 91, 3º C, 47013 Valladolid.

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

D.A. DE LUIS, R. ALLER, L. CUÉLLAR, J.J. TORTOSA, E. ROMERO, D. BELLIDO Y C. TERROBA.

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 atherosogenesis 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: plasma (activator inhibitor type 1 (PAI-1), thrombin-antithrombin 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), factor VIIa (normal range 5-85 Mu/ml) was determined by enzyme immunoassay with a commercial kit (Staclot, Asnieres-sur-Seine, France).

Haemostasis assessment

Plasminogen activator inhibitor type 1 (PAI-1) (normal range c. 10 U/ml) and tissue plasminogen activator (t-PA) (normal range 1-12 ng/ml) were determined by enzyme immunoassay, (TintElize PAI Unica, Sweden). Thrombin-antithrombin III complex (TAT) (normal range 1-10 ng/ml) was determined by enzyme immunoassay with a commercial kit (Flaunyost TAT icon, Marburg, Germany). Von Willebrand antigen (vW) (normal range 50-160%) was determined by enzyme immunoassay (George King Bio-Medical, Inc., Overlandpark, KS, USA). Protein C and S were determined by coagulometry, (normal range 70-150% for both). Thrombomodulin (normal range 15-55 ng/L), D dimer (normal range 2.5-2.87 mg/L), plasma-antithrombin (FPA) (normal range 99-368 ng/L), and prothrombin activation fragment F1+2 (F12) were determined by enzyme immunoassay with a commercial kit (Boehringer Mannheim, Almere, The Netherlands).

Factor VIIa (normal range 5-85 MUI/ml) was determined by enzyme immunoassay with a commercial kit (Staclot, Assnieres-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 Electrocardiograph) and analyzed by an electrocardiologist. The presence of any of the following findings was considered suggestive of coronary heart disease: T wave inversion, ST segment depression and Q waves. Patients with signs or symptoms of cerebrovascular disease were evaluated with a CNS computed tomography. The final diagnosis was reviewed by a neurologist.

Peripheral vascular disease was clinically defined by the presence of intermittent claudication, absent or weakened peripheral pulses, or both. Retinopathy was documented by standard fundus eye examination and diagnosed on the presence of microaneurysms, venous dilatation, cotton-wool spots, neovascularization of the optic disc, or hemorrhages. Clinical neuropathy was defined by an abnormal neurologic examination, consistent with the presence of peripheral sensorimotor neuropathy. Nephropathy was defined by the presence of urinary albumin excretion of 30 mg or more per 24 hours.

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 Fischer’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 overweight no 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, 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 pro-coagulant parameters in diabetic patients, with decrease in anti-coagulant parameters in diabetic patients.
De Luis DA, et al. High risk haemostasis patterns in overweight patients with type 2 diabetes mellitus

a significant increase in prothrombin activation fragment \( F1+2 \) and factor \( \text{VIII} \). Values of \( \text{PAI} \) and \( \text{TAT} \) did not have statistical differences. Fibromodulin parameters showed significant differences in \( \text{TPA} \) and \( \text{D dimer} \) (increased and \( \text{PAP} \) decreased), without differences in \( \text{FvW} \). A decrease in anticoagulant 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 \( (\text{BMI} < 25) \) and without microangiopathy (51.1%), but no differences were found. In a correlation analysis between HbA1c and haemostasis parameters, only protein \( C \) and \( \text{PAI} \) 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 \( \text{FvW} \) 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\(^8\) who had an increase in \( \text{TAT} \) levels. In our study no significant differences in \( \text{TAT} \) levels were detected between overweight diabetic and control subjects, but \( \text{F}1+2 \) and activated factor \( \text{VII} \) were increased in diabetic patients, showing a hypercoagulable state. In diabetic patients\(^9\), it has been shown that fibromodulin parameters, such as \( \text{PAI}-1 \) and \( \text{t-PA} \) antigen were strongly related to insulin resistance, whereas the link with factor \( \text{VII} \) and other procoagulant parameters remained weak. These alterations might contribute to increase cardiovascular mortality in diabetes. For example, Motishita et al\(^10\) showed significantly higher levels of \( \text{TAT} \), fibromodulin and \( \text{PAI}-1 \) in 22 diabetics patients with coronary heart disease than 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 \( C \) inhibiting system could be abnormal\(^11\). These abnormalities of anticoagulant system might constitute a potential trigger for haemostatic activation. Gabarra et al\(^17\) demonstrated alterations in overweight diabetic patients in the plasma levels of fibrinogen, \( \text{F1+2} \), 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 fibrin/fibronectin system in overweight diabetic patients, such as a significant increase in \( \text{D dimer} \). Increased levels of plasminogen activator (PA)-1 might be involved in the pathogenesis of the vascular complications of diabetes mellitus. However, Mansfield et al\(^18\) showed low PA-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 an intrinsic

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