

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 of 61.3 ± 12.3 years, body mass index (BMI) 27.2 ± 3.9 kg/m² and duration of diabetes 8.4 ± 6.7 years. A group of twenty three voluntary controls chosen at random (blood donors) (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 (diabetics and controls) underwent the following examinations; plasminogen 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), plasmin-antiplasmin (PAP), and prothrombin 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 haemostasis and clinical parameters.

Results. Overweight diabetics patients showed an increment in procoagulant parameters (F12 1.38 ± 0.4 vs 1.21 ± 0.25 nmol/l, $p < 0.05$; VII(a) 94.6 ± 48 vs 81.7 ± 28 (MU/ml); $p < 0.05$), and a decrease in fibrinolytic parameters (PAP 262.9 ± 107.5 vs 348.5 ± 143 ng/l; $p < 0.05$), and anticoagulant parameters (Thrombomodulin 27.4 ± 11.7 vs 45.1 ± 21.7 ng/ml; $p < 0.05$), with an increased in D dimer levels (DD 22.3 ± 26.8 vs 9.7 ± 5.4 g/l; $p < 0.05$) and t-PA 12.6 ± 5.1 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 HbA_{1c} 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 was also performed between BMI and haemostasis parameters and, only FvW was correlated with BMI ($r = 0.32$; $p < 0.05$). No correlations were found between haemostasis parameters with BMI and HbA_{1c} 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: $61,3 \pm 12,3$ años, índice de masa corporal (IMC) $27,2 \pm 3,9$ kg/m² y duración de la diabetes $8,4 \pm 6,7$ 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 $27,6 \pm 3,1$ 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 tisular del plasminógeno (t-PA), antígeno Von Willebrand (vW), proteína C (PC), proteína S (PS), trombomodulina (TH), factor VII activado, dímero D (DD), plasmína-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 micro y macroangiopatía. En ambos grupos, se realizó un análisis de correlación entre los parámetros clínicos y los hemostáticos.

Resultados. Los pacientes diabéticos con sobrepeso evidenciaron un incremento en los factores procoagulantes (F12 $1,38 \pm 0,4$ frente a $1,21 \pm 0,25$ nmol/l; $p < 0,05$); VII[a] $94,6 \pm 48$ frente a $81,7 \pm 28$ (MU/ml); $p < 0,05$), y un descenso en los parámetros fibrinolíticos (PAP $262,9 \pm 107,5$ frente a $348,5 \pm 143$ ng/l; $p < 0,05$), así como en los parámetros anticoagulantes (trombomodulina $27,4 \pm 11,7$ frente a $45,1 \pm 21,7$ ng/ml; $p < 0,05$), con un incremento en los niveles de dímero D (DD $22,3 \pm 26,8$ frente a $9,7 \pm 5,4$ g/l; $p < 0,05$) y (t-PA $12,6 \pm 5,1$ frente a $7,4 \pm 3,1$ 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 (HbA_{1c}). 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 HbA_{1c} 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.

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

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INTRODUCTION

Diabetes mellitus is an independent risk factor for the development of atherosclerosis¹. 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 molecules²⁻⁵. More recently, some authors have shown that diabetic patients had a hypercoagulable state⁶.

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 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 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), plasmin-antiplasmin (PAP), prothrombin activation fragment F1+2 (F12). Blood samples for coagulation testing were collected into 31.3 g/L trisodium 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 1 (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 Umeå, Sweden). Thrombin/antithrombin III complex (TAT) (normal range 1.0-4.1 ng/l) was determined by enzyme immunoassay with a commercial kit (Enzygnost TAT micro, 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 14-55 ng/ml), D dimer (normal range: 2.5-97.5 g/l), plasmin-antiplasmin (PAP) (normal range 99-368 ng/L), and prothrombin activation fragment F1+2 (F12) (normal range 0.4-1.1 nmol/L) were determined by enzyme immunoassay with a commercial kit (Boehringer Mannheim, Almere, The Netherlands). Factor VIIa (normal range 5-85 Mu/ml) was determined by enzyme immunoassay with a commercial kit (Staclot, Asnières-sur-Seine, France)⁷⁻⁹.

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 Electronics) and evaluated by a cardiologist. The presence of any of the following findings was considered suggestive of coronary heart di-

TABLE 1. Clinical characteristics of overweight diabetic patients and controls

	Diabetic patients (n = 23)	Control subjects (n = 23)	p
Age (years)	61.3 ± 12.3	62 ± 13	NS
Sex (male/female)	16/7	15/8	NS
BMI (kg/m ²)	27.2 ± 3.9	27.6 ± 3.1	NS
Diabetes duration (years)	8.4 ± 6.7	–	
HbA _{1c} (%)	8.9 ± 2.4	–	
Macroangiopathy (%)	12.8%	0	
Microangiopathy (%)	48.9%	0	

Microangiopathy (retinopathy and/or nephropathy and/or neuropathy).

TABLE 2. Differences in hemostasis parameters in diabetic and control patients

	Diabetic patients (n = 23)	Control subjects (n = 23)	p
Procoagulant parameters			
AF1+2 (nmol/L)	1.38 ± 0.4	1.21 ± 0.25	< 0.05
PAI (U/ml)	25.3 ± 24.9	37.3 ± 51.7	NS
TAT (ng/L)	3.52 ± 2.8	5.63 ± 4.5	NS
VII (a)(MU/ml)	94.6 ± 48	81.7 ± 28	< 0.05
Fibrinolysis parameters			
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
FvW (%)	188 ± 57.1	109.8 ± 27.4	NS
DD (g/l)	22.3 ± 26.8	9.7 ± 5.4	< 0.05
Anticoagulant parameters			
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

sease: T wave inversion, ST segment depression and Q waves. Patients with signs or symptoms of cerebrovascular disease were evaluated with a CNS computerized 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 or hemorrhages. Clinical neuropathy was defined by an abnormal neurological 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 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 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 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 pro-coagulant parameters in diabetic patients, with

a significant increase in prothrombin activation fragment F1+2 and factor VII(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 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 (48.9%) 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 control¹⁰ 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 patients¹¹, 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, Morishita et al¹² showed significantly higher levels of TAT, fibrinogen and PAI-1 in 22 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/S inhibiting system could be abnormal¹³. These abnormalities of anticoagulant system might constitute a potential trigger for haemostatic activation: Gabazza et al¹⁴ 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 levels¹⁵. Increased levels of plasminogen activator-1 (PAI-1) might be involved in the pathogenesis of the vascular complications of diabetes mellitus. However, Mansfield et al¹⁶ 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 al¹⁷ in diabetics patients achieving good control after 3 months of therapy intensification, observed a significant reduction in protein S and c4b-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 subjects¹⁸, 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 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 prethrombotic 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 subjects¹⁹.

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 cytokines²⁰.

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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