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


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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 (15 males/8 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 ± 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 control) underwent the following examinations: plasma fibrinogen activator inhibitor type 1(PAI-1), thrombomodulin/thrombin 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 postthrombin 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 diabetic patients showed an increment in plasma fibrinogen (F12) 1.38 vs 1.21 ± 0.25 mmol/l; p < 0.06; Von Willebrand factor (vW) 81.7 ± 28 (MU/ml); p < 0.05), and a decrease in fibrinolytic parameters thrombin - anti thrombin III complex (TAT) 410.7 ± 348.5 ± 143 ng/l; p < 0.05), and in the 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 ± 28.9 vs 9.7 ± 5.4 µg/l; p < 0.05) and PAP 262.9 ± 107.5 frente a 348.5 ± 143 ng/l; p < 0.05). No se encontraron correlaciones entre los parámetros fibrinolíticos (PAP 262.9 ± 107.5 frente a 348.5 ± 143 ng/l; p < 0.05) y el 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 tisular del plasminógeno (t-PA), anticoagulante Von Willebrand (vW), proteina C y proteína S. En los pacientes diabéticos no hubo diferencia en función de la ausencia o presencia de micro o macroangiopatía. En ambos grupos, se realizó un análisis de correlación entre los parámetros clínicos y los hemostáticos.

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

Keywords: Type 2 diabetes mellitus. Haemostasis. Overweight.
Diabetes mellitus is an independent risk factor for the development of atherosclerosis\(^1\). The possible mechanisms are unclear. It is posulated 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\(^2\). More recently, some authors have shown that diabetic patients had a hypercoagulable state\(^3\).

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 antihypoglycemic 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-mimogen activator inhibitor type 1 (PAI-1), thrombinoan/thrombinogen 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 activator inhibitor type 1 (PAI-1), factor VII (a), TAT (micro), F1+2 (F12). Blood samples for coagulation testing were collected into 3 ml of trisodium citrate solution between 07:00 and 09:00 am, after the subjects had fasted for 12 h. Samples were centrifugated 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 (TinElize PAI Unica, Sweden). Thrombinoan/thrombinogen III complex (TAT) (normal range 1-10.4 ng/ml) was determined by enzyme immunoassay (Tinaclot TAT iatros, 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) was determined by enzyme immunoassay (Diagnostica Stago, Asnieres-sur-Seine, France)\(^4\).

#### Chronic diabetic complications assessment

All diabetic patients were checked in the Clinic for chronic complications. Ischemic heart disease was assessed by anamnesis and in addition, a 12-lead resting electrocardiogram was recorded in quiet 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-

### 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 pro-coagulant parameters in diabetic patients, with

#### 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 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.
a significant increase in prothrombin activation fragment F1+2 and factor VIII(a). Values of PAI and TAT did not have statistical differences. Fibronectin 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 BMI (8/9) and without macroangiopathy (51.1%), but no differences were found. In a correlation analysis between HbA1c, antithrombotic parameters, only protein C and TAT 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 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 22 diabetics 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 abnormal. These abnormalities of anticoagulant system might constitute a potential trigger for haemostatic activation. Gabazza 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 macro or microangiopathy 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 inhibitor 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-angioopathy, and haemostasis could act in a second step, so that there was a lack of relationship between diabetic complications and haemostasis parameters. For example Alhunis et al. in diabetic patients achieving good control after 3 months of therapy, showed a significant reduction in protein S and cD-binding protein, however, no differences could be observed in other parameters and HbA1c did not show any correlation with plasma antigentic levels or functional activies 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 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 prothrombotic state demonstrated in relatives of type 2 diabetic patients, who in 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**