Fibrinogen Plasma Levels as a Marker of Thrombin Activation in Diabetes

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This study attempted to verify the existence of a correlation between fibrinogen, a major cardiovascular risk factor in diabetes, and indexes of thrombin generation and action, prothrombin fragment 1 + 2 (F1 + 2), and D-dimer (D-D), in a group of diabetic subjects compared with a matched control group. Forty insulin-dependent diabetes mellitus patients and 30 matched healthy control subjects participated in this study. The subjects were tested for the following parameters: fibrinogen, prothrombin F1 + 2, D-D, fasting glycemia, and HbA_{1c}. In addition, 5 diabetic subjects who maintained stable fibrinogen plasma levels >300 mg/dl for at least 6 months before the study were treated with 12,500 U/day subcutaneous heparin for 7 days. Diabetic subjects showed increased levels of fibrinogen, prothrombin F1 + 2, and D-D plasma levels. Simple linear regression analysis detected a positive correlation between fibrinogen and prothrombin F1 + 2, D-D, and glycosylated HbA_{1c}. In the five diabetic subjects treated with heparin fibrinogen, prothrombin F1 + 2 and D-D levels decreased at the end of the treatment. All these parameters returned to baseline after 7 days of washout. These data indicate that fibrinogen plasma levels are correlated to parameters of thrombin activation in plasma in diabetic patients and suggest that high fibrinogen plasma levels might be a risk marker for cardiovascular disease in diabetes because it is an expression of an existing thrombophilia.
***Diabetes* 43:430–32, 1994**

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Received for publication 14 June 1993 and accepted in revised form 13 October 1993.

F1 + 2, fragments 1 + 2; D-D, D-dimer; CVD, cardiovascular disease; BMI, body mass index; NDDG, National Diabetes Data Group; CV, coefficient of variation.

Several prospective studies have demonstrated that high fibrinogen plasma levels are associated with an increased risk of ischemic heart disease; the impact of fibrinogen as an independent predictor of cardiovascular disease (CVD) is similar to that of the major recognized risk factors (1,2). Similar results have been demonstrated even in diabetes (3). However, the pathophysiological mechanisms linking elevated fibrinogen levels to increased risk of CVD are not clear.

Increased thrombin formation unrelated to acute episodes has been reported to occur in the majority of patients with ischemic heart disease (4,5). Prothrombin F1 + 2, a good marker of thrombin generation in plasma (6), and D-dimer (D-D), the principal breakdown fragment of fibrin (7), are considered good tools to reveal an existing thrombophilia.

In this study, we aimed to verify the existence of a correlation between fibrinogen and these indexes of thrombin generation and action in a group of diabetic subjects compared with a matched control group.

RESEARCH DESIGN AND METHODS

Forty insulin-dependent diabetic subjects (18 males and 22 females, 29 ± 5.4 years of age, body mass index [BMI] 27.9 ± 3.2 kg/m^2, duration of diabetes 7.4 ± 2.6 years, mean insulin dosage 0.7 ± 0.3 IU · kg^{-1} · day^{-1}, means ± SD), selected according to National Diabetes Data Group (NDDG) criteria (8), gave informed consent to participate in this study. Diabetic patients were on diet plus insulin therapy. No other drugs had been used by all the subjects during the 3-month period before the study. None of the subjects had histories of acute or chronic infections, myocardial infarction, stroke, or other cardiovascular events or ECG signs of active ischemic heart disease. All diabetic subjects were normoalbuminuric (albumin excretion rate 4.1 ± 1.7 μg/min).

Thirty healthy normal subjects, matched for sex (14 males and 16 females), age (28 ± 7.4 years of age), and BMI (26.8 ± 2.1 kg/m^2) with a normal oral glucose tolerance test according to NDDG criteria (8), served as the control group.

Study design. The subjects were tested for the following parameters: fibrinogen, prothrombin F1 + 2, D-D, fasting glycemia, and HbA_{1c}. In addition, 5 subjects (25.7 ± 1.5 years of age, 3 males and 2 females) who maintained stable fibrinogen plasma levels >300 mg/dl for at least 6 months before the study were treated with 12,500 U/day subcutaneous heparin for 7 days. The above-mentioned parameters were evaluated at the start of the heparin administration, after 7 days of treatment, and after 7 days of washout period.

Biohumoral determinations. After an overnight fast (≥12 h) and after the subjects were in the supine position for 10 min, a sample of venous blood was obtained from an antecubital vein without stasis. To study coagulation parameters, blood was collected through a silicone-treated needle (diameter, 2 mm) and was allowed to flow freely into silicone-treated glass tubes where it was mixed with 10% of its volume of 0.1 M trisodium citrate. The blood was immediately centrifuged at 1,700 *g* for 20 min at 4°C and frozen at −80°C until assayed.

Plasma fibrinogen was measured functionally by the method of Rossi et al. (9) in an automatic coagulometer autoanalyzer (Instrumentation Laboratory, Lexington, MA). The intra- and interassay coefficient of variations (CVs) were 3.1 and 4.0%, respectively.

Prothrombin F1 + 2 plasma levels were evaluated by ELISA according to Pelzer et al. (10). The intra- and interassay CVs were 5.0 and 5.9%, respectively. D-D was measured immunoenzymatically according to Rylatt et al. (11). For this method the intra- and interassay CVs were 4.4 and 5.6%, respectively. Plasma glucose was measured by glucose oxidase method. HbA_{1c} was evaluated by affinity chromatography (12).

Statistical analysis. Statistical analysis of data was accomplished by means of the BMDP statistical software package. Statistical evaluation was performed by simple regression analysis and unpaired Student's *t* tests. To evaluate the effect of heparin administration in the five diabetic subjects, the matched Student's *t* test was used. All statistical tests were two-tailed. *P* values <0.05 were regarded as statistically significant.

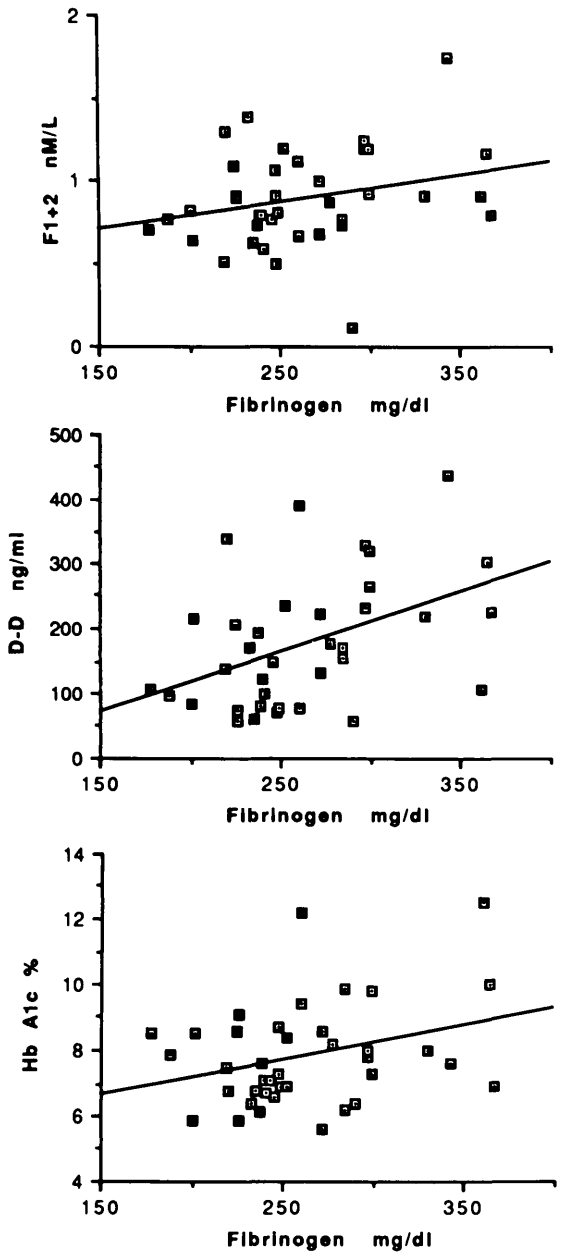

FIG. 1. Simple linear correlation between plasma fibrinogen levels, prothrombin F1 + 2, D-D, and HbA_{1c} in the studied diabetic subjects.

RESULTS

Diabetic subjects showed increased fibrinogen (261.0 ± 46.0 vs. 190.3 ± 30.4 mg/dl, $P < 0.001$), prothrombin F1 + 2 (0.92 ± 0.26 vs. 0.60 ± 0.12 nM, $P < 0.001$), and D-D (174.2 ± 99.3 vs. 130.0 ± 54.0 ng/ml, $P < 0.03$) plasma levels. Simple linear regression analysis detected a positive correlation between fibrinogen and prothrombin F1 + 2 ($r = 0.37$, $P < 0.02$), D-D ($r = 0.44$, $P < 0.007$), and HbA_{1c} ($r = 0.31$, $P < 0.05$; Fig. 1). In the 5 diabetic subjects treated with heparin, fibrinogen (325.4 ± 41.1 vs. 255.2 ± 32.3 mg/dl, $P < 0.05$), prothrombin F1 + 2 (0.87 ± 0.1 vs. 0.63 ± 0.2 nM, $P < 0.001$), and D-D (236 ± 51.8 vs. 175.2 ± 48.5 ng/ml, $P < 0.01$) significantly decreased. All these parameters returned to baseline after 7 days of washout (fibrinogen, 332.5 ± 48.2 mg/dl; prothrombin F1 + 2, 0.86 ± 0.2 nM; D-D, 225.8 ± 48.65 ng/ml).

DISCUSSION

Although epidemiological studies have long demonstrated the strong and often independent direct correlation between high fibrinogen plasma levels and CVD, the mechanism by which fibrinogen would act is still unclear. On the other hand, in most patients with ischemic heart disease, an increased thrombin formation has been measured (4,5). Similarly, increased fibrinogen levels and increased thrombin activation have been frequently reported in diabetes (13–15). One might suspect the link

between plasma fibrinogen concentration and CVD to be a thrombosis-prone status; however, no clear evidence exists of such a connection in the literature. Markers of thrombin activation are now easily available, which make it possible to document the existence of thrombophilia; of them, two of the most reliable are prothrombin F1 + 2 and D-D. These two represent the quantity of thrombin generated in plasma (6) and the amount of fibrin breakdown (7) produced in circulation, respectively. In this study, we examined a group of diabetic subjects with the aim of investigating the relationship between fibrinogen and these two markers of thrombin activation. By simple regression analysis, a positive correlation between fibrinogen and coagulation activation markers (prothrombin F1 + 2 and D-D) and HbA_{1c} was found.

These data indicate that fibrinogen plasma levels are correlated to the thrombin activation in plasma and that metabolic control may influence this phenomenon. Additional information with regard to the relationship between fibrinogen and thrombin activation might be provided by the data obtained from short-term treatment with heparin of five diabetic subjects. As might be expected after heparin treatment, a reversible reduction of the plasma concentration of prothrombin F1 + 2 and D-D occurred. Interestingly, however, heparin treatment also determined a reversible reduction of the concentration of fibrinogen. These results agree with a preliminary report showing that heparin administration lowers both fibrinogen and fibrinopeptide A levels, a good index of fibrin formation (16), in subjects with ischemic heart disease (17) and in diabetic subjects (18). We would like to offer a possible explanation of this finding. Prothrombin F1 + 2 and fragment D, of which D-D is an expression, modulates fibrinogen synthesis in the liver (19,20). Therefore, an increased prothrombin F1 + 2 and fragment D formation linked to thrombin generation might stimulate fibrinogen overproduction by the liver. If this holds true, the simultaneous decrease of fibrinogen, D-D, and prothrombin F1 + 2 obtained as a result of heparin administration gives support to the hypothesis of the existence of a positive feedback between thrombus formation, fibrinolysis, and fibrinogen production. In conclusion, we suggest that high fibrinogen plasma levels might be a risk marker of CVD in diabetes because of its expression of an existing thrombophilia.

ACKNOWLEDGMENTS

We are indebted to Carla Bulfoni, Nazia Modesti, and Lucia Zilli for technical assistance.

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