Altered Platelet Activating Factor Metabolism in Insulin Dependent Diabetes Mellitus

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Abstract

Diabetes mellitus is associated with several abnormalities of platelet function. Recent studies have shown that the blood level of platelet activating factor (PAF), a potent inducer of platelet aggregation, is elevated in insulin dependent diabetes mellitus (IDDM) and remains unchanged in non-insulin dependent diabetes mellitus (NIDDM) patients. However, the mechanism of this increase in PAF levels has not been determined. In this study we have measured the activity of plasma PAF acetyihydrolase (an enzyme that regulates PAF levels) and lipoprotein levels in control subjects and diabetic patients. The data presented show that plasma PAF acetyihydrolase activity is significantly decreased in IDDM and is not altered in NIDDM patients. The lipoprotein levels were similar in control and diabetic subjects and there was no correlation between lipoprotein levels and PAF acetylhydrolase activity. These results suggest that the elevated levels of PAF in IDDM patients could be due to a decrease in plasma PAF acetyihydrolase activity (JPMA 45:122,1995).

Introduction

Patients with diabetes mellitus are known to have an increased risk for coronaiy heart disease and other vascular disorders[1,2]. Patients with poorly controlled diabetes usually have several abnormalities of lipid and lipoprotein metabolism[3,4]. Increased peroxidation of low density lipoproteins (LDL) leading to foam cell formation, fatty streaks and plaque formation in the arterial wall is considered as the main mechanism in the pathogenesis of atherosclerosis[5]. Another majorcontributing factor in this process is the hyper-reactivity of platelets which can lead to increased platelet adhesion and aggregation[6]. Platelet activating factor (PAF) is a phospholipid which is formed by platelets, leukocytes, mast cells and vascular endothelial cells in response to several chemical and immune stimuli[7]. PAF has a wide spectrum of biological actions which include enhanced platelet aggregation, activation o(mononuclear cells, increased vascular permeability, hypotension, contraction of smooth muscles and alterations in the intermediary metabolism[7]. The biological functions of PAF are regulated by PAF acetylhydrolase which cleaves PAF to lyso-PAF (inactive product) by hydrolyzing its acetyl moiety[7]. PAF acetylhydrolase is associated with both high and low density lipoproteins and it is suggested that the enzyme activity may correlate with lipoproteins levels[8]. Recent studies have shown that the blood levels of PAF are elevated in insulin dependent diabetes mellitus (IDDM) and remain unchanged in non-insulin dependent diabetes mellitus (NIDDM) patients[9]. However, it is not known whether this alteration in IDDM patients is due to an increase in the synthesis or a decrease in the degradation of PAF. Therefore, in this study we have measured the activity of PAF acetylhydrolase enzyme in plasma obtained from control, 1DDM and NIDDM patients. We have also measured serum lipoprotein levels and have looked at their correlation with PAF acetylhydrolase activity in control and diabetic subjects.

Subjects and Methods

Subjects

Age and sex matched diabetic patients and control subjects were recruited from the Diabetic and Executive Clinics of the Aga Khan University Hospital, Karachi. The diabetic patients who had impaired renal function or albuminuria, clinical evidence of cardiovascular disease or on concurrent medication with aspirin or non-steroidal anti-inflaniniatoty drugs were excluded from the study. An informed consent was taken from all patients and control subjects before withdrawing the blood for the study. Venous blood was obtained from all subjects after an overnight fast for the measurement of serum glucose and lipid profile. Glucose levels were measured by using a glucose analyzer whereas serum triglycerides, total cholesterol, LDL and HDL levels were determined by standard enzymatic procedures as described earlier[10,11].

PAF Acetylhydrolase Activity

PAF acetyihydrolase activity was measured by the method of Blank et al[12]. Briefly, blood samples were mixed with 3.8 (w/v) sodium citrate solution (9:1) and centrifuged at 1,200 g for 20 minutes and plasma was separated. The enzyme activity was assayed by incubating 150 ul of plasma with unlabeled PAF (100 uM), 0.1 uCi [3H] PAF (specific activity 110 Ci/mmol) in Tris-HC1 (30 mM, pH 7.4) at 37°C for 15 minutes in a shaking water bath. Each incubation was carried out in duplicate. After 15 minutes the reaction was stopped by adding 0.4 ml of 1 M citric acid. The lipids were extracted by addition of chloroform methanol (2:1 v/v) and dried under nitrogen. The dried samples were reconstituted in chloroform and applied to silica gel G thin layer chromatography plates. The plates were developed in a solvent system containing chloroform: methanol: acetic acid: water(100:60: 16:8 v/v) to a distance of 17 cm. The radioactive zones were located and quantified by the use of a Berthold TLC linear analyzer and chromatography data system çModel LB 511, Berthold, Germany) as described earlier[13] . The level of significance between control and patient population was calculated by using the student's t-test. P values less than 0.05 were considered statistically significant.

Results

Table. Serum glucose, triglycerides, total cholesterol, LDL and HDL cholesterol levels in control and diabetic subjects.

Group	Glucose mg/dl	Triglycerides mg/dl	Total Cholesterol mg/dl	LDL-Chol mg/dl	HDL-Chol mg/dl
Control	100±04	117±7	190±12	128±11	38±3
NIDDM	268±40**	167±15*	193±14	131±8	39±2
IDDM	294±24**	124±22	169±14	118±11	35±2

Each value represents mean±SEM of 8 determinations.
*P<0.01: level of significance for difference between control subjects and diabetic patients.
**p<0.05: level of significance for difference between control subjects and NIDDM patients.

The data presented in Table show the metabolic profile of normal subjects and diabetic patients. As

anticipated, fasting blood glucose levels (mg/dl, mean±SEM; n=8) were higher (p<0.0 1) in patients with IDDM (294±24) and NIDDM (268±40) as compared control group (100±04). The serum triglyceride levels were increased by 42% inNIDDM patients (controls 117±7 v/s NIDDM 167±15; p

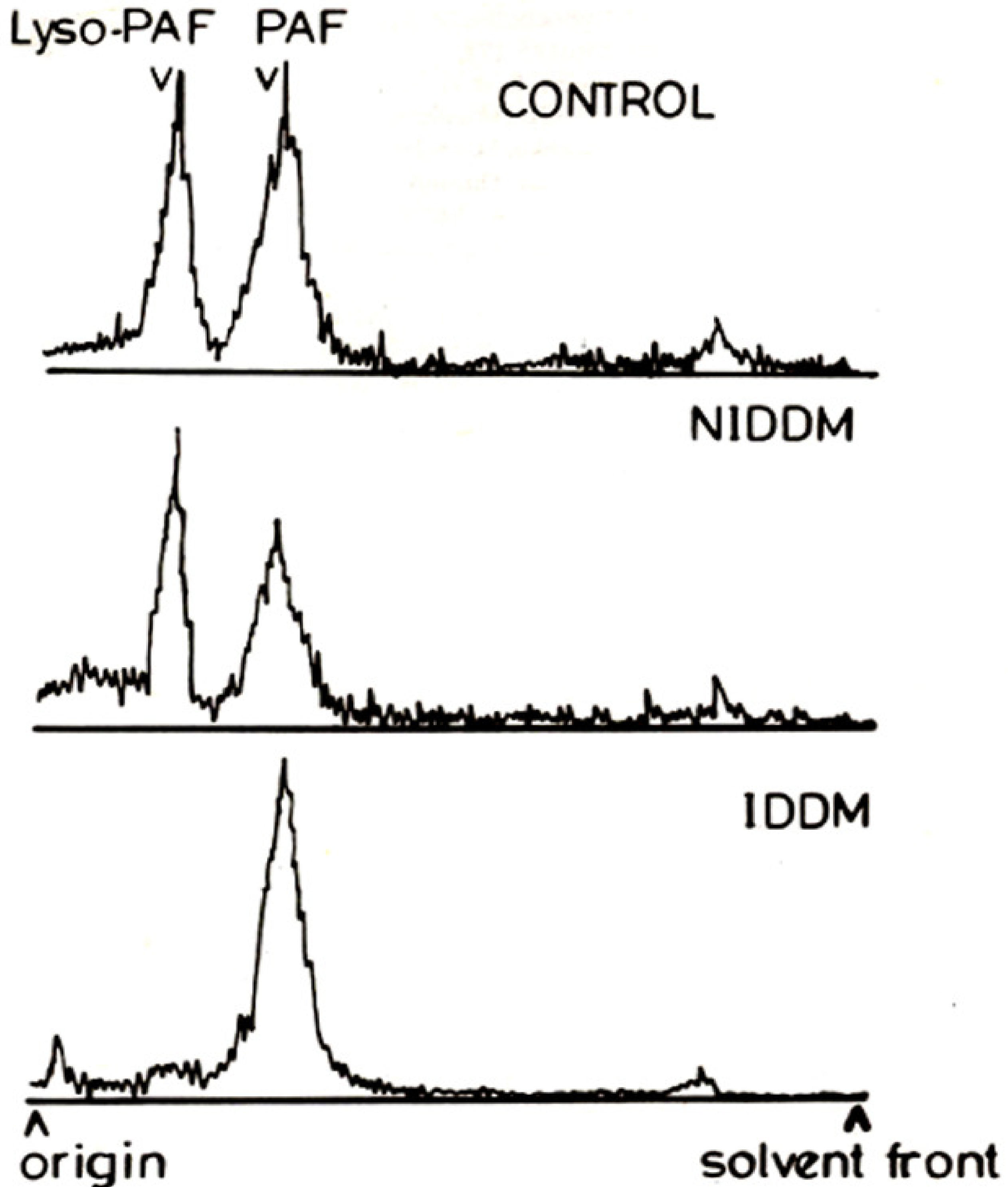

Figure 1. A representative radiochromatographic scan showing the profile of plasma PAF acetylhydrolase metabolites in control, NIDDM and IDDM patients.

Figure 1 represents atypical radiochromatographic scan obtained after thin layer chmmatography of the

products of the reaction for PAF acetylhydrolase activity. It shows a marked decrease in the formation of lyso-PAF (product of PAF degradation) in IDDM patients, whereas no similar change was detected in NIDDM patients.

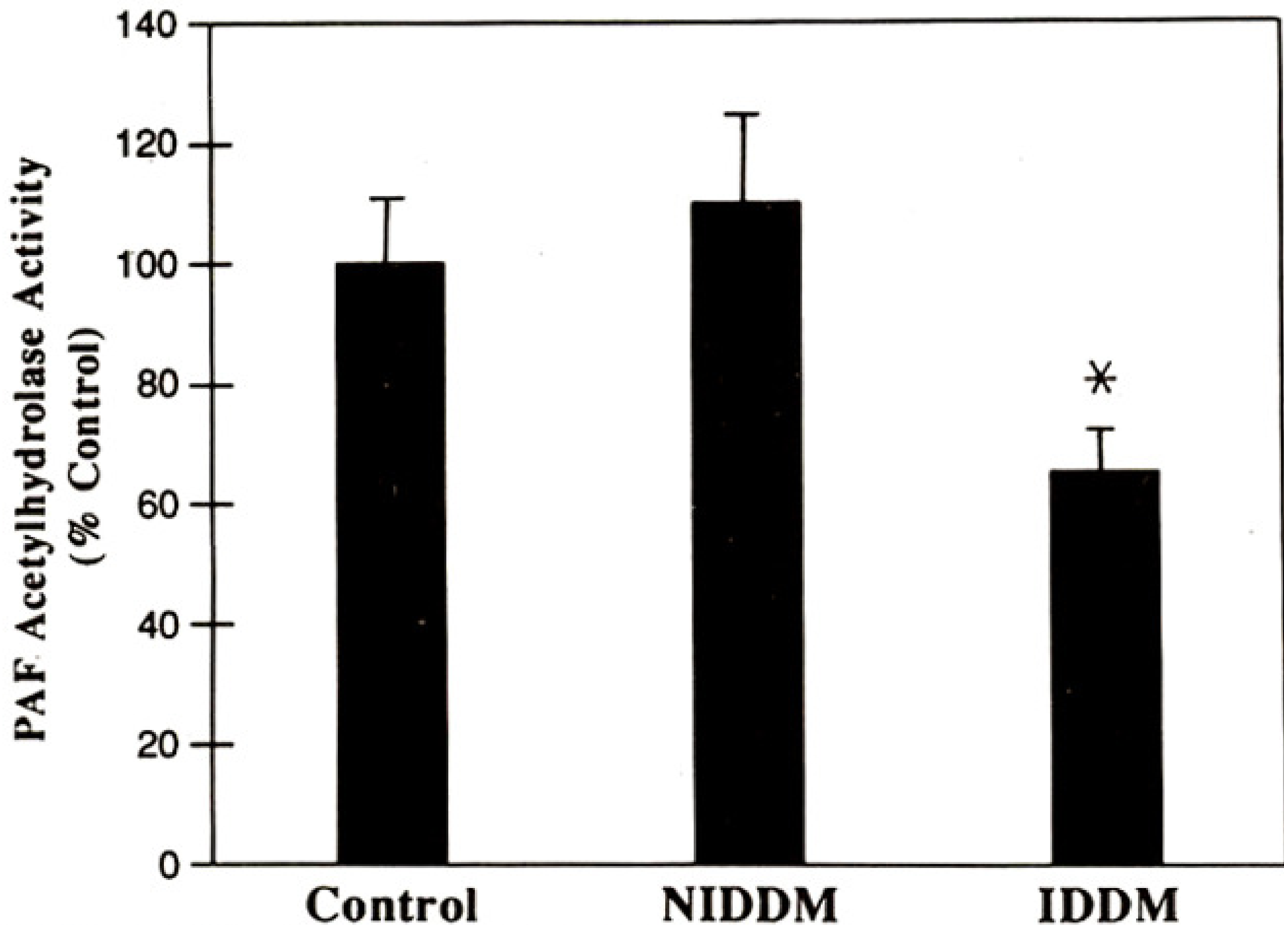

Figure 2. Plasma PAF acetylhydrolase activity in control, NIDDM and IDDM subjects. The number of subjects in each group is 8 and each assay was carried out in duplicate as described under methods.

Figure 2 presents the data on plasma PAF acetyihydrolase activity which is calculated on the basis of radioactivity incorporated into the peak of lyso-PAF formed. There was a 35% decrease in the activity of PAF acetylhydrolase in the plasma of IDDM patients when compared to control subjects (p

Discussion

Platelet activating factors has several physiological functions and also acts as a pathological mediator in allergy, inflammation, asthma and vascular disorders[7,8]. Recent studies have shown that the serum levels of PAF are elevated in IDDM patients[9]. The increase in PAF levels could be due to increased biosynthesis or decreased degradation of PAF. The data presented in this study demonstrate that there is a decrease in the activity of PAP acetyihydrolase in patients with IDDM. A decrease in the PAP acetylhydrolase activity would indicate that the degradation of PAF would be slowed leading to an increase in the serum levels of PAP resulting in an enhancement of its effects. This is supported by the reports that diabetic human platelets show hypersensitivity to PAP in both aggregation as well as in phosphatidic acid production[14]. The plasma PAF acetyihydrolase has recently been shown to prevent oxidative modification of LDL[15]. A decrease in its activity may enhance oxidation of LDL and promote

atherogenesis. Plasma PAP acetylhydrolase is associated with lipoprotein fractions and it s activity may correlate with LDL levels[8]. In our study the levels of total cholesterol, HDL and LDL were similar among control and diabetic subjects and there was no correlation between lipoprotein levels and PAP acetyihydrolase activity. Our results indicate that an alteration in the lipoprotein metabolism is not necessarily required for a change in PAP acetylhydrolase activity. This is further supported by the work of Satoh et al[16] who have shown that PAF acetylhydrolase activity is increased in patients with essential hypertension who have normal plasma lipoprotein levels. Thus, inadditiontolipoproteins, other factors may also be involved in the regulation of PAP acetyihydrolase activity. A decrease in PAP acetylhydrolase activity in IDDM patients will eventually result in enhanced effects of PAF. This in turn can influence the actions of PAP in diabetes. PAF has been shownto induce hepatic glycogenolysis[17]. Anincrease in PAF levels in diabetics can aggravate hyperglycemia. PAF also increases hepatic fatty acid and triglyceride synthesis[18] and thus it may be partly responsible for the hypertriglyceridemia seen in diabetic patients. Finally, the increased responsiveness of platelets of diabetic patients to various aggregating agents including PAP may be responsible for the increased incidence of vascular complications which are commonly found in diabetes[6].

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