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ORIGINAL ARTICLE

Macroprolactinemia, like hyperprolactinemia, may promote platelet activation

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Abstract Insulin resistance, which provides a convenient milieu for platelet activation, has been closely associated with atherosclerotic disorders. Although it often accompanies hyperprolactinemia, findings conflict concerning its clinical impact in macroprolactinemia. In order to investigate the relationship between hyperprolactinemia and platelet activation evidenced by ADP-stimulated P-selectin expression on flow cytometry, we studied hyperprolactinemic, macroprolactinemic, and normoprolactinemic subjects. Thirty-four hyperprolactinemic and 44 age- and body mass index-matched euprolactinemic premenopausal women were included. They were matched regarding insulin sensitivity status, waist circumference, blood pressures, and plasma lipids. In order to detect macroprolactinemia among hyperprolactinemic cases, prolactin was measured before and after polyethylene glycol (PEG) precipitation in patients' sera. P-selectin expression was significantly higher in the hyperprolactinemic group ($P = 0.001$), and 41.2% of them exhibited macroprolactinemia. Expression of P-selectin was comparable between the macroprolactin-negative (monomeric hyperprolactinemia; $n = 20$) and -positive ($n = 14$) subgroups ($P = 0.90$). Both subgroups showed greater expression compared with normoprolactinemic controls ($P = 0.014$ and 0.005, respectively). Platelet activation accompanies the atherosclerotic disorders closely associated with insulin resistance. Among groups matched with regard to insulin-sensitivity markers, both monomeric hyperprolactinemia and macroprolactinemia appeared to promote platelet activation.

Keywords Hyperprolactinemia · Macroprolactinemia · Platelet activation · Insulin resistance

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Introduction

Hyperprolactinemia is often caused by physiological or pathological conditions—pregnancy and lactation or lactotrophic adenoma, for example—and other phenomena that interfere with dopamine secretion. Recently, prolactin has been shown to affect insulin sensitivity by stimulating insulin secretion and regulating adipocytokine release [1]. Hyperinsulinemia and impaired glucose tolerance have been detected among hyperprolactinemic patients with or without pituitary tumors [2, 3]. Insulin resistance also has been linked with changes in blood coagulation homeostasis, such as decreased fibrinolysis and hypercoagulation [4].

A member of the selectin family, P-selectin (also designated PADGEM, GMP-140, and CD62P) is expressed on activated platelets. P-selectin mediates rolling of leukocytes on activated endothelium and promotes development of atherosclerotic lesions. Adhesive platelet interactions mediated by P-selectin are important in both hemostatic

and inflammatory processes [5]. Plasma concentrations of adhesion molecules are increased in patients with established cardiovascular risk factors such as obesity, dyslipidemia, hypertension, and Type 2 diabetes mellitus [6–9]. Identified as a novel cofactor for platelet activation, prolactin also increases P-selectin expression and platelet aggregation in a dose-dependent manner, and the short isoform of the prolactin receptor has been detected on platelets [10, 11].

Because hyperprolactinemia and insulin resistance both affect platelet activation, we wished to examine the association between serum prolactin levels and platelet activation among groups of hyperprolactinemic and euprolactinemic subjects matched with respect to insulin-sensitivity markers. Further, prolactin can be subclassified based on molecular mass into monomeric prolactin (23 kDa), big prolactin (50–60 kDa), and big, big prolactin (macroprolactin, a large antigen–antibody complex of 150–170 kDa). Because previous reports regarding the biological activity of macroprolactinemia are conflicting [12–14], we also assessed the possible relationship between macroprolactinemia and platelet activation.

Materials and methods

Subjects

The Baskent University Ethics Committee for Human Studies approved the study protocol, and all the participants provided written informed consent. We recruited 34 premenopausal women with a serum prolactin level >628.5 mU/l who had been admitted to our outpatient endocrinology clinic between October 2006 and November 2007. Forty-four age- and body mass index-matched healthy premenopausal volunteers with normoprolactinemia were included as control group.

Exclusion criteria included the presence of diabetes mellitus, dyslipidemia, hypertension, hypothyroidism, end-stage renal disease, treatment with any drug that might interfere with serum prolactin, and diseases that could contribute to hyperprolactinemia. Participants had no abnormality other than hyperprolactinemia, and none took medication regularly. All were nonsmokers. Formal tests of hypothalamopituitary function yielded results within normal limits.

Table 1 Characteristics of participants with hyperprolactinemia and normoprolactinemia

	Hyperprolactinemia ($n = 34$)	Normoprolactinemia ($n = 44$)	P
Age (years)	30.79 ± 7.84	29.32 ± 7.79	0.411
Body mass index (kg/m^2)	26.71 ± 5.32	25.16 ± 5.66	0.22
Waist circumference (cm)	81.57 ± 11.08	81.51 ± 12.15	0.98
Glucose at 0 min (mg/dl)	87.29 ± 10.07	85.07 ± 7.81	0.27
Glucose at 120 min (mg/dl)	104.61 ± 30.34	97.56 ± 24.32	0.27
High-density lipoprotein (mg/dl)	57.32 ± 13.02	52.64 ± 12.96	0.12
Triglycerides (mg/dl)			
Mean	94.79	93.35	0.16
Median	87.0	68.0	
Minimum–maximum	44–198	33–344	
Metabolic syndrome (*n*/*n* %)	3/31 (8.8)	3/41 (6.8)	0.53
Prolactin level (mU/l)			
Mean	1845.89	339.44	0.0001
Median	1524.57	324.38	
Minimum–maximum	691.8–3989.52	69.56–584.78	
Fasting insulin level (μIU/ml)			
Mean	9.72	9.22	0.96
Median	8.0	8.1	
Minimum–maximum	2.6–28.10	2.9–27.20	
HOMA-IR			
Mean	2.21	1.95	0.81
Median	1.70	1.62	
Minimum–maximum	0.46–7.29	0.55–6.58	

Continuous variables are expressed as mean ± SD

HOMA-IR homeostasis model assessment of insulin resistance

Table 2 P-selectin expression

	Macroprolactin (−) ($n = 20$)		Macroprolactin (+) ($n = 14$)		Control ($n = 44$)	
	ADP-stimulated (%)	Non-stimulated (%)	ADP-stimulated (%)	Non-stimulated (%)	ADP-stimulated (%)	Non-stimulated (%)
Minimum	0.4	1	0.3	3.7	0.5	1
Maximum	6.6	76.2	2.8	54.7	3.5	23.2
Median	1.3	13.6	1.3	15.3	1.6	6.7

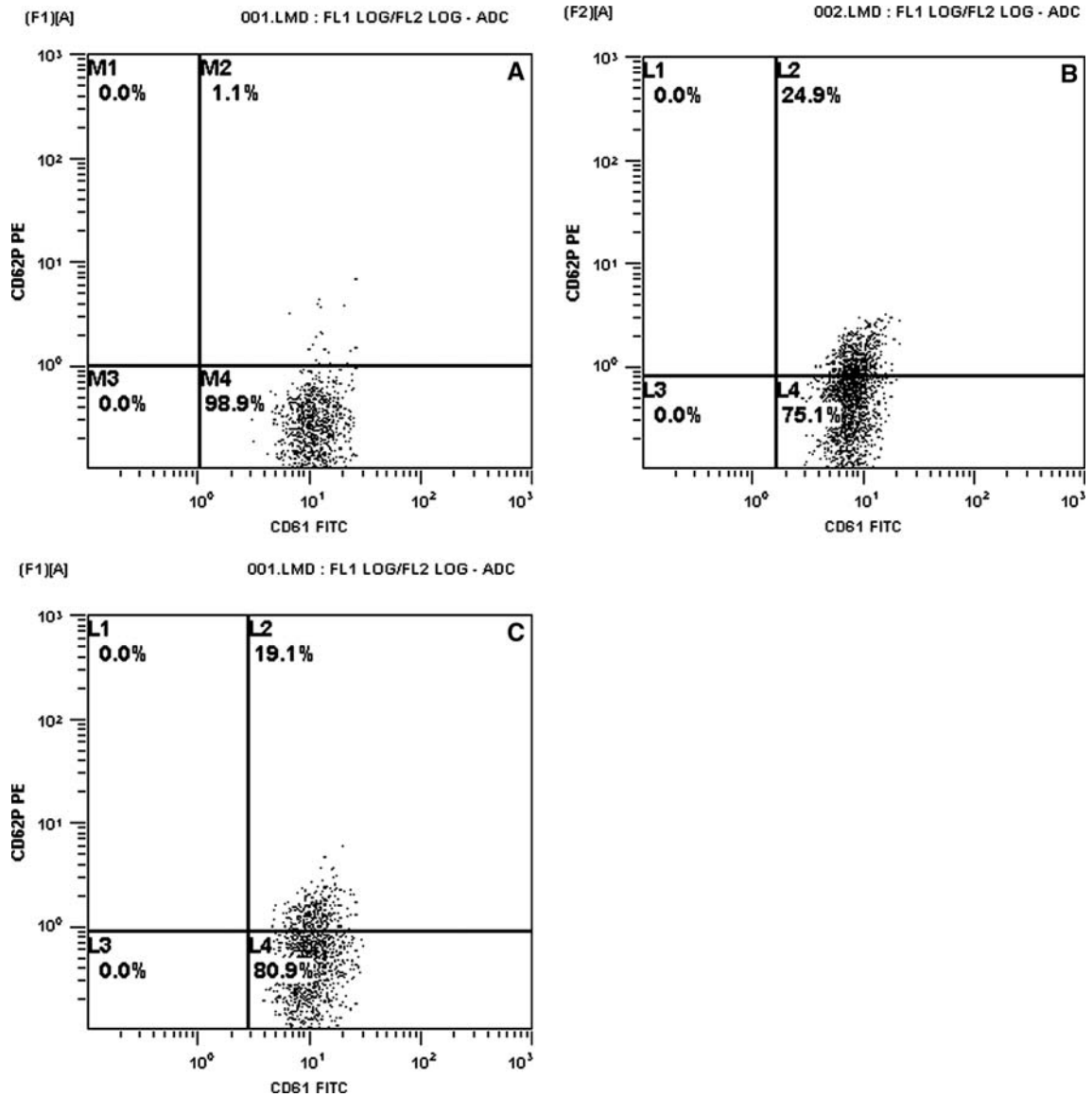

Fig. 1 Typical flow-cytometric plots for platelet activation (P-selectin expression), showing side scatter and positive labeling with the platelet-specific identifier (anti-CD61 antibodies)

Risk factor assessment

After each subject rested in a seated position for ≥10 min, two blood pressure measurements were obtained from the right arm using a mercury sphygmomanometer, and the two readings were averaged. Height, weight, and waist circumference at the umbilicus were measured in light clothing without shoes.

After fasting overnight, all the patients underwent an oral glucose tolerance test (with 75 g of glucose) at about 8 AM the next morning. Fasting levels of insulin, total cholesterol, triglyceride, and high-density lipoprotein

Table 3 Magnetic resonance imaging findings

Finding (n[%])	Macroprolactinemia (−) ($n = 20$)	Macroprolactinemia (+) ($n = 14$)
Microadenoma	11 (55)	5 (35.7)
Macroadenoma	3 (15)	0
Partial empty sella	2 (10)	1 (7.1)
Normal	4 (20)	8 (57.1)

χ^2, $P = 0.065$

cholesterol (HDL) were also measured. Insulin resistance was calculated with the homeostasis model assessment of insulin resistance (HOMA-IR) formula: [fasting insulin (U/ml) × fasting glucose (mg/dl)]/405 [15].

Definition of metabolic syndrome

The criteria of the International Diabetes Federation (IDF) were used to define metabolic syndrome [16]: central obesity (waist circumference of ≥94 cm in men or ≥80 cm in women) and at least two of the following criteria: (a) serum triglyceride level ≥150 mg/dl, (b) serum HDL level <40 mg/dl in men or <50 mg/dl in women, (c) blood pressure ≥130/85 mmHg, or (d) fasting serum glucose level ≥100 mg/dl.

Assays

Serum glucose levels were analyzed with the glucose oxidase method. Homogeneous enzymatic colorimetric assays were used for serum HDL and low-density lipoprotein cholesterol (LDL) measurements, as were enzymatic colorimetric assays for triglycerides. A Roche Modular DP device was used for the analyses mentioned above. Fasting insulin concentrations were determined by a microparticle enzyme immunoassay (AxSYM, Abbott Diagnostics Division, Abbott Park, IL).

Prolactin and macroprolactin assays

Prolactin was measured according to the manufacturer's protocols with a chemiluminescent microparticle immunoassay (Architect Ci8200, Abbott Diagnostics Division, Abbott Park, IL). The intra-assay coefficients of variation (CVs) were 0.223 and 3.2%, the corresponding inter-assay CVs were 0.310 and 4.4%. The reference range was 25.2–628.53 mU/l. Samples from hyperprolactinemic patients were subjected to PEG (8000-BioBasic 3185 B10) to detect the presence of macroprolactin [17]. In brief, 250 µl of serum, mixed with an equal volume of PEG, 250 g/l in phosphate-buffered saline (137 mmol/l sodium chloride; 10 mmol/l sodium phosphate) at pH 7.4, was incubated for 10 min at room temperature. The mixture was centrifuged for 30 min at 3500 rpm. Prolactin was measured both in untreated serum and in the supernatant. The difference between prolactin concentrations in untreated and PEG-treated sera was expressed as prolactin recovery. Diagnosis of macroprolactinemia was regarded as certain if prolactin recovery in a serum sample was below 40%.

Flow-cytometric platelet analyses

Citrated whole blood from subjects with hyperprolactinemia and from healthy volunteers was diluted in phosphate-buffered 0.5% bovine serum albumin (Sigma, St. Louis, MO). The platelet count was adjusted to ∼20,000 platelets/µl. Initially, direct activation of the diluted platelets without ADP stimulation was measured by flow cytometry. Nonactivated, diluted whole blood (20 µl) was incubated at room temperature in darkness for 20 min after adding 10 µl of anti-CD61-FITC (Beckman Coulter, Marseilles, France) and 10 µl of anti-CD62P-PE (Beckman Coulter). In the second step (activation), 20-µl aliquots of the platelet suspension and 10 µmol/l ADP (final concentration) were incubated at 37°C for 5 min. After 5 min, the monoclonal antibodies—10 µl of anti-CD61-FITC and 10 µl of anti-CD62P-PE—were added. This suspension was incubated at room temperature for 20 min in darkness.

After each incubation with antibodies, an EPICS XL-MCL flow cytometer (Beckman Coulter, Miami, FL) and EXPO 32 ADC software were used for analyses. Platelets were identified by binding of anti-CD61 antibodies. After excluding nonspecific binding, the degree of activation was determined by binding of anti-CD62P antibodies. P-selectin expression was expressed as the percentage of platelets positive for the molecule. The normal range for ADP-stimulated P-selectin expression by platelets was determined using blood from 100 healthy blood donors. Typical flow cytometric plots for P-selectin staining are shown in Fig. 1.

Statistical analyses

SPSS software (Statistical Product and Services Solutions, version 12.0, SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Data are expressed as means ± SD, unless indicated. A value for $P < 0.05$ was considered statistically significant. Parametric variables were compared by using t-tests, and categorical variables were compared using χ^2 tests.

Results

Age, body mass index, waist circumference, prevalence of metabolic syndrome, and variables related to metabolic

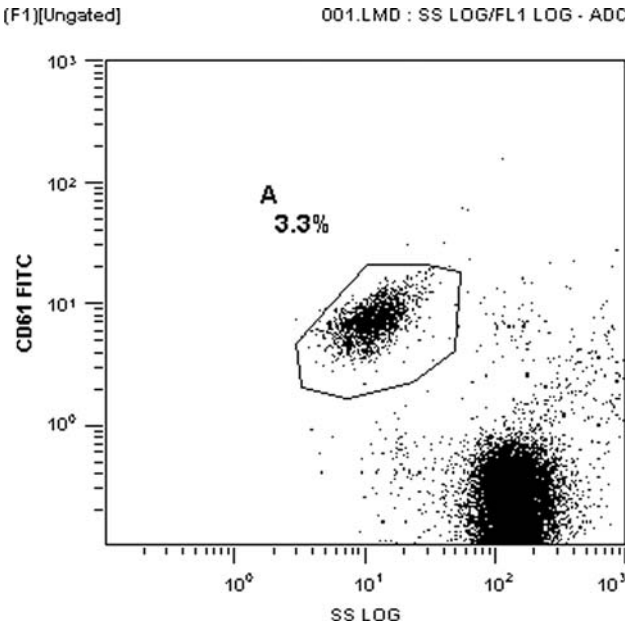

Fig. 2 Flow-cytometric plots of platelet activation, as measured by monoclonal anti-CD62P antibodies, in a normoprolactinemic subject (**a**), a patient with monomeric hyperprolactinemia (**b**), and a macroprolactinemic patient (**c**). ADP-stimulated expression was greater in both monomeric hyperprolactinemic patients and those with macroprolactinemia compared with controls ($P = 0.005$ and $P = 0.003$, respectively), but expression did not differ significantly between the hyperprolactinemic and macroprolactinemic subgroups ($P = 0.90$)

syndrome did not differ significantly between hyperprolactinemic ($n = 34$) and euprolactinemic ($n = 44$) groups (Table 1). Differences in fasting insulin levels and homeostasis model of insulin resistance (HOMA-IR) calculations between groups likewise were not statistically significant. When patients were grouped as macroprolactin-negative, macroprolactin-positive, or euprolactinemic, again, no differences were detected. Macroprolactinemia was detected in 14 (41.2%) of the hyperprolactinemic patients.

ADP-stimulated and non-stimulated P-selectin expression findings are shown in Table 2. ADP-stimulated expression was significantly greater in the hyperprolactinemic group compared with the control patients ($P = 0.001$). ADP-stimulated expression was greater in both macroprolactinemia-negative and -positive hyperprolactinemic subgroups compared with controls ($P = 0.005$ and $P = 0.003$, respectively), but expression did not differ significantly between the two subgroups ($P = 0.90$; Figs. 2a–c).

Some patients' prolactin values remained elevated after polyethylene glycol (PEG) precipitation over 40%. They were considered to have high monomeric prolactin values in addition to macroprolactinemia. There was no difference in platelet activation between macroprolactin-positive and -negative groups when these patients were regarded as macroprolactin-negative.

Signs of macroprolactinemia-negative and -positive hyperprolactinemic subjects—irregular menses, galactorrhea, infertility, alopecia, hirsutism, hyperprolactinemia, and incidentally detected pituitary adenomas—did not differ at admission ($P > 0.05$).

Magnetic resonance imaging (MRI) results, in patients with hyperprolactinemia, are given in Table 3. There were no significant differences in this small subgroup according to the presence or absence of macroprolactinemia.

Discussion

Platelet activation is considered a central mechanism in arterial thrombogenesis and in the pathophysiology of ischemic stroke. Wallaschofski et al. [18] detected high plasma prolactin and leptin concentrations, and increased P-selectin expression, in patients with ischemic stroke or transient ischemic attack. They also showed a significant, positive correlation between prolactin levels and enhanced ADP-stimulated P-selectin expression. Hyperprolactinemia was proposed to be a possible risk factor for stroke by mediating a thrombogenic effect through enhanced platelet reactivity [18]. They also proposed that increased prolactin levels could be a coactivator of ADP-stimulated P-selectin expression in acute coronary syndromes [19]. However, a very recent report noted that prolactin did not effect platelet activation [20]. The controversy has been attributed to several possible technical explanations [20].

Levels of plasma adhesion molecules are elevated in patients with obesity, dyslipidemia, hypertension, and Type 2 diabetes, all being established risk factors for cardiovascular diseases [6–9]. Activated platelets also have been identified in the circulating blood of patients with coronary artery disease [8, 21, 22].

Our study and control groups were matched regarding the factors known to influence insulin sensitivity, such as age, body weight, and fasting glucose level. Considering the information from the existing literature and this study, we propose that prolactin has a direct effect of on platelets.

This study, in keeping with previous studies, shows a positive correlation between hyperprolactinemia and platelet activation upon ADP stimulation. Although reports concerning the clinical impact of macroprolactinemia are conflicting [12–14], macroprolactinemia, like 23-kDa hyperprolactinemia, also appears to be associated with platelet activation.

Several investigators have examined the bioactivity of macroprolactin using in vitro assays, but results of these studies differed, showing increased, normal, or reduced

prolactin activity in sera from patients with macroprolactinemia [23, 24]. The conflicting findings could be attributed to differences in prolactin assays. Moreover, macroprolactin, a complex of monomeric prolactin with a large molecular mass, might have difficulty in exiting from the capillaries, resulting in lower effective bioavailability [24, 25].

Our results support the literature proposing the idea of weak in vivo activity of macroprolactin. This issue is particularly important because macroprolactinemia is not uncommon. Indeed, and in agreement with other articles [12], the prevalence of macroprolactinemia in our hyperprolactinemic population was 41.2%.

The clinical presentation of macroprolactinemia in some patients has been reported to be oligomenorrhea or amenorrhea, galactorrhea, or infertility, as often seen with hyperprolactinemia. Some authors have proposed that occurrence of these symptoms together with macroprolactinemia might be coincidental [26, 27]. However, among the 106 patients with macroprolactinemia studied by Valette-Kasic et al. [28], several had these symptoms, and 4 of the patients had histologically proven prolactinoma. In this study, we detected no difference in symptoms at admission between the macroprolactinemic and monomeric hyperprolactinemic patients.

The numbers of subjects harboring an adenoma or partial empty sella were similar between the macroprolactinemic and monomeric hyperprolactinemic subgroups in our study. Mounier et al. [29] suggested that prolactinoma might be associated with macroprolactinemia; five of their 13 patients with prolactinoma had macroprolactinemia, and the clinical and biological characteristics of the groups with and without macroprolactinemia were similar. An article by Olukoga and Kane described 1 adenoma found by pituitary imaging among the three macroprolactinemic patients [30].

In conclusion, platelet activation is involved in the pathogenesis of atherosclerotic disorders that are strongly related to insulin resistance. In this study, between two groups of patients matched for insulin-sensitivity markers, hyperprolactinemia per se was shown to increase platelet activation. Our data also indicate the impact of macroprolactinemia on platelet activation. We believe that this new finding may highlight the importance of treating macroprolactinemia. Further studies are needed to clarify the metabolic consequences of macroprolactinemia.

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