The Journal of International Medical Research
2007; 35: 704 – 708

Lack of Platelet Activation Reflected by Circulating Soluble Glycoprotein V in Pre-eclampsia

K Acar[1], A Sucak[2], Y Beyazit[3], G Genc[2], IC Haznedaroglu[4], S Aksu[4] and N Danisman[2]

[1]Department of Haematology, and [2]Department of Perinatology, Zekai Tahir Burak Women's Health, Education and Research Hospital, Ankara, Turkey; [3]Department of Internal Medicine, and [4]Department of Haematology, Hacettepe University Medical School, Ankara, Turkey

Pre-eclampsia (PE) is a human pregnancy-specific disorder of unknown aetiology. Although the quantitative relationship between platelet aggregation in PE is not clearly defined yet, we aimed to investigate the possible relationship between PE and platelet glycoprotein V (GPV), which is an integral platelet membrane protein involved in the function of the GPIb-V-IX receptor. Fifty patients with PE and 37 normotensive pregnant women (controls) were enrolled in this study. Fasting blood samples were collected and soluble GPV (sGPV) levels were determined using a commercially available enzyme immunoassay. No statistically significant difference in sGPV was found between PE patients and control subjects. There was no correlation between sGPV and platelet counts or between pregnancy duration and platelet counts. Further clinical and experimental investigations are needed to elucidate the pathological processes involved in the development of PE in complicated pregnancies.

KEY WORDS: Pre-eclampsia; Pregnancy; Blood pressure; Platelet counts; Soluble glycoprotein V

Introduction

The change from the non-pregnant to the pregnant state in females is associated with activation of the haemostatic system, which is an integral part of an inflammatory reaction.[1 – 5] Enhanced haemostasis is even more pronounced when pregnancy is complicated by pre-eclampsia (PE)[1] and PE occurs in 6 – 8% of all pregnancies worldwide. Research on the biomarkers of vascular endothelium and leucocyte activation has suggested that PE may arise from a maternal systemic inflammatory response associated with endothelial dysfunction.[6] Platelet hyperfunction could also complicate the pathobiological processes in PE, according to the findings of several investigations into thrombocytes and their metabolites in PE.[1,3,7 – 9] Whether 'platelet hyperfunction' is a cause or effect in PE remains to be investigated, however, since endothelial dysfunction may also lead to 'secondary' platelet activation.

Platelet glycoprotein V (GPV) is an integral membrane protein of platelets that is involved in the function of the GPIb-V-IX receptor.[10] The circulating soluble form of GPV (sGPV) serves as a biomarker of *in vivo* platelet activation.[10–12] The aim of this study was to assess circulating sGPV concentrations in patients with PE. Since sGPV indicates ongoing platelet activation, sGPV levels may demonstrate primary platelet activation in PE patients. Elucidation of the function of platelets is important because antiplatelet drugs have been suggested for the clinical management of PE patients.[13]

Patients and methods

STUDY POPULATION

Patients with PE and normotensive pregnant subjects (controls) were enrolled in the study. PE was diagnosed when the blood pressure was ≥ 140/90 mmHg for the first time during pregnancy after 20 weeks of gestation, and proteinuria was ≥ 300 mg/day or 30 mg/l (1+ dipstick) in random urine samples. A normal normotensive pregnancy was defined as a singleton pregnancy, with a diastolic blood pressure of ≤ 85 mmHg and no proteinuria. Patients with HELLP syndrome (haemolytic anaemia, elevated liver enzymes and low platelet count) and intrauterine exitus were excluded from the study. The study was performed in Zekai Tahir Burak Women's Health, Education and Research Hospital in accordance with the Declaration of Helsinki and each participant gave written informed consent.

LABORATORY ANALYSES

Fasting blood samples were collected from a large peripheral vein of each study subject without using a tourniquet. The first 2.0 ml of blood was discarded, then 4.5 ml was drawn into a Diatube-H CTAD (sodium citrate, theophylline, adenosine and dipyridamole) tube (Becton Dickinson, Plymouth, UK). The tube was immediately placed on ice and was centrifuged within 1 h at 2500 ***g*** at 2 – 8 °C for 20 min. The supernatant was centrifuged again at 2500 ***g*** at 2 – 8 °C for 20 min to prepare platelet-depleted plasma, which was stored at –80 °C until the thrombosis markers were measured. Soluble GPV levels were determined using a commercially available enzyme immunoassay (Asserachrom® Soluble GPV, Stago, Asnières, France). The assay was performed according to the manufacturer's instructions.

STATISTICAL ANALYSES

Statistical analyses were performed with SPSS® version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Results are given as the mean ± SD. Student's *t*-test was used to analyse any differences between the two groups. Pearson's correlation coefficient was used for correlation analysis. A *P*-value < 0.05 was considered to be statistically significant.

Results

Fifty patients with PE and 37 normal normotensive pregnant women (controls) were enrolled in this study and their clinical characteristics are shown in Table 1. There were no significant differences between the two study groups with respect to age, duration of pregnancy (weeks), haemoglobin level, leucocyte counts and sGPV levels. Although the platelet counts were normal in both groups, those of the PE patients were significantly lower than the control subjects ($P < 0.05$). Correlation analyses showed no association between sGPV levels and platelet counts, sGPV levels and blood pressure, or between pregnancy duration and platelet counts. We did,

TABLE 1:
Clinical characteristics and soluble glycoprotein V levels of pre-eclamptic patients and control normotensive pregnant subjects

	Pre-eclampsia patients (n = 50)	Normotensive pregnant subjects (n = 37)	P-value
Age (years)	26.74 ± 5.68	26.70 ± 4.70	
[range]	[17 – 39]	[18 – 38]	NS
Week of pregnancy	32.92 ± 3.37	31.81 ± 4.14	NS
Haemoglobin (g/dl)	11.64 ± 1.62	11.26 ± 1.21	NS
Leucocyte count (x10^3/mm^3)	11.5 ± 3.5	12.6 ± 3.2	NS
Platelet count (x10^3/mm^3)	213 ± 73	250 ± 86	< 0.05
SBP (mmHg)	150.80 ± 16.14	115.14 ± 12.82	< 0.001
DBP (mmHg)	98.80 ± 9.39	72.16 ± 8.54	< 0.001
sGPV (ng/ml)	50.29 ± 12.43	48.32 ± 10.49	NS

Values are mean ± SD. NS, not significant; SBP, systolic blood pressure; DBP, diastolic blood pressure; sGPV, soluble glycoprotein V.

however, find a negative correlation between systolic/diastolic blood pressure and platelet counts (r^2 = –0.222 and –0.243, $P < 0.05$).

Discussion

In this study, there were no significant differences in the sGPV levels between PE patients and normal normotensive pregnant subjects, although the platelet counts of PE patients were significantly lower than those of the controls. Since sGPV is a biomarker of platelet activation,[10 – 12] the pathophysiology of PE may not be explained solely by the hyperfunction of thrombocytes. Previous studies, that particularly focused on P-selectin, proposed that platelet activation is prominent in PE.[7,8] P-selectin is a marker located at the junction between the endothelium and platelets.[14] Vascular endothelial dysfunction and neutrophil activation have been well documented in PE,[1,6,15] and the endothelial expression and regulation of platelet activating receptors have also been described.[16] Platelets from PE patients have been found to exhibit no significant thrombopoietin potentiation,[17] which is quite important in platelet activation.[18] Moreover platelet activation suppressors, such as adenosine, may compensate and tend to prevent excessive platelet activation in PE.[8] Taken together, platelet function in PE seems to be related to the interactions of numerous vascular endothelial and leucocyte molecules. The findings of our present study with sGPV in PE suggest that the platelets of PE patients do not tend to be primarily activated. Their functions seem to be affected by the complicated ongoing events of systemic generalized inflammation, vascular endothelial dysfunction and leucocyte hyperfunction.[6,19 – 21]

The preventive use of antiplatelet drugs is a matter of debate in PE.[13] The rationale for using antiplatelet drugs, such as low-dose aspirin, in PE is based on the findings that deficient intravascular production of prostacyclin, which is a vasodilator, and excessive production of thromboxane, which is a vasoconstrictor and stimulant of platelet aggregation, were evident in PE.[13] A meta-analysis of 59 trials (37 560 women with PE)

revealed that there may be a 17% reduction in the risk of PE associated with the use of antiplatelet agents.[13] This meta-analysis also demonstrated that, although there was no statistical difference in the relative risk based on maternal risk, there was a significant increase in the absolute risk reduction of PE for high-risk compared with moderate-risk women.[13] There were no statistically significant differences between treatment and control groups for many other outcomes.[13] Currently little is known, therefore, about which women with PE are most likely to benefit, when antiplatelet agents should be started and at what dose they should be used.[13] The findings of our present study with sGPV in PE suggest that there may be at least a subpopulation of PE patients in whom platelet activation, if it occurs, is not the primary event. Their disease management, therefore, should focus on the systemic generalized inflammation, vascular endothelial dysfunction and leucocyte hyperfunction,[6,19 – 21] rather than taking an antiplatelet approach.

The haemostatic changes that occur during pregnancy are strictly regulated by numerous regulatory molecules of primary and secondary haemostasis that are active at times of health or disease.[4,5,19,22] These changes may be due to the ongoing maternal systemic inflammatory response associated with endothelial dysfunction.[6] The findings of our present study suggest that the platelets of PE patients might not be the only components involved in the pathological haemostasis characteristic of PE. The enhanced haemostasis observed in PE patients appears to result from upregulated inflammation, vascular endothelial dysfunction and leucocyte hyperfunction.[6,19 – 21] Coagulation proteins, natural anticoagulant molecules, profibrinolytic enzymes and antifibrinolytic regulators should all be considered, therefore, when investigating the pathological haemostatic processes observed in PE, and in the anticoagulant disease management of PE patients in the clinic.

Further clinical and experimental investigations are required in order to understand fully the pathological processes involved in the development of PE in the complications of pregnancy. It is hoped that further research will lead to the development of improved clinical strategies for the management of PE in pregnant women.

Conflicts of interest

No conflicts of interest were declared in relation to this paper.

• Received for publication 13 May 2007 • Accepted subject to revision 4 June 2007
• Revised accepted 6 August 2007

References

1 Holthe MR, Lyberg T, Staff AC, *et al*: Leukocyte-platelet interaction in pregnancies complicated with preeclampsia. *Platelets* 2005; **16:** 91 – 97.
2 Holthe MR, Staff AC, Berge LN, *et al*: Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. *Am J Obstet Gynecol* 2004; **190:** 1128 – 1134.
3 Pekonen F, Rasi V, Ammala M, *et al*: Platelet function and coagulation in normal and preeclamptic pregnancy. *Thromb Res* 1986; **43:** 553 – 560.
4 Haznedaroglu S, Ozcan T, Malkoc S, *et al*: Hemostatic markers in preterm labor. *Thromb Res* 1997; **86:** 89 – 90.
5 Haznedaroglu S, Ozcan T, Karaaslan Y, *et al*: Fibrinolytic activation with intrauterine fetal death. *NZ Med J* 1998; **111:** 130.
6 Poston L: Endothelial dysfunction in pre-eclampsia. *Pharmacol Rep* 2006; **58**(suppl): 69 – 74.

7 Lok CA, Nieuwland R, Sturk A, *et al*: Microparticle-associated P-selectin reflects platelet activation in preeclampsia. *Platelets* 2007; **18:** 68 – 72.
8 Yoneyama Y, Suzuki S, Sawa R, *et al*: Plasma adenosine levels and P-selectin expression on platelets in preeclampsia. *Obstet Gynecol* 2001; **97:** 366 – 370.
9 Socol ML, Weiner CP, Louis G, *et al*: Platelet activation in preeclampsia. *Am J Obstet Gynecol* 1985; **151:** 494 – 497.
10 Ravanat C, Freund M, Mangin P, *et al*: GPV is a marker of *in vivo* platelet activation – study in a rat thrombosis model. *Thromb Haemost* 2000; **83:** 327 – 333.
11 Atalar E, Haznedaroglu IC, Kilic H, *et al*: Increased soluble glycoprotein V concentration during the acute onset of unstable angina pectoris in association with chronic cigarette smoking. *Platelets* 2005; **16:** 329 – 333.
12 Blann AD, Lanza F, Galajda P, *et al*: Increased platelet glycoprotein V levels in patients with coronary and peripheral atherosclerosis – the influence of aspirin and cigarette smoking. *Thromb Haemost* 2001; **86:** 777 – 783.
13 Duley L, Henderson-Smart D, Meher S, *et al*: Antiplatelet agents for preventing pre-eclampsia and its complications. *Cochrane Database Syst Rev* 2007; issue 2: article CD004659.
14 Ertenli I, Kiraz S, Arici M, *et al*: P-selectin as a circulating molecular marker in rheumatoid arthritis with thrombocytosis. *J Rheumatol* 1998; **25:** 1054 – 1058.
15 Uszynski W, Uszynski M: Two hypotheses about preeclampsia: endothelium injury and inflammation. *Ginekol Pol* 2003; **74:** 237 – 245.
16 Wang Y, Gu Y, Lucas MJ: Expression of thrombin receptors in endothelial cells and neutrophils from normal and preeclamptic pregnancies. *J Clin Endocrinol Metab* 2002; **87:** 3728 – 3734.
17 Carroll RC, Owers RL, Elder RF, *et al*: Lack of thrombopoietin potentiation of platelet collagen activation in the first trimester is associated with preeclampsia. *Thromb Res* 2005; **116:** 51 – 54.
18 Haznedaroglu IC, Goker H, Turgut M, *et al*: Thrombopoietin as a drug: biologic expectations, clinical realities, and future directions. *Clin Appl Thromb Haemost* 2002; **8:** 193 – 212.
19 Estelles A, Gilabert J, Aznar J, *et al*: Changes in the plasma levels of type 1 and type 2 plasminogen activator inhibitors in normal pregnancy and in patients with severe preeclampsia. *Blood* 1989; **74:** 1332 – 1338.
20 Eskenazi B, Fenster L, Sidney S: A multivariate analysis of risk factors for preeclampsia. *JAMA* 1991; **266:** 237 – 241.
21 Mahaba HM, Ismail NA, El Damaty SI, *et al*: Pre-eclampsia: epidemiology and outcome of 995 cases. *J Egypt Public Health Assoc* 2001; **76:** 357 – 368.
22 Ozturk MA, Haznedaroglu IC, Turgut M, *et al*: Current debates in antiphospholipid syndrome: the acquired antibody-mediated thrombophilia. *Clin Appl Thromb Haemost* 2004; **10:** 89 – 126.

Author's address for correspondence
Dr K Acar
Department of Haematology, Zekai Tahir Burak Women's Health, Education and Research Hospital, Ankara, Turkey.
E-mail: acarkadir@yahoo.com