

Original Article

GPIb α reflects the development and progress of the patients with severe preeclampsia

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Abstract: Preeclampsia (PE) is a pregnancy-specific syndrome that occurs in a previously normotensive woman. Some data suggested that the activation parameters of platelets in preeclampsia. The aim of this study is to determine whether the levels of GPIb α and GPIIb for patients with preeclampsia were enhanced after cesarean section. In this study, detecting levels of GPIb α and GPIIb by flow cytometry (FCM). The venous blood of 48 severe preeclampsia women, 16 mild preeclampsia and 22 normotensive women, were collected before operation and 72 hours after the operation. Blood samples were obtained also from 20 non-pregnant women. Results: The level of GPIb α of the normotensive pregnancy was lower than the control group, but there was no significance ($P > 0.05$). The level of GPIb α of the severe preeclampsia group was much lower than other groups ($P < 0.01$). In the severe preeclampsia group, the level of GPIb α of postoperative patients was higher than preoperative patients ($P < 0.01$). There was no significance of GPIIb levels between each group ($P > 0.05$). In conclusion, GPIb α was an important index of reflecting the change of severe preeclampsia. Detecting the levels of GPIb α plays an important role in observing the development of this disease and guiding clinical treatment.

Keywords: Preeclampsia, platelet glycoprotein, flow cytometry, platelet aggregation

Introduction

Preeclampsia (PE) is a pregnancy-specific syndrome that occurs in a previously normotensive woman. It occurs in about 5 to 10% of pregnancies and continues to be a major cause of maternal and perinatal morbidity and mortality [1]. It is a multisystem disease of unknown etiology, and the underlying pathogenic mechanism appears to be a complex interaction of the placental and maternal tissues [2] leading to generalized endothelial function in the normal shift of hemostatic equilibrium toward hypercoagulability. Alternation in coagulation, fibrinolysis, platelet, and vascular endothelial function are believed to play an important role in the pathogenesis of preeclampsia. A recent study by Robb et al. [3] looked at the influence of normal pregnancy and P-EC on platelet activation. They demonstrated a progressive increase in in-vivo platelet activation during normal gestation. This did not seem to be a feature of established P-EC, however, the finding are in contrast

to a lot of previous studies showing pronounced expression of platelet activation markers including CD32, CD61, CD42a, CD62P and CD63 in P-EC. Both of these studies [4, 5] also showed evidence of platelet activation in normal pregnancy when compared with non-pregnant women, but other flow cytometry studies have been unable to demonstrate this phenomenon [6-8]. Thus, the precise determination of the extent of platelet activation both in normal pregnancy and in each of the preeclampsia patients remains ill-defined.

Most of the data suggested that the activation parameters of platelets in preeclampsia, several studies involved on the resting platelets and got paradoxical results [9-11]. The aim of this study was to determine whether platelet surface glycoprotein GPIb α and GPIIb were enhanced by preeclampsia. We also wanted to investigate whether the platelet surface change at the third trimester of normal pregnancy or

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glycoproteins GPIb α (cd42b) and GPIIb (cd41) occurred after cesarean section.

Materials and methods

This study was carried out on 48 severe preeclampsia pregnant females admitted to the hospital between September 2007 and July 2009. 16 cases had mild preeclampsia; 22 uncomplicated pregnant females and 20 non-pregnant females were included as control groups. The cases were selected from the Obstetrics and Gynecology Department of the second Hospital of Jilin University. Consent was obtained from all women before inclusion in the study. Patient age ranged between 20-37 years, with a gestational age between 32-39 weeks. All cases were primigravida with singleton pregnancy. Exclusion criteria included chronic hypertension, hemostatic abnormalities, cancer, diabetes, obesity, and cardiovascular, autoimmune, renal, and hepatic diseases.

We diagnosed cases of preeclampsia according to the criteria proposed by the American College of Obstetricians and Gynecologists. Preeclampsia was defined as the presence of hypertension associated with proteinuria after the 20th week of gestation in women known to be normotensive. Mild preeclampsia was defined as a blood pressure of at least ≥ 140 mm Hg (systolic) and/or 90 mm Hg (diastolic) on two occasions of up to 4-6 hours apart. The blood pressure was measured on the right arm and with the patient always in the seated position. Proteinuria was defined as urinary excretion of 300 mg or more of protein in 24 hours. Severe PE was defined as systolic blood pressure ≥ 160 mm Hg or diastolic blood pressure ≥ 110 mm Hg on at least 2 consecutive occasions, 4-6 hours apart; and proteinuria 2 g/l or at least 3+ protein by dipstick. The normotensive pregnant women had systolic/diastolic blood pressure of 120/80 mm Hg and no history of hypertension or proteinuria. The non-pregnant women had neither clinical alterations nor a history of PE or hypertension [12]. Cesarean section had been performed in all of the pregnant patients.

Gestational age was determined from the last normal menstrual period and confirmed by fetal biometry at the routine ultrasound scan at 16-20 weeks, or first trimester measurement of crown rump length.

Reagent and instrument

CD42b-FITC (Becton-Dickinson), CD41-PE (Biollegend) Flow cytometry (FACS AriaTM cell sorter, BD).

Blood samples

For the preeclampsia women the first collect blood time was after admission to the hospital, but before drug usage. For the normotensive women the first collect blood time was before the operation. For the non-pregnant women the first collect blood time was a random time. Except for non-pregnant women, the second collect blood time was within 72 hours after the cesarean section for the other groups. Venous blood was collected from the patient's antecubital fossa with a 21-gauge needle without tourniquet. An EDTA vacutainer blood-collecting tube (Becton Dickinson, Rutherford, BNJ) was filled first to obtain an automated platelet count. Any platelet counts that generated an analyzer "flag" were recounted manually. For flow cytometric analysis, whole blood samples were placed into 109 mmol/L sodium citrate anticoagulant tubes puisne (1:9). 10 μ l CD42b-FITC monoclonal antibody and 10 μ l CD41-PE monoclonal antibody was added to this test tube. 5 μ l whole blood and 50 μ l phosphate buffered saline (PBS) was added into the control tubes and the test tubes, and they were placed in a dark room for 20 minutes at room temperature. 4-8°C precooled 1% paraformaldehyde 1 ml was then added, to be detected by the flow cytometry within 6 hours.

Flow cytometry

For FCM forward angle scatter (FSC), side angle scatter (SSC) and fluorescence detection signal FITC, the PE was set to logarithmic (Log) mode. The flow rate was set low in order to reduce cell adhesion. According to FSC and SSC light scattering signals, we set the platelet gates on the basis of the scatter distribution, and we properly adjusted FITC and PE compensation. In CD41-PE/SSC dot plot draw platelet gates, each time we measured more than 10,000 platelets. CD42b-positive platelets in platelet results are expressed as a percentage of total positive results.

Statistical analysis

BD FACSD software Flow cytometric data was collected from BD FACSDiva software. Analysis

Table 1. Levels of blood pressure and platelet GPIb α and GPIIb of each group before caesarean section

Groups	No	Blood pressure (mmHg*)			GPIb α (%)	GPIIb (%)
		Systolic	Diastolic	MAP		
Non-pregnant	20	98.66 \pm 5.84	73.38 \pm 6.45	83.83 \pm 6.73	97.01 \pm 1.28	97.87 \pm 0.62
Normotensive	22	113.28 \pm 7.45	73.01 \pm 7.72	85.47 \pm 6.67	96.66 \pm 1.62	97.97 \pm 0.57
Mild preeclampsia	16	142.98 \pm 12.87	95.94 \pm 10.18	112.47 \pm 9.42	96.15 \pm 1.65	97.82 \pm 0.72
Severe preeclampsia	32	169.23 \pm 23.28	110.87 \pm 14.80	133.28 \pm 13.45	89.17 \pm 4.62 ^{①,②,③}	98.33 \pm 1.15

*1 mmHg = 0.133 kPa; ①compared with non-pregnant group, $F = 13.0276$, $t' = -9.0593$, $t' (0.01) = 2.7568$, $P < 0.01$; ②compared with normotensive group $F = 8.1331$, $t' = -8.4467$, $t' (0.01) = 2.7572$, $P < 0.01$; ③compared with mild preeclampsia $F = 7.84$, $t' = -7.6287$, $t' (0.01) = 2.7852$, $P < 0.01$.

was performed with SPSS software, version 13 (SPSS Inc Chicago, IL). All results were expressed as mean \pm SD. A P -value < 0.05 was considered statistically significant. Qualitative unpaired clinical data were compared between two or three groups by student's tests. Comparisons between two groups were performed by Student's t -test or Mann-Whitney U -tests.

Results

Severe preeclampsia and control characteristics

All of the groups were of similar age, parity and gestational age. Blood samples from pregnant controls and women with preeclampsia were drawn at the same time of gestation with a median in both groups of 38 weeks. No women were in labor.

Expression of GPIb α on platelets

Lower percentages of GPIb α (CD42b) expressing microparticles could be detected in the blood of severe preeclampsia. There was a significant statistical difference compared to other groups (**Table 1**; **Figure 1**, $P < 0.01$). Although the percentage of GPIb α in mild preeclampsia women was lower than that in normotensive and non-pregnant women, no significant difference was detected ($P > 0.05$). There was no difference between the normotensive group and non-pregnant group ($P > 0.05$).

The percentages of (CD41) expressing microparticles in all of the groups were no different ($P > 0.05$) (**Table 1**).

GPIb α and GPIIb expression in different groups

The platelet surface density of GPIb α after operation was higher in mild preeclampsia and

normotensive women than before the cesarean section, but it has no different significance ($P > 0.05$). The platelet surface density of GPIb α after the operation was significantly higher in samples from severe preeclampsia than before the operation; there was difference between before and after the operation ($P < 0.01$). No significant differences were seen in GPIIb level of every group between before the operation and after the operation (**Table 2**, $P > 0.05$).

Discussion

Preeclampsia is a disorder of a leading cause of maternal and perinatal morbidity and mortality worldwide. Some research has shown that the aggregation/adhesion/activation of platelets were important issues for the preeclampsia development. The activation-dependent decrease in the binding of MoAbs to the platelet GPIb α -IX-V complex may be a sensitive marker [13]. Our study detected the changes of platelet surface glycoprotein in different groups and discussed the clinical significance of the glycoprotein.

Most of the circulating platelets were in a resting state; they couldn't adhere to each other and adhere to the vessel endothelium. But when the resting platelets were activated, the platelets surface glycoprotein GPIb α exhibited conformational changes and distribution, then combined with the respective protein receptor to trigger the adhesion and activation. The GPIb α was encoded by gene mapping to chromosomes 17p12 [14]. GP Ib is the major subunit and presents binding sites for most extracellular ligands involved in platelet adhesion to von Willebrand factor (vWF) on the injured vascular wall [15, 16]. It played an important role on platelet aggregation and adhesion during the formation of thrombus.

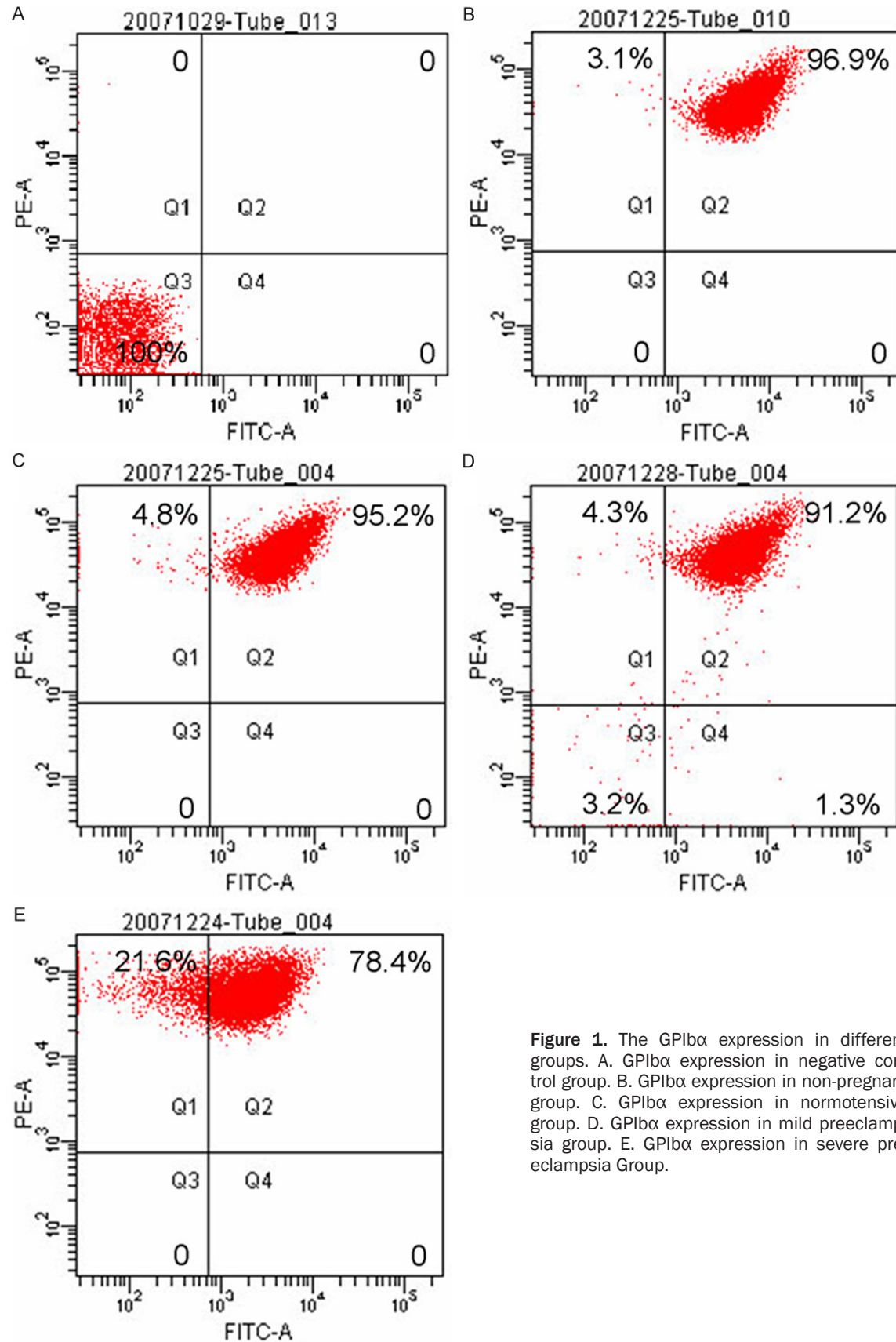


Figure 1. The GPIb α expression in different groups. A. GPIb α expression in negative control group. B. GPIb α expression in non-pregnant group. C. GPIb α expression in normotensive group. D. GPIb α expression in mild preeclampsia group. E. GPIb α expression in severe preeclampsia Group.

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Table 2. Levels of platelet GPI b $\alpha\alpha$ and GPIIb of each group before and after caesarean section ($\bar{x} \pm s$)

Group	No	GPIb α		GPIIb	
		Before	After	Before	After
Normotensive	22	96.66 \pm 1.62	97.13 \pm 1.17	97.97 \pm 0.57	97.78 \pm 0.98
Mild preeclampsia	16	96.1 \pm 1.65	96.82 \pm 1.19	97.82 \pm 0.72	97.88 \pm 1.21
Severe preeclampsia	32	89.17 \pm 4.62	96.67 \pm 1.27 ^①	98.33 \pm 1.15	98.57 \pm 1.03

① Compared with before the caesarean section operation, $P < 0.01$.

Some studies have confirmed that GPIb α expression reduction was in mellitus [17], chronic renal failure [18], and coronary artery disease [16]. Gonzalez-Quintero et al. found that [19, 20] in vivo levels of both CD31C/42b and CD62 in EMP were elevated significantly in patients with preeclampsia and patients with gestational hypertension when they were compared with control subjects. In our study the CD42b (GPIb α) was significantly reduced in severe preeclampsia compared to other groups. This was different to the Thomas et al.'s study [21]. Some research had confirmed the CD42b (GPIb $\alpha\alpha$) expression decreased was a sensitive marker of platelet activation [14, 22]. The explanation might be hypothesized as follows: 1, when the blood vessel wall was damaged in severe preeclampsia, subendothelial structures were exposed to flowing blood. Collagen was the most abundant thrombogenic protein present in the subendothelial matrix. The GP Ib-V-IX receptor complex and its main ligand-von Willebrand Factor (vWF) resulting in a shear-mediated structural change in the molecule that allowed GP Ib to bind to the vWF A1 domain. This was considered to be primarily an adhesive interaction characterized by a fast dissociation rate that slowed down platelets [16]. If this adhesion on endothelial cells had a certain repair, this might further lead to platelet aggregation, and small artery lumen caused both microthrombus changes also increased thrombotic tendency, increased tissue organ ischemia and hypoxia. 2 Systemic vascular dysfunction or vasospasm could lead to blood flow state changes. At that time the shearing force flow was far higher than physiological levels; it caused the platelet aggregation and rapid activation. This process was generally considered that the mechanism of accumulation of high shear forces first platelet GPIb α /IX adhesion to vWF interaction occurs, then GPIIb/IIIa and the

role of vWF factor, platelet aggregated form a solid. Severe preeclampsia patients were with seriously systemic small artery spasm, thus formed abnormally high levels of blood flow shear stress environment, which might directly induce platelet adhesion and aggregation. Our data showed that GPIb $\alpha\alpha$ reduction in severe preeclampsia was significant while GPIb $\alpha\alpha$ reduction observed in the mild preeclampsia change was not significant. We analyzed the presence of small artery spasm in mild preeclampsia patients without endothelial cell injury, and given appropriate antispasmodic comprehensive treatment, status recovery is possible. For some severe preeclampsia patients associated with platelet GPIb $\alpha\alpha$ decreasing levels, the patients were in states with vascular endothelial cell injury, platelet activation, adhesion and aggregation of consumption, and prothrombotic state. As we expected 72 hours postpartum GPIb $\alpha\alpha$ levels were significantly higher than antepartum in severe preeclampsia patients, reached normal maternal levels. It showed that with the termination of pregnancy, fetal placental tissue discharge, and elimination of all the factors causing endothelial injury, vascular endothelial injury rapidly relieved, platelet activation state improved, and thrombosis tendency which might exist prenatal and microvascular thrombosis status gradually improved.

This study showed that expression of GPIIb in each group was high status, no significant difference was detected ($P > 0.05$). Therefore, perhaps GPIIb (CD41) was more resistant to the pathophysiologic mechanisms of preeclampsia. So it could be further confirmed GP IIb was a constant marker in flow cytometry, and it was also a useful marker to delineate platelet range.

Flow cytometry is an emerging technology from the last decade. It is eminently suited to study

the expression of platelet surface receptors both qualitatively as well as quantitatively. It can serve as a useful marker for the documentation of in vivo platelet activation, and thus, forewarn the risk of thromboembolism in patients with preeclampsia. Therefore, we can use this kind of technique to monitor the severity of preeclampsia.

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Disclosure of conflict of interest

None.

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References

- [1] Roberts JM, Cooper DW. Pathogenesis and genetics of preeclampsia. *Lancet* 2001; 357: 53-56.
- [2] Gui S, Ni S, Jia J, Gong Y, Gao L, Zhang L, Zhou R. Inconformity of CXCL3 plasma level and placenta expression in preeclampsia and its effect on trophoblast viability and invasion. *PLoS One* 2014; 9: e114408.
- [3] Robb AO, Din JN, Mills NL. The influence of the menstrual cycle, normal pregnancy and preeclampsia on platelet activation. *Thromb Haemost* 2000; 103: 372-378.
- [4] Janes SL, Goodall AH. Flow cytometric detection of circulating activated platelets and platelet hyper-responsiveness in pre-eclampsia and pregnancy. *Clin Sci* 1994; 86: 731-739.
- [5] Ulutas KT, Dokuyucu R, Sefil F, yengil E, Sumbul AT, Rizaoglu H, Ustun I, Yula E, Sabuncu T, Gokce C. Evaluation of mean platelet volume in patients with type 2 diabetes mellitus and blood glucose regulation: a marker for atherosclerosis? *Int J Clin Exp Med* 2014; 7: 955-961.
- [6] Gibson B, Hunter D, Neame PB, Kelson JG. Thrombocytopaenia in preeclampsia and eclampsia. *Semin Thromb Hemost* 1982; 8: 234-247.
- [7] Gatti L, Tenconi PN, Guarneri D, Bertulesi C, Ossola MW, Bosco P. Hemostatic parameters and platelet activation by flow cytometry in normal pregnancy: a longitudinal study. *Int J Clin Lab Res* 1994; 24: 217-219.
- [8] Star J, Rosene K, Ferland J, Dileone G, Hogan J, Kestin A. Flow cytometric analysis of platelet activation throughout normal gestation. *Obstet Gynecol* 1997; 90: 562-568.
- [9] Holthe MR, Staff AC, Berge LN, Lyberg T. Different levels of platelet activation in preeclampsia, normotensive pregnant, and non-pregnant women. *Am J Obstet Gynecol* 2004; 190: 1128-1134.
- [10] Star J, Rosene K, Ferland J, DiLeone G, Hogan J, Kestin A. Flow cytometric analysis of platelet activation throughout normal gestation. *Obstet Gynecol* 1997; 90: 1-8.
- [11] Hagberg IA, Lyberg T. Blood platelet activation evaluated by flow cytometry: optimised methods for clinical studies. *Platelets* 2000; 11: 137-150.
- [12] Tumer JA. Diagnosis and management of preeclampsia: an update. *Int J Womens Health* 2010; 30: 327-337.
- [13] Michelson AD. Flow cytometry: a clinical test of platelet function. *Blood* 1999; 87: 4925-4936.
- [14] Wenger RH, Wicki AN, Kieffer N. The 5' flanking region and chromosomal localization of the gene encoding human platelet membrane glycoprotein GPIIb/IIIa. *Gene* 1989; 85: 517-524.
- [15] Thijs T, Nuytens BP, Deckmyn H, Broos K. Platelet physiology and antiplatelet agents. *Clin Chem Lab Med* 2010; 48: s3-s13.
- [16] Knight CJ, Panesar M, Wright C, Clarke D, Butowski PS, Patel D, Patrinely A, Fox K, Goodall AH. Altered platelet function detected by flow cytometry. Effects of coronary artery disease and age. *Arterioscler Thromb Vasc Biol* 1997; 17: 2044-2053.
- [17] Han Y, Wang Z, Wang A. Alterations of platelet membrane glycoproteins in patients with diabetes mellitus and their clinical significance. *Zhonghua Xue Ye Xue Za Zhi* 1999; 20: 127-129.
- [18] Moal V, Brunet P, Dou L, Morange S, Sampol J, Berland Y. Impaired expression of glycoproteins on resting and stimulated platelets in uraemic patients. *Nephrol Dial Transplant* 2003; 18: 1834-1841.
- [19] Gonzalez-Quintero VH, Smarkusky LP, Jiménez JJ. Elevated plasma endothelial microparticles: preeclampsia versus gestational hypertension. *Am J Obstet Gynecol* 2004; 191: 1418-1424.
- [20] Gonzalez-Quintero VH, Jiménez JJ, Jy W, Mauro LM, Hortman L, O'Sullivan MJ, Ahn Y. Elevated plasma endothelial microparticles in preeclampsia. *Am J Obstet Gynecol* 2003; 189: 589-593.

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- [21] Kuhne T, Ryan G, Blanchette V, Semple JW, Hornstein A, Mody M, Chang W, McWhirter L, Freedman J. Platelet-surface glycoproteins in healthy and preeclamptic mothers and their newborn infants. *Pediatr Res* 1996; 40: 876-880.
- [22] Erturk A, Cure E, Parlak E, Cure MC, Yuce S, Kizilkaya B. Serum resistin levels may be new prognostic factor of Crimean-congo hemorrhagic fever. *Int J Clin Exp Med* 2014; 7: 3536-3542.