



Research article

Circulating microparticles in preeclampsia

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ABSTRACT

Objective: The purpose of this study was to assess the microparticles serum levels as a predictor of maternal and fetal complications in preeclampsia.**Materials and methods:** In this cross sectional study, 50 pregnant women with preeclampsia (mild preeclampsia [n=29] and severe preeclampsia [n=21]) and 50 normotensive matched control group were recruited. Maternal blood endothelial microparticles (EMP), platelet microparticles (PMP), hemoglobin, hematocrit, platelet count, liver enzymes, and total bilirubin levels were assessed in all patients.**Results:** EMP count was significantly higher in preeclampsia group compared with controls. Moreover, significantly higher EMP levels were observed in patients with severe preeclampsia compared with mild preeclampsia. There was a significant negative correlation between platelet count and EMP. Also, EMP was correlated positively with AST and bilirubin levels. EMP levels was significantly higher in cases with neonatal death (p=0.04). No significant correlation was observed between PMP and EMP levels.**Conclusion:** EMP levels tend to be increased with severity of preeclampsia. Moreover, higher EMP levels are seen in cases with fetal death.**Key words:** Preeclampsia; endothelial microparticles; maternal complications; fetal outcome.Received: 11.8.2009
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Introduction

Preeclampsia is a pregnancy-specific disease characterized by maternal hypertension that is thought to be preceded by endothelial cell activation and an inappropriate inflammatory response. In Egypt, the national survey for maternal mortality ranked (pre)eclampsia as the second cause of maternal death accounting for 16.7% of maternal deaths [1]. The exact cause of preeclampsia is unclear but this disease is known to be induced by a placental factor and it is hypothesized that oxidative stress may also contribute to its pathogenesis [2].

Preeclampsia is characterized by obstruction of the spiral arteriolar lumen by atherosclerosis that may impair placental blood flow. It is thought that these changes cause significant decrease in placental perfusion that eventually leads to the preeclampsia syndrome [3, 4]. Current concepts about the pathogenesis of preeclampsia include endothelial dysfunction, inflammatory activation; oxidative stress and predisposing maternal factors provide targets for well-

designed nutritional investigation [5]. Important evidence exists to implicate endothelial cell injury to the pathophysiology of this pregnancy-specific disease. Endothelial cells shed vesicles in to the circulation on activation or apoptosis. These vesicles, termed endothelial microparticles (EMP), contain cytoplasmic components and negatively charged phospholipids bearing some of the cell surface proteins. Elevated EMP have been shown in thrombotic and immunologic disorders such as thrombotic thrombocytopenic purpura, multiple sclerosis, coronary heart disease, and in women with preeclampsia [6, 7].

In this study, we aimed to determine the significance of serum EMP levels with relation to severity of preeclampsia and fetal outcome.

Materials and Methods

Setting and patient selection

This study was conducted at Kasr El-Eini Women's Hospital between March 2008 and January 2009. Fifty pregnant



Table 1. Characteristics of patients.

	Mild preeclampsia (N=29)	Severe preeclampsia (N=21)	Controls (N=50)	P
Age	27.5±5.6	27.4±5.0	25.1±3.8	0.14
Gravida	2 (1-4)	2 (1-3)	1 (1-4)	0.11
Parity	1 (0-3)	1 (0-2)	0 (0-2)	0.10
<i>Laboratory workup</i>				
Systolic blood pressure (mmHg)	143.6±5.7	162.8±15.3	104.3±6.3	
Diastolic blood pressure (mmHg)	93.4±4.7	113.6±4.8	66.8±4.8	
Hemoglobin (g/dl)	10.1±1.2	9.6±1.5	10.3±0.6	0.11
Hematocrit	30.3±2.5	29.0±2.5	30.0±1.4	0.13
Platelets count (× 10 ⁹ /l)	269.8±56.3 ^a	227.2±41.0 ^b	317.3±42.7 ^c	<0.001*
AST (IU/dl)	22.2±21.2 ^a	51.1±26.4 ^b	10.9±3.7 ^c	<0.001*
ALT (IU/dl)	24.0±32.1 ^a	28.1±11.1 ^a	10.4±1.6 ^b	0.01*
Bilirubin (mg/dl)	0.6±0.1 ^a	0.7±0.0 ^a	0.1±0.0 ^b	<0.001*
Proteinuria (urine dipstick)				
+1	19 (65.5%)	0	-	
+2	10 (34.5%)	0	-	
+3	0	12 (57.1%)	-	
+4	0	9 (42.9%)	-	
<i>Neonatal parameters</i>				
Birthweight (g)	2708±344 ^a	2281±584 ^b	3327±289 ^c	<0.001*
GA at delivery (wk)	37.2±1.6 ^a	35.3±2.7 ^b	38.5±1.1 ^c	<0.001*
Apgar score 5 min.	9 (3-9) ^a	8 (4-9) ^b	9 (7-9) ^a	<0.001*
Neonatal outcome				
Uneventful	26 (89.6%)	11 (52.3%)	50 (100.0%)	
Death	1 (3.4%)	6 (28.5%)	0	0.01
NICU admission	2 (6.9%)	3 (14.2%)	0	
Respiratory distress syndrome	0	1 (4.7%)	0	
<i>Particle counts</i>				
EMP (count/μl)	8799 (6054-26622) ^a	12952 (9424-43586) ^b	6584 (1260-9777) ^c	<0.001*
PMP (count/μl)	6780 (1884-20984)	8469 (612-15500)	6282 (1279-21272)	0.957

* Statistically significant (p<0.05) overall p values.

a,b,c; groups with different superscripts denotes statistically significant differences in *post hoc* tests (p<0.05).

Notes: Values are expressed as mean ± SD, median (minimum-maximum), or frequency (%)

GA, gestational age; NICU, Neonatal intensive care unit; EMP, Endothelial microparticles; PMP, platelet microparticles

women who are diagnosed to have preeclampsia and 50 normotensive pregnant women as matched control group were recruited to the study. Gestational age was confirmed by dating sonography in all patients.

Preeclamptic patients were further divided into subgroups as mild and severe preeclampsia (blood pressure ≥160/110 mmHg, proteinuria ≥3 g/24 h). Patients with chronic hypertension, superimposed preeclampsia, pregestational diabetes mellitus, history of recurrent pregnancy loss, renal or hepatic disease, and smokers were excluded from the study. The control group included pregnant women of the same gestational age with normal obstetric follow-up.

Essays

A 10 ml of venous blood sample was with drawn from all cases. Five ml from each sample was used to measure hemoglobin concentration, hematocrit level, AST, ALT, total bilirubin and platelet count. The remaining 5 ml was frozen

and used to extract serum for assaying microparticle levels using a flow cytometric assay of EMP and PMP, with the use of fluorescent monoclonal antibodies anti-CD 31 and anti-CD 42 at once.

Statistical analysis

Comparisons among the groups were performed with one-way ANOVA and Kruskal-Wallis tests for parametric and nonparametric data, respectively. *Post hoc* analyses were performed by using Student's t test and Mann Whitney U test with Bonferroni corrected p values. For comparing categorical data, Chi square test was performed. Yates correction was used when the frequency is less than 10.

Statistical calculations performed using Microsoft Excel version 7 (Microsoft Corp., Redmond, WA) and SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL) software. Results were reported as mean ± standard deviation (SD), median (minimum-maximum), or frequency (%).

Results

Study results and patient characteristics were presented in **Table 1**. The clinical characteristics of the three groups, by definition, systolic and diastolic blood pressures were higher in severe preeclampsia group compared to mild cases, and both groups were higher compared to the control group. There was no significant difference in hemoglobin concentration and hematocrit value between the three studied groups. Severe preeclampsia group had a platelet count significantly lower than mild preeclampsia and control groups. Also, mild preeclampsia group had significantly lower platelet count compared to the control group. AST, ALT and bilirubin concentration were significantly higher in severe preeclampsia in comparison to control group. AST and bilirubin were significantly higher in mild preeclampsia in comparison to control group.

Severe preeclamptic cases delivered significantly earlier than those with mild preeclampsia and control women. Delivery was also significantly earlier in mild preeclampsia compared to control group. Similarly, birth weight was significantly lower in severe preeclampsia group than the other two groups and in mild preeclampsia group in comparison to control group. Apgar score after 5 minutes was significantly lower in severe preeclampsia group compared to the other two groups. Six neonates in severe preeclampsia group died. One neonatal death was recorded in the mild preeclampsia group, while no deaths occurred in the control group neonates. Neonatal mortality is significantly higher in severe preeclampsia compared to mild preeclampsia group ($p = 0.01$, **Table 1**).

EMP count was significantly higher in severe preeclampsia group compared to the other two groups and in mild preeclampsia group in comparison to control group (**Table 1, Figure 1**). On the other hand, PMP count showed no significant difference between the three studied groups. There was no correlation between EMP and PMP ($r=0.066$, $p=0.58$) (**Figure 2**). There was a significant negative correlation between platelet count and EMP. Also, EMP was correlated positively to AST and bilirubin concentration. Meanwhile, PMP count was not correlated to laboratory parameters. EMP was significantly higher in cases that suffer neonatal death in comparison to those with living neonates ($p=0.04$). There was no significant difference in neonatal death with regard to PMP levels.

Discussion

Microparticles (MP) are small membrane fragments first described in 30 years ago as 'platelet dust'. MP are generated as a result of cell activation or apoptosis following disturbance of cell membrane architecture. They thought to be function as biological vectors [8]. PMP contain significant amount of procoagulant and proinflammatory platelet activating factor (PAF). Similarly, EMP were shown to contain part of von Willebrand factor (vWF) that can induce platelet aggregation [9].

The number of MP from a specific cellular origin might be increased in a specific disease process. However, the constituents of MP are also known to be changed as a result of mechanism of origin. EMP formed as a result of endothelial activation were phenotypically and quantitatively different from that of apoptosis [10]. Pirro *et al.* [11]

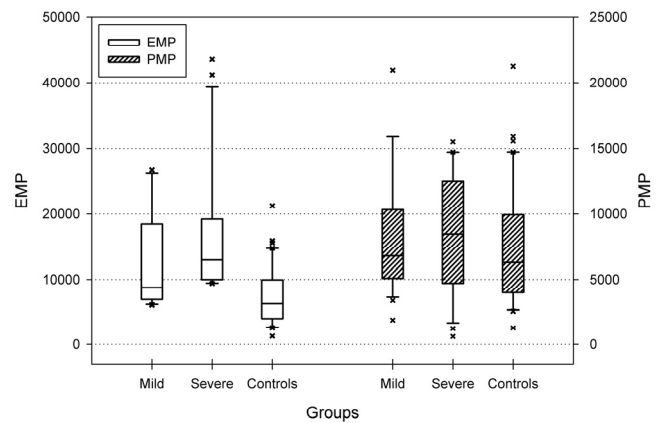


Figure 1. Endothelial microparticles (EMP) and platelet microparticles (PMP) values among groups.

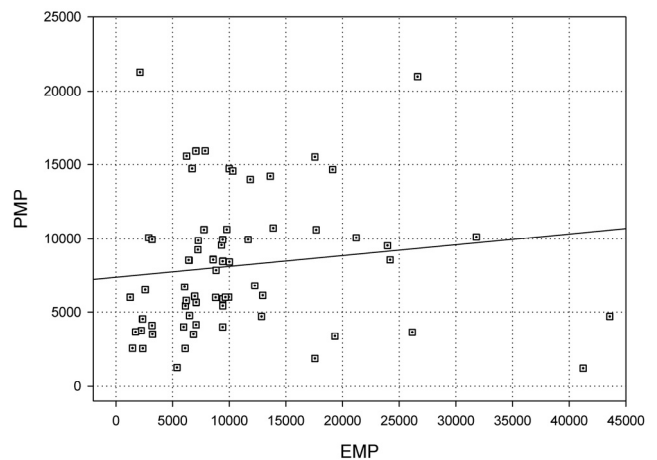


Figure 2. No significant correlation was observed between endothelial microparticle (EMP) and platelet microparticle (PMP) levels ($r=0.066$, $p=0.58$).

showed that the increased ratio of CD 31+/CD41 EMP is a marker of atherosclerosis in hypercholesterolemia. In this respect, although we failed to demonstrate significant increase in the number of PMP in three groups their function in pathophysiological mechanism might be different. Previous studies were reported similar result with respect to the number of PMP in preeclampsia [6, 7].

On the other hand, significantly increased EMP in preeclamptic patients support the endothelial injury theory [12-14]. EMP tend to increase with the severity of clinical syndrome (**Figure 1**).

MP in preeclamptic women (prMP) shown to contain PMP, EMP and also syncytial MP (SMP) that originates from placental syncytiotrophoblasts. prMP were shown to stimulate the release of nitric oxide and variety of mediators that modulate endothelial function and cause oxidative stress. This and other pathways of oxidative stress cause destabilization of placental syncytiotrophoblasts to release SMP [8]. Soluble fms-like tyrosine kinase-1 (sFlt-1, VEGFR-1) and soluble endoglin (sEng) are secreted from syncytium and cause endothelial dysfunction by inhibiting

VEGF and TGF- β 1[15]. These placental factors plays important role in the development of clinical syndrome [16].

Conclusion

In this study we demonstrated that the number of EMP is significantly increased with the severity of clinical syndrome and also associated with the adverse fetal outcome. Further studies are required to elucidate the underlying mechanisms of these findings.

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