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Original Article

Circulating endothelial cells (CECs) and E-selectin: Predictors of preeclampsia

Ferdous Mehrabian[1], Sayed Mohammad Hashemi Jazi[2], Shaghayegh Haghjooy Javanmard[3], Mahshid Kaviani[4], Vida Homayouni[5]

Abstract

BACKGROUND: Circulating endothelial cells (CECs) and E-selectin are known as sensitive and specific markers of endothelial dysfunction. This study investigated whether CECs and E-selectin are surrogate biomarkers of preeclampsia and if measurement of CECs and E-selectin, early in the third trimester, could be a means of predicting preeclampsia.

METHODS: In this prospective, descriptive-analytic study, rollover test was performed on 523 pregnant women during 28-30 weeks of gestation. CECs were measured by anti-CD 146-driven immunomagnetic isolation in women with positive rollover test. They were followed up prospectively until delivery without any active intervention. Women with and without preeclampsia were determined. The number of CECs and level of E-selectin were compared in the two studied groups.

RESULTS: From the 47 pregnant women with positive rollover test who were selected and followed up, 22 individuals were diagnosed with preeclampsia while the remainder were normotensive. Mean CEC numbers was significantly higher in preeclamptic women than normal pregnancies (24.7 cells/mL vs. 13 cells/mL). The best cut-off point for CEC numbers was 6.5 with a sensitivity of 78.9% and a specificity of 69.1%. The level of E-selectin was significantly higher in mothers with preeclampsia ($p < 0.05$).

CONCLUSIONS: Higher levels of CECs and E-selectin in women with positive rollover test who developed preeclampsia prior to onset of the complication were predictive of preeclampsia. However, larger studies are needed to confirm these findings.

KEYWORDS: Preeclampsia, Endothelial Dysfunction, CECs, E-Selectin.

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Preeclampsia is considered as one of the most common leading causes of feto-maternal morbidity and mortality worldwide.[1] It is not only related with high rate of obstetric morbidity and mortality, but also places the mother at increased risk for developing cardiovascular diseases in future.[2]

Preeclampsia is characterized by hypertension and proteinuria developing after 20 weeks of gestation and can manifest as late as 4-6 weeks postpartum. The clinical presentations of preeclampsia are hypertension and proteinuria, with or without pathologic edema.[3]

The incidence of preeclampsia has been estimated as 3-14% of all pregnancies worldwide.[4] The incidence of the disease has been reported to be 4-18% and 6.8% in developing countries and Iran, respectively.[5,6]

Though many studies have described the pathologic events responsible for the disease, the underlying mechanisms of the disease are not known completely. It seems that many

1- Associate Professor, Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
2- Associate Professor, Department of Cardiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
3- Assistant Professor, Department of Physiology, Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
4- Resident, Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
5- PhD Student, Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
Corresponding author: Shaghayegh Haghjooy Javanmard
E-mail: shaghayeghhaghjoo@yahoo.com

more studies are needed to elucidate the etiology of the disease.[7,8]

Recent investigations have suggested vascular endothelial dysfunction as the most important component of the pathophysiology of preeclampsia.[9] Many studies have indicated relations between preeclampsia and markers of endothelial dysfunction, such as von Willebrand factor, endothelin, soluble vascular cell adhesion molecule, thrombomodulin, cellular fibronectin, thrombus precursor protein (TpP),[10] as well as increased growth factor activity and circulating endothelial cells (CECs) in pregnant women.[11]

Among the mentioned markers of endothelial dysfunction and injury, CECs are known as a sensitive and specific novel marker.[12] Many investigators have confirmed CECs involvement in different vascular disorders including preeclampsia.[13]

Soluble E-selectin, as one of the circulating adhesion molecules, is a member of the selectin family. It is specifically expressed on the surface of stimulated endothelial cells.[14] Plasma E-selectin concentration may be a marker of endothelial dysfunction or activation. It is increased in the maternal circulation during pregnancy and recent evidences indicated its further elevation in some complications of pregnancy such as preeclampsia.[15-17]

Currently, there is no widely accepted screening test for predicting preeclampsia. The development of an accurate biomarker for predicting preeclampsia would improve care by allowing closed prenatal monitoring, earlier recognition of preeclampsia, expeditious administration of steroid for fetal lung maturity and appropriate antihypertensive therapy. Such biomarker would also allow for investigation of targeted strategies for preeclampsia prevention. The purpose of this study was to evaluate the accuracy of measurement of CECs and E-selectin early in the third trimester as a means to predict preeclampsia.

Methods

In this prospective, descriptive-analytic study, 523 pregnant women, who received prenatal care in Alzahra and Shahid Behesti Hospitals in Isfahan, were selected by convenient sampling method from June 2009 until June 2010. Selected pregnant women underwent rollover test during 28-30 weeks of gestation. Pregnant women with renal disease, infection, previous transplantation, collagen vascular disease and chronic hypertension were excluded from the study. Women with positive rollover test were enrolled in the study. The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences and written informed consents were obtained from all studied patients.

The numbers of CECs were measured in selected women. The subjects were followed up prospectively until delivery without any active intervention. Maternal and perinatal outcomes were defined in accordance with the American College of Obstetrics and Gynecology guidelines.[18] Women with and without preeclampsia were determined and the level of CECs and E-selectin were compared in the two studied groups (Figure 1).

Clinical assessments

- *Positive rollover test:* The rollover test was considered positive if diastolic blood pressure increased 20 mmHg when the patient moved from the left lateral recumbent to supine position.[19]
- *Preeclampsia:* Preeclampsia was defined as the development of a systolic blood pressure of 140 mmHg or greater, and/or a diastolic blood pressure of 90 mmHg or greater and proteinuria of 300 mg or greater per 24 hours after 20 weeks of pregnancy, or urine dipstick 1+ or greater on 2 occasions for at least 4 hours. Blood pressures were measured 2 times for each patient at least 4 hours apart.[20] The blood pressure was taken with the patient's arm supported on the level of the heart. Diastolic pressure was reported as phase V Korotkoff.[21]

Laboratory measurements

Immunomagnetic bead method: We measured CECs according to a previously validated technique.[22,23] Therefore, one milliliter of

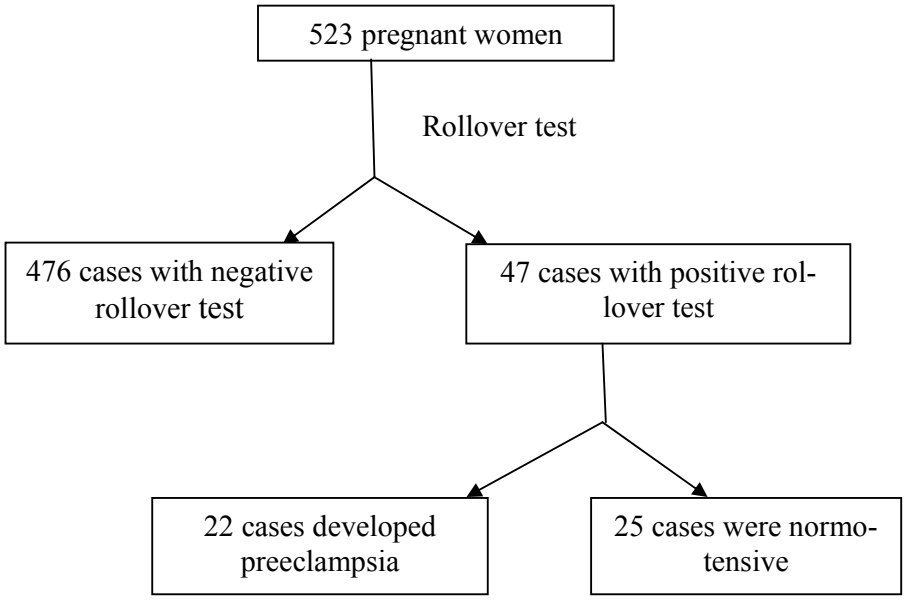

Figure1. Flow diagram of patient participation through the study

thoroughly mixed venous blood was collected in an ethylenediaminetetraacetic acid (EDTA) tube while the first 7 ml of blood drawn were discarded to avoid contamination by endothelial cells from the punctured vessel wall. The blood sample was mixed with 1 ml lysis buffer and incubated at room temperature for 10 minutes. The suspension was centrifuged for 10 minutes and the cell pellet was resuspended in 1 ml of phosphate buffered saline (PBS) solution. Then, 20 µl of Fc-receptor blocking agent (Miltenyi, Germany) were added and incubated for 20 minutes. Followed by further incubation, one hundred microliters of anti-CD146 coated microbeads per 10^7 cells were then added to this mixture and incubated for 15 minutes at 4°C. Bead-bound cells were then separated from non-bound cells using a combination of washing with buffer solution and magnetic separation (Miltenyi, Germany). One hundred microliters of prepared Ulex europaeus lectin solution (2 mg/ml) (Sigma, Germany) was then added to the clean cell/bead suspension and gently mixed for ≥ 30 minutes at room temperature (22–24°C) in darkness. The solution was then washed three times in a magnet.

The resulting rosetted cells and beads were finally resuspended in 125 µl of PBS for counting under epifluorescence microscopy (Zeiss, Welwyn Garden City, UK) in a Nageotte counting chamber. Cells were defined as CECs if they demonstrated rosetting with ≥ 4 CD146–immunobeads, were approximately 20–50 µm in diameter with distinctive cell morphology, and stained positively with endothelial specific Ulex europaeus lectin. Serum levels of E-selectin were assessed by Human sE-selectin Platinum enzyme-linked immunosorbent assay (ELISA) kits produced by eBioscience according to the manufacturer's instructions.

Statistical analysis

We hypothesized that increased numbers of CECs early in the third trimester are associated with an increased risk of developing preeclampsia. Therefore, the numbers of CECs in preeclamptic and normal patients were compared using student's t- test. P values less than 0.05 were considered statistically significant. In order to determine the appropriate cutoff point of CECs number for identifying the risk of preeclampsia, the sensitivity, specificity, and

likelihood ratio were calculated at different levels of CECs using receiver operating characteristics (ROC) curve. Data analysis was performed in $SPSS_{15}$ (SPSS Inc., Chicago, USA).

Results

In this study, a total of 47 women with positive rollover test were selected out of 523 subjects. The individuals were followed up and 22 pregnant women were diagnosed with preeclampsia while the remainder were normotensive. The characteristics of women with and without preeclampsia are presented in table 1. As this table shows, Spearman's correlation revealed a positive correlation between age of mothers and preeclampsia ($r = 0.258$; $p = 0.5$).

The sensitivity, specificity and predictive characteristics of different levels of CECs and E-selectin based on the ROC curve are presented in table 2. The ROC curve analysis, applied to markers values, showed the best diagnostic profile for CECs and E-selectin with an area under the curve (AUC) of 0.81 and 0.89, respectively. The best cutoff points were 6.5 cells/ml for CECs and 14.6 ng/ml for E-selectin. The sensitivity, specificity and accuracy of both CECs and E-selectin were 88.9%, 83.3% and 86.1%, respectively.

Discussion

In this study, mean level and predictive values of CECs and E-selectin in preeclamptic and non-preeclamptic pregnant women were evaluated. The results of the current study indicated that the number of CECs and level of serum E-selectin in preeclamptic women were significantly higher than normotensive pregnant women.

As mentioned, preeclampsia is related to an imbalance of circulating angiogenic factors which results in endothelial malfunction and vasospasm.[24] Many studies supported the endothelial origin of preeclampsia. According to these studies, impaired placentation results in releasing different soluble factors in maternal circulation which consequently increases endothelial permeability possibly through protein kinase C-dependent pathways. These soluble factors can also change the expression of adhesion molecules and calcium content of endothelial cells.[21]

Several markers of endothelial damage,

Table 1. The characteristics of women with and without preeclampsia

	Normotensive women (n = 25)	Preeclamptic women (n = 22)	P-value
Age (years)	27.9 ± 5.6 (20-38)	26.8 ± 5.4 (20-36)	NS
Parity	1.9 ± 1.2 (1-5)	1.5 ± 0.7 (1-3)	NS
Gestational age at the time of positive rollover test (weeks)	29.9 ± 2.0 (28-31)	30.1 ± 1.2 (28-32)	NS
Gestational age at delivery (weeks)	38.6 ± 1.9 (33-40)	35.1 ± 2.0 (30-39)	< 0.05
Type of delivery			
- Normal vaginal delivery	17 (68.0%)	5 (22.7%)	< 0.05
- Cesarean section	8 (32.0%)	17 (77.3%)	< 0.05
Mean birth weight (g)	2927.5 ± 495 (1800-3700)	2236 ± 513 (1000-3100)	< 0.05
Proteinuria	1 (4.0%)	4 (18.2%)	< 0.05
Previous preeclampsia	3 (12.0%)	1 (4.5%)	NS
History of			
- Polycystic ovarian disease	1 (4.0%)	1 (4.5%)	NS
- Gestational diabetes mellitus	1 (4.0%)	1 (4.5%)	NS
- other diseases	0 (0%)	0 (0%)	NS
Systolic blood pressure (mmHg)	110.5 ± 11.5 (100-123)	173.3 ±28 (125-210)	< 0.05
Diastolic blood pressure (mmHg)	63 ±7 (60-80)	103 ±14 (73-132)	< 0.05
E-selectin (ng/ml)	7.0 ± 1.5	30.9 ± 3.8	< 0.05
Median of CECs numbers (cells/mL)	13 (1-40)	24.7(2-100)	< 0.05

All values are expressed as mean ± SD (range) or number (%).

Table 2. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different levels of CECs and E-selectin using receiver operating characteristics (ROC) curve

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
E-selectin	79	95.5	93.8	84
CECs	83.3	72.2	75	71.3

such as thrombomodulin, soluble von Willebrand factor (svWF) and tumor necrosis factor-α, and their efficacy in determining endothelial dysfunction have been studied previously. Though measuring soluble endothelial markers, such as thrombomodulin, is easy, it could be influenced by different factors including renal function. In addition, measuring svWF and thrombomodulin is not reliable enough due to the fact that these factors could not discriminate between activation and damage.[25,26]

In the current study, we evaluated CECs numbers as an indicator of endothelial malfunction among preeclamptic women. Evidence supports the efficacy of CECs numbers in this regard and it is considered as a reliable marker for endothelial damage in many diseases. Recently, use of this marker has become more widespread due to new methods which facilitate the enumeration of CECs.[27,28]

The findings of this study indicated that median of CECs numbers was significantly higher in preeclamptic pregnant women than in normotensive ones. Our results were in accordance with the study of Grundmann et al. that evaluated the relation between CECs and preeclampsia using enumeration of CECs with anti-CD146–driven immunomagnetic isolation. They enumerated CECs in pregnant women before delivery and 1 and 3-5 days after delivery and reported that the mean value of CECs numbers was significantly higher in preeclampsic women (88 cells/ml) than normal pregnancies (16 cells/m) and healthy non-pregnant women (12 cells/ml). The number of CECs in preeclampsic women decreased significantly after delivery. They also reported a significant correlation between CECs and systolic blood pressure in preeclampsic women and concluded that CECs can be considered as a novel marker of endothelial damage in preeclampsia.[27]

In another study in Turkey, Canbakan et al. evaluated the number of CECs in patients with preeclampsia using anti-CD146 antibody similar to our study. They showed that the numbers of CECs were significantly higher in preeclamptic women (13.2 ± 5.2 cells/ml) than hypertensive patients (6.9 ± 0.8 cells/ml), healthy pregnant women (5.2 ± 1.4 cells/ml), and non-pregnant controls (4.0 ± 1.8 cells/ml). They indicated a significant correlation between CECs and systolic and diastolic blood pressure and concluded that CECs number was a valuable marker in evaluating the endothelial dysfunction in preeclampsia.[13]

In contrast, Strijbos et al. investigated the levels of CECs numbers in normotensive and severe preeclamptic pregnancies and indicated that the number of CECs was not significantly different between preeclamptic women and the control group and all the numbers were in the normal range. Since other markers including thrombomodulin, E-selectin and endoglin were significantly higher in preeclamptic women, they concluded that the role of endothelial dysfunction and activation in preeclampsia was more significant than the actual endothelial damage characterized by the increased numbers of CECs.[29]

Although the mentioned studies confirmed the correlation between CECs numbers and preeclampsia, they recommended that the prognostic value of this marker in preeclampsia should also be investigated. In the current study, we determined the predictive value of CECs numbers in the diagnosis of preeclampsia early in the third trimester. The results indicated that CECs numbers during the early period of third trimester have a predictive value for later diagnosis preeclampsia. The cutoff point was calculated as 6.5 cells/ml.

Another endothelial marker which was studied in this study was E-selectin. Our findings

showed that the serum concentrations of E-selectin were significantly higher in preeclamptic pregnant women. This marker was found to have appropriate sensitivity and specificity.

In China, Xing et al. indicated that E-selectin had an important role in shallow trophoblast invasion, the major pathological change of preeclampsia.[30]

In a recent study in Greece, the level of E-selection was compared in preeclamptic and control subjects. E-selectin was found to increase significantly in preeclamptic women in antepartum and postpartum period. They concluded that endothelial dysfunction may be one of the key processes in the pathogenesis of preeclampsia in pregnancy and the links between preeclampsia and future cardiovascular diseases.[31] In line with the findings of this study, Kim et al. indicated that serum levels of E-selectin were significantly higher in both mild and severe preeclampsia than normal pregnancy. However, there were no statistical differences in the levels of E-selectin between mild and severe preeclampsia.[32] While Bretelle et al. reported similar results,[16] Bersinger et al. suggested that serum levels of E-selectin were within normal pregnancy ranges among preeclamptic women.[33]

The limitation of this study was the small sample size. However, this is the first report of measuring maternal serum CECs and E-selectin levels as a predictor of preeclampsia. We demonstrated that maternal serum CECs and E-selectin levels increase prior to preeclampsia onset in women with positive rollover test. Further studies with larger sample sizes are required to validate these results and estimate the sensitivity and specificity of these markers for preeclampsia screening.

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Conflict of Interests
Authors have no conflict of interests.

Authors' Contributions
FM designed the study, coordinated and carried out the clinical investigation and participated in manuscript preparation. MHJ provided assistance in the design of the study. SHJ carried out the experiments, performed the statistical analysis and participated in manuscript preparation. MK provided assistance in the clinical investigation and statistical analysis. VH carried out the experiments. All authors have read and approved the content of the manuscript.

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