Pregnancy Hypertension 17 (2019) 1–4

Contents lists available at ScienceDirect

Pregnancy Hypertension

journal homepage: www.elsevier.com/locate/preghy

Soluble E-selectin, platelet count and mean platelet volume as biomarkers for pre-eclampsia

Hend El-Sayed Hassan[a], Hanan Azzam[a], Maha Othman[b,*], Mohammad Hassan[c], Tarek Selim[a]

[a] Clinical Pathology Dept., Italy Elbarood General Hospital, El Behera, Egypt
[b] Biomedical and Molecular Sciences Dept., Faculty of Medicine, Queen's University, Canada
[c] Gynaecology & Obstetrics Dept., Faculty of Medicine, Mansoura University, Egypt

ABSTRACT

Objectives: Preeclampsia remains a major cause of maternal mortality and morbidity worldwide with increased risk for cardiovascular disease later in life. Many previous studies have examined several biomarkers including E-selectin. We aimed to assess the role of sE-selectin together with platelet count and mean platelet volume (MPV) as biomarkers for the prediction of preeclampsia.
Study design and major outcome measures: This case-control study included 85 pregnant women; 40 healthy (mean age 27.1 ± 4.8 years) and 45 with preeclampsia (mean age 26.8 ± 6.7 years), recruited at the third trimester of pregnancy and subjected to full clinical and laboratory testing. This included complete blood picture, urine analysis and plasma sE-selectin using ELISA.
Results: A significant decrease in platelet count ($P = 0.003$), and a significant increase in MPV ($P < 0.001$) were seen in patients versus controls. Plasma sE-selectin levels were significantly higher in patients versus control ($P = 0.002$). ROC curve showed the best cut-off values for sE-selectin was 64.3 ng/mL, with 58% sensitivity 80.0% specificity. Positive predictive value was 76.5; negative predictive value was 62.7 and accuracy was 68.2 with a statically significant area under curve ($P = 0.002$). Platelet count, MPV and sE-selectin were significantly associated with PE in univariate analysis. In multivariate analysis, only MPV and sE-selectin were independent risk factors for PE development. Higher MPV or sE-selectin were associated with PE development ($P < 0.001$).
Conclusion: The simultaneous use of sE-selectin and platelet count and volume may help earlier recognition of preeclampsia and thus appropriate and more efficient therapy. Larger studies are likely to help verify data and justify wider application of these markers.

1. Introduction

Preeclampsia (PE) is a multi-system disorder of pregnancy, which is characterized by hypertension and proteinuria that develop after 20 weeks of gestation in previously normotensive women [1]. PE remains a major cause of maternal, fetal and neonatal morbidity and mortality all over the world.

The exact pathogenesis of preeclampsia remains unclear, however, the origin of the condition is known to be residing in the placenta rather than the fetus [2–4]. In most cases, the pathology involves placental insufficiency, which may be associated with diffuse placental thrombosis, inflammation and vasculopathy, and/or abnormal trophoblastic invasion of the endometrium. It has been proposed that generalised endothelial dysfunction occurring in the mother, with impaired trophoblastic invasion of the maternal placental bed are the initial events in this syndrome [5,6]. In addition, several studies have examined the role of platelets including mean platelet volume in the pathophysiology of hypertension [7,8].

Over the years several biophysical and biochemical markers for preeclampsia have been examined but so far an ideal one is yet to be identified. Furthermore, routine screening is not available and may not be justified. While uteroplacental Doppler US is valuable in detecting the impaired placental perfusion, its predictive value has not been sufficiently high to justify using it in routine screening [9]. Other markers for placental dysfunction, maternal inflammatory response, endothelial dysfunction and coagulopathies were examined. Some of these markers include angiogenic factors such as VEGF, P1GF, sflt-1, sEng, P-selectin, cell-free fetal DNA, ADAM12, placental protein 13 (PP-13), Pentraxin 3 (PTX3) and pregnancy-associated plasma protein A (PAPP-A) [10]. Endothelial dysfunction markers such as VCAM-1, ICAM-1 seem to be critical as it is associated with vasospasm which is considered central in the pathogenesis of preeclampsia [11].

E-selectin or endothelial-leukocyte adhesion molecule 1(ELAM-1) is a 115 kDa endothelial transmembrane glycoprotein and are the only endothelial cell-specific markers indicating endothelial cell activation. E-selectin expression can be induced by various cytokines, subsequently, it partly "sheds" from endothelium into the plasma, and can be measured as a soluble form [12]. Soluble E-selectin was found to be increased in the maternal circulation during pregnancy and recent evidences indicate further elevation in some complications of

* Corresponding author at: Department of Biomedical and Molecular Sciences, School of Medicine, Queen's University, Kingston, Ontario K7L 3N6, Canada.
E-mail address: othman@queensu.ca (M. Othman).

https://doi.org/10.1016/j.preghy.2019.04.008
Received 7 March 2018; Received in revised form 20 April 2019; Accepted 25 April 2019
Available online 26 April 2019

pregnancy such as preeclampsia [13,1].

This study aimed to evaluate the potential combined role of soluble E-selectin, platelet count and mean platelet volume in the pathogenesis of preeclampsia in an attempt to determine their value as biomarkers for the disease.

2. Patients and methods

This case-control study included 85 pregnant women; 40 healthy pregnant women and 45 women with preeclampsia, All patients were selected from the outpatient clinic of Obstetric and Gynecological Department in Mansoura University Hospital. The study was approved by the local ethics committee at Mansoura University Hospital. Informed consents were obtained from all the study participants.

Preeclampsia was defined as hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg after 20 weeks' gestation) and proteinuria (≥300 mg in a 24 h urine collection or one dipstick measurement of ≥1 +) according to the American College of Obstetricians and Gynecologists definitions guidelines [14].

Exclusion criteria were history of hypertension, hepatic or renal disease, cardiac disease, diabetes mellitus, established atherosclerosis, malignancy, autoimmune disease, systemic infection, recent major surgery or trauma, alcohol consumption, and cigarette smoking. No medication, such as antiplatelet, anticoagulant or antihypertensive drugs were used within 14 days prior to blood withdrawal.

All cases were subjected to full clinical assessment, abdominal ultrasonography, and laboratory testing including complete blood picture, liver and kidney function tests, and urine analysis (Spot urine test or 24 h collection). Blood withdrawal was performed in the third trimester of pregnancy.

2.1. Blood sampling

Peripheral blood was withdrawn by venipuncture with minimal stasis using a 21-gauge needle between 0900 and 1000 h from all patients after overnight fasting and following a 30 min in resting position. Blood was collected at the third trimester during the antennal follow up visits. The first 3 ml of blood was collected in a K2 EDTA tube (Becton Dickinson, Franklin Lakes, New Jersey, USA) for blood counts and additive-free tubes to obtain serum for liver and kidney function tests. A sample (4.5 ml) of blood was collected into vacutainers containing 3.8% sodium citrate (Becton Dickinson) and mixed at ratio of 1 part citrate to 9 parts blood. For E-selectin, blood samples were centrifuged for 15 min at approximately 5000 rpm, and were stored in two aliquots at −20 °C.

2.2. Laboratory assessments

Complete blood picture including platelet count and MPV, serum creatinine, and urine analysis including urine protein (spot urine test or 24 h collection) were performed for all participants, using standard techniques.

2.3. Measurement of plasma sE-selectin

Plasma sE-selectin was determined using ELISA (eBioscience Eyssenecksstrasse 460322 Frankfurt am Main, Germany) according to the manufacturer's instruction. Normal range: 17.5–88.1 ng/ml with a sensitivity of 0.3 ng/ml.

3. Results

The present study included 45 pregnant suffering from preeclampsia (patient group). In addition, 40 healthy age- matched pregnant were included as control group. The clinical characteristics and demographics for patients and control group are provided in Table 1.

Table 1
Clinical characteristics and demographics in the patient group compared with the control group.

Parameters		Control group (n = 40)	Patient group (n = 45)	P
Maternal age (years)		27.15 ± 4.742	26.82 ± 6.672	0.793
Gestational age (Weeks)		32.67 ± 5.075	33.09 ± 3.872	0.667
Gravidity	Primigravida N (%)	9 (22.5)	18 (40)	0.105
	Multigravida N (%)	31 (77.5)	27 (67.5)	
Parity	Nullipara N (%)	11 (27.5)	25 (55.6)	0.009
	Multipara N (%)	29 (72.5)	20 (44.4)	
Blood pressure (mmHg):				
Systolic (mean ± SD)		109.62 ± 9.015	147.56 ± 11.90	< 0.001*
Diastolic (mean ± SD)		73.50 ± 7.355	96.00 ± 10.31	< 0.001*

* P is significant.

The patients' ages ranged from 18 to 43 years old with mean age 26.82 ± 6.67 years compared with matched pregnant control group with ages ranged from 18 to 37 years old with mean age 27.15 ± 4.74 years. There were no significant differences in gestational age, gravidity between total PE cases when compared to control subjects (P > 0.05) but a significant difference was found in parity P = 0.009. As expected there were significant increase in systolic and diastolic blood pressure inpatient group compared with control group (P < 0.001).

Eighty nine percent of our population were delivered by Caesarean section and only 11% were by vaginal delivery. There was a 92% livebirth in all deliveries.

Table 2 shows the laboratory analyses, There were no statistical differences in total leukocyte count or hemoglobin concentration between the two groups. There was a significant decrease in platelet count in patient group versus controls P = 0.003), whereas there was significant increase in MPV in patient group versus controls (P < 0.001). Proteinuria ≥ 2 was considered significant and comparing absent or non-significant versus significant proteinuria; patients had significant differences when compared to control subjects (P < 0.001).

Plasma sE-selectin levels were significantly higher in patient group versus control group. The mean E-selectin level was 47.24 ng/mL ± 28.821 for patient group compared to 68.49 ng/mL ± 33.315 in the control (P = 0.002) Fig. 1.

Receiver operator curve (ROC) showed that the best cut-off values established for E-selectin was 64.26 ng/mL, with a sensitivity of 57.8% and a specificity of 80.0%. Positive predictive value (PPV) was 76.5; negative predictive value (NPV) was 62.7 and accuracy was 68.2%. The area under the curve was 0.693 (95% CI = 0.579–0.806, P = 0.002).

We then used uni- and multivariate analysis to evaluate the sE-Selectin and other laboratory elements as predictors of preeclampsia

Table 2
Laboratory findings in the patient group compared with the control group. WBC (white blood cell), MPV (mean platelet volume).

Laboratory data	Control group (n = 40)	Patient group (n = 45)	P
WBC ($\times 10^9$/L)	8.43 ± 2.56	8.54 ± 2.84	0.847
Hemoglobin concentration (g/dl)	10.01 ± 2.04	10.29 ± 1.12	0.924
Platelet count ($\times 10^9$/L)	229.25 ± 65.7	191.4 ± 48.16	0.003*
MPV (fL)	9.32 ± 0.95	10.86 ± 1.42	< 0.001*
Proteinuria:			
Patients no (%)			
Absent or non significant	40 (100)	5 (11.1)	< 0.001*
Significant	0 (0)	40 (88.9)	

* P is significant.

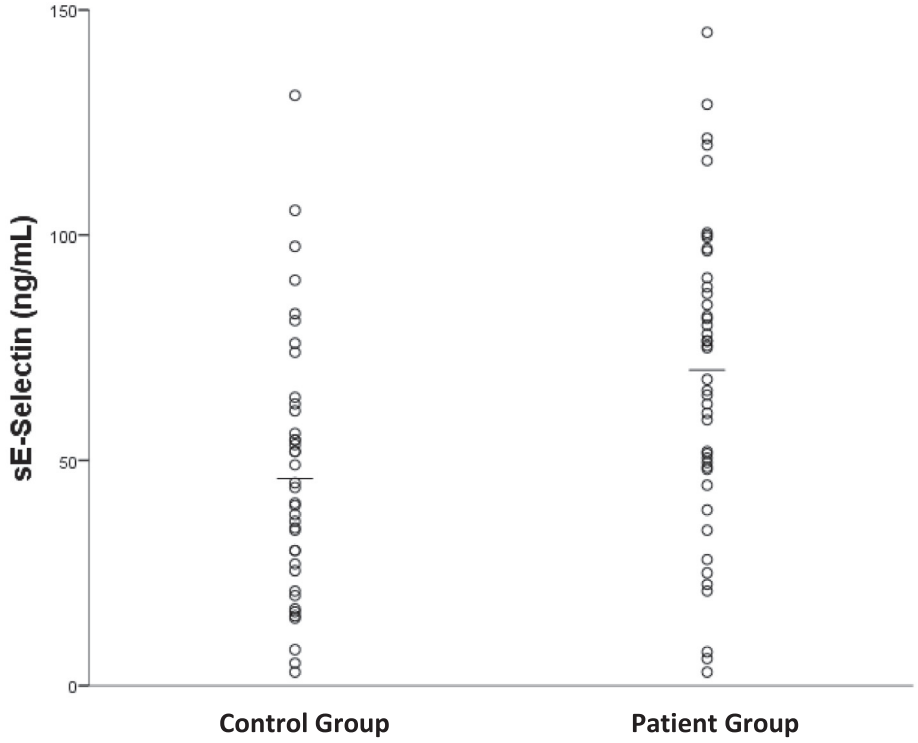

Fig. 1. sE-selectin level in the patient group compared with the control group.

Table 3
Uni and multivariate analysis for prediction of preeclampsia within healthy pregnant women.

	Univariate				Multivariate			
	P	OR	95% C.I		P	OR	95% C.I	
Nullipara	0.192	0.519	0.194	1.391	–	–	–	–
Platelet count ($\times 10^9$/L)	0.006*	0.988	0.979	0.996	0.768	1.002	0.990	1.014
MPV (fL)	< 0.001*	2.768	1.340	9.713	< 0.001*	1.759	1.388	4.892
sE-Selectin (ng/mL)	0.004*	1.022	1.007	1.038	0.005*	1.027	1.008	1.046

* P is significant.

within healthy pregnant women. Applying nulliparous state, platelet count, MPV, ands E-selectin as predictors of PE, platelet count, MPV and sE-selectin showed significant association with PE in univariate analysis. However, in multivariate analysis, only MPV and sE-selectin were independent risk factors for PE development. Higher MPV or sE-selectin were associated with PE development ($P < 0.001$, OR = 1.759, 95% CI = 1.388–4.892; $P = 0.005$, OR = 1.027, 95% OR = 1.008–1.046 respectively). Data are shown in Table 3.

4. Discussion

Preeclampsia is a major cause of maternal and perinatal mortality with estimated incidence of 3–14% of all pregnancies worldwide [15]. Women with preeclampsia are at an increased risk for obstetric complications such as preterm delivery [16], and also subsequent cardiovascular disease later in life [17]. While many previous studies have documented relations between preeclampsia and markers of endothelial dysfunction, there is no widely accepted screening test for predicting preeclampsia. E-selectin has been examined as a biomarker for the disease but not within the context of evaluation of other previously documents markers such as platelet count or volume.

In this study we show using univariate and multivariate analysis that at least MPV and sE-selectin can be independent risk factors for PE development. We provide an evidence that sE-selectin together with platelet count and volume can be used in combination as markers for predicting preeclampsia.

Endothelial dysfunction has been proven in the pathogenesis of preeclampsia. The assessment of endothelial function any require costly and invasive methods. However, circulating soluble forms of adhesion molecules and selectins can be an attractive and relatively easy marker to assess. The state of inflammation and oxidative stress occurring during pregnancy can activate vascular endothelial cells and this increase expression of adhesion molecules and selectins [16]. Platelet's role in oxidative stress and thrombosis has also been documented. Furthermore, platelet count and volume have been assessed in relation to preeclampsia [18]. Our data goes in line with previous studies confirming E-selectin is a marker for preeclampsia [19,20].

The novelty in this study is the simultaneous use of a combination of sE-selectin and platelet count and volume as markers for prediction of preeclampsia. These simple and relatively inexpensive markers can be used for close prenatal monitoring, help earlier recognition of preeclampsia in high risk cases and thus allow appropriate antihypertensive therapy and speedy administration of steroid for fetal lung maturity if needed. Further insight into the pathophysiological role of sE-selectin in preeclampsia may facilitate a targeted strategy for preeclampsia prevention. We indeed recognize the limitation of the study due to the relatively small number of patients and we recommend larger studies to establish a foundation for wider use of these markers.

References

[1] C.W. Redman, I.L. Sargent, Latest advances in understanding preeclampsia, Science 308 (5728) (2005) 1592–1594.

[2] S. Hahn, A.K. Gupta, C. Troeger, C. Rusterholz, W. Holzgreve, Disturbances in placental immunology: ready for therapeutic interventions? Springer, Semin. Immunopathol. 27 (4) (2006) 477–493.

[3] B. Huppertz, Thefeto-maternal interface: setting the stage for potential immune interactions, Semin. Immunopathol. 29 (2) (2007) 83–94.

[4] E. Jauniaux, L. Poston, G.J. Burton, Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution, Hum. Reprod. Update 12 (2006) 747–755.

[5] T.H. Hung, J.N. Skepper, D.S. Charnock-Jones, G.J. Burton, Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia, Circ. Res. 90 (12) (2002) 1274–1281.

[6] N. Soleymanlou, I. Jurisica, O. Nevo, Molecular evidence of placental hypoxia in preeclampsia, J. Clin. Endocrinol. Metab. 90 (7) (2005) 4299–4308.

[7] L. Gang, Z. Yanyan, Z. Zhongwei, D. Juan, Association between mean platelet volume and hypertension incidence, Hypertens. Res. 40 (2017) 779–784.

[8] E. Varol, S. Akcay, A. Icli, H. Yucel, E. Ozkan, D. Erdogan, M. Ozaydin, Mean platelet volume in patients with prehypertension and hypertension, Clin. Hemorheol. Micro. Circ. 45 (2010) 67–72.

[9] A. Conde-Agudelo, J. Villar, M. Lindheimer, World Health Organization systematic review of screening tests for preeclampsia, Obstet. Gynecol. 104 (6) (2004 Dec) 1367–1391.

[10] S. Grill, C. Rusterholz, R. Zanetti-Dällenbach, S. Tercanli, W. Holzgreve, S. Hahn, O. Lapaire, Potential markers of preeclampsia, Reprod. Biol. Endocrinol. 7 (1) (2009) 70–84.

[11] S.Y. Kim, H.M. Ryu, J.H. Yang, M.Y. Kim, H.K. Ahn, H.J. Lim, J.S. Shin, H.J. Woo, S.Y. Park, Y.M. Kim, J.W. Kim, E.H. Cho, Maternal serum levels of VCAM-1, ICAM-1andE-selectin in preeclampsia, J. Korean Med. Sci. 19 (5) (2004) 688–692.

[12] S. Blankenberg, S. Barbaux, L. Tiret, Adhesion molecule and atherosclerosis, Atherosclerosis 170 (2003) 191–203.

[13] C.F. Krieglstein, D.N. Granger, Adhesion molecules and their role in vascular disease, Am. J. Hypertens. 14 (2001) 44S–54S.

[14] D. Woelkersa, J. Barton, P. von Dadelszenc, Baha Sibai, The revised 2013 ACOG definitions of hypertensive disorders of pregnancy significantly increase the diagnostic prevalence of preeclampsia, Pregnancy Hypertens. 5 (1) (2015) 38.

[15] B. Sibai, G. Dekker, M. Kupferminc, Preeclampsia, Lancet 365 (9461) (2005) 785–799.

[16] X. Chen, T.O. Scholl, Maternal biomarkers of endothelial dysfunction and preterm delivery, PLoS One 9 (1) (2014) e85716.

[17] J.T. Drost, H.E.M. Angela, M. SuzanneHolewijn, A.B. Leo, J.E. Joosten, T. Yvonne, van der Schouw, G. Jacquelinede, Novel cardiovascular biomarkers in women with a history of early preeclampsia, Atherosclerosis 237 (1) (2014) 117–122.

[18] P. Pignatelli, F. Violi, Platelet oxidative stress and thrombosis, Thromb. Res. 129 (3) (2012) 378–381.

[19] F. Mehrabian, S.H.M. Jazi, S.H. Javanmard, M. Kaviani, V. Homayouni, Circulating endothelial cells (CECs) and E-selectin: predictors of preeclampsia, J. Res. Med. Sci. 17 (1) (2012) 15–21.

[20] Z.B. Tuzcu, E. Asicioglu, M. Sunbul, B. Ozben, H. Arikan, M. Koc, Circulating endothelial cell number and markers of endothelial dysfunction in previously preeclamptic women, Am. J. Obstet. Gynecol. 213 (4) (2015 Oct) 533.e1-7.