Majalah Kedokteran Sriwijaya
Th. 53 Nomor 2, April 2021

MATERNAL SERUM ENDOCAN AS A POTENTIAL PREECLAMPSIA MARKER

Peby Maulina Lestari

Department of Obstetrics and Gynecology, Mohammad Hoesin Hospital, Faculty of Medicine Universitas Sriwijaya, Palembang

Email: peby_faris@yahoo.com

ABSTRACT

Preeclampsia remains the leading cause of maternal and neonatal morbidity and mortality worldwide. The imbalance of interactions between the placenta, immunity and the maternal cardiovascular system also plays a role in preeclampsia. Early onset preeclampsia (POD) and advanced onset preeclampsia (POL) are thought to have different pathomechanisms, causing different clinical symptoms. One theory of preeclampsia is the occurrence of endothelial dysfunction. Endocan as a specific endothelial protein is also thought to have an effect. Various studies on the role of Endocan in cardiovascular disease have also been carried out, while its role in preeclampsia that occurs in pregnant women is still very minimal. This study aims to determine the role of Endocan as a specific endothelial protein in preeclampsia. The study design was cross-sectional. A total of 72 subjects (24 subjects each in the POD, POL and normotensive pregnancy groups) were selected by consecutive sampling. Enzym Linked Immunoabsorbant Assay (ELISA) was performed to assess the Endocan concentration. In general, there is no significant difference in the characteristics of research subjects. Endocan concentrations were found to be higher in the POD group, while in the POL and normotensive pregnancy groups there was no significant difference. Endocan can be considered as a potential marker of preeclampsia, especially early onset.

Keywords: Endocan, endothelium, preeclampsia

1. INTRODUCTION

Preeclampsia is a specific disorder that occurs in pregnancy, characterized by hypertension and proteinuria after 20 weeks' gestation, in women who were previously normotensive (Walker, 2000; Robert, 2001). Preeclampsia affects 2–8% of pregnancies, and is a major cause of maternal and fetal morbidity and mortality, as well as causes the second largest maternal mortality worldwide (Huppertz B, 2008). The etiology of preeclampsia remains unclear, but it is thought that various factors, including placental ischemia, endothelial cell dysfunction, vasospasm, inflammation, inadequate angiogenesis and oxidative stress play a role in its development. Disruption of trophoblast invasion in early pregnancy will cause placental dysfunction and damage to the endothelium of blood vessels. In endothelial dysfunction there is an imbalance between humoral and cellular factors that will affect the structure and function of the endothelial wall. Risk factors that influence the occurrence of endothelial dysfunction include hypertension, dyslipidemia, smoking, age and obesity (Petrák et al, 2006; Widlansky et al, 2003; Ganz et al, 2003). In preeclampsia, endothelial dysfunction, if it

continues, will eventually affect the maternal hemodynamic system and lead to changes in maternal heart morphometry, especially in women who already have risk factors in their blood vessels.

Circulating Endothelial Cells (CEC) and other endothelial cell factors are often used as markers of endothelial cell activation / dysfunction. Several previous studies have shown an increase in CEC and other markers of endothelial cell damage such as soluble vascular cell adhesion molecule-1 (sVCAM-1), E-selectin and Endocan (Possomato-vieira & Khalil 2016).

Endocan is a specific endothelial cell molecule in humans, which was first discovered in 1999. Endocan is released by vascular endothelium into the circulation and plays an important role in regulating various processes, including adhesion, inflammation, and tumor progression. Endocan levels in serum will increase in several conditions related to endothelial activation or dysfunction (Yuksel et al, 2015).

In preeclampsia, there is extensive inflammation of the various organs involved, which will lead to excretion endocan. Endocan is a new marker that is thought to be associated with the incidence of hypertension. The role of Endocan as a marker in the incidence of hypertension has been tested in several studies, but the role of Endocan as a response to endothelial dysfunction in preeclampsia is still very limited.

Based on the time of diagnosis, there are terms early onset preeclampsia (<34 weeks) and late onset preeclampsia (> 34 weeks). Early onset preeclampsia is said to be associated with poor placentation, IUGR, and long-term cardiovascular effects than late-onset preeclampsia, whose pathogenesis is more related to maternal metabolic and cardiovascular factors that can induce endothelial dysfunction. The endothelium is the target organ in the pathophysiology of preeclampsia. Therefore, it is estimated that the maternal serum concentration of Endocan can be used as a marker of preeclampsia.

2. METHOD

This study is a cross-sectional comparative study with a screening technique, namely consecutive sampling. The research was conducted at Muhammad Hoesin Hospital Palembang and RS Hermina Palembang from October 2017 to January 2018. In this study, the population was all pregnant women who came with severe preeclampsia to Muhammad Hoesin Hospital Palembang and Hermina Palembang Hospital during the study period. The inclusion criteria were divided into two groups, namely the preeclampsia group and the normotensive pregnant women group. In the preeclampsia group the inclusion criteria were pregnant women who had severe preeclampsia (POD: gestational age> 20 weeks - <34 weeks; POL: gestational age> 34 weeks), single live intrauterine pregnancy, normal body temperature, and willing to participate in the study with signed an informed consent. Whereas for the normotension group, the inclusion criteria were gestational age> 37 weeks, not yet in labor, normotension, no history of cardiovascular disease and no history of other medical disorders. Subjects with a history of heart disease or currently suffering from cardiovascular disease, previous history of kidney disease, a history of liver dysfunction, a history of haematological disease and coagulation disorders, and using anticoagulant drugs, chronic antihypertensive and thrombolytic or not willing to be included in the study were excluded from the study.

The sample size was calculated using the numerical data sample size estimation formula for the mean difference of the 2 independent groups. There were 72 samples in this study, with the distribution

of each of the 24 samples in the POD, POL and normotensive groups.

Each study subject was subjected to standard examinations according to the preeclampsia protocol (blood pressure, proteinuria, complete blood chemistry, liver and kidney function tests and assessment of neurological deficits). Subjects who meet the inclusion criteria will then be given informed consent to collect blood for serum Endocan examination. 10 mL of venous blood samples for research subjects were taken for Endocan examination. The blood sample taken was inserted into an ethylenediamine tetracetic acid (EDTA) tube for DPL examination, a serum separator tube (SST) tube for PP 13 and Endocan examinations. Blood samples (for PP 13 and Endocan were centrifuged at 1500 g for 10 minutes and stored at -80ºC). Measurement of maternal endocan serum concentrations was carried out using the ELISA technique using the Human Endothelial-Cell Specific Molecule-1 (ESM-1) ELISA kit (Aviscera Bioscience, Inc). From the results of this measurement, data will be obtained in units of ng / mL. The examination was carried out at the Prodia Laboratory. This study has passed the ethical review of the Faculty of Medicine, University of Indonesia, number 953 / UN2.F1 / ETIK / 2017. The research data is recorded in the form provided. Data will be recorded in the master table, verified, edited, coded and analyzed using the Stata version 15 program. The statistical analysis of the data from this study used One Way ANOVA and Kruskal Wallis with a significance level of 0.05.

3. RESULT

The results showed that there are significant differences in the mean gestational age in early onset preeclampsia, late onset and normotensive pregnancy in the 3rd trimester of pregnancy. The differences in mean age, height, BSA, and parity were not statistically significant in early onset preeclampsia, late onset and normotensive pregnancy in the 3rd trimester of pregnancy (Table 1).

Table 1. Characteristics of Research Subjects

Characterictic	Early Onset	Late Onset	Control	p value
Age (years)	31.56 ± 7.47	29.37 ± 6.18	30.23 ± 2.32	0.381
Height (cm)	154.41 ± 4.76	153.96 ± 4.93	154.58 ± 5.93	0.907
Weight (kg)	72.89 ± 10.05	79.96 ± 10.14	69.04 ± 5.11	0.286
BSA (m^2)	1.76 ± 0.14	1.74 ± 0.14	1.72 ± 0.08	0.423
Parity	1.33 ± 1.30	1.11 ± 1.34	1.15 ± 0.54	0.746
Gestational age (weeks)	31.37 ± 1.96	37.81 ± 1.90	38.00 ± 0.00	<0.001

*BSA: *body surface area* (m^2), One Way ANOVA test, p value < 0.05

Meanwhile, the maternal serum concentrations of Endocan in both early onset and late onset preeclampsia compared to controls are presented in Table 2. The results of analysis using Kruskal Wallis showed that the difference in Endocan was not statistically significant in early onset preeclampsia, late onset and normotensive pregnancy in the 3rd trimester of pregnancy. However, it appears that Endocan levels in the two preeclampsia groups are relatively the same and higher than in normotensive pregnancies.

Table 2. Concentrations of Maternal Endocan Serum

Marker	Early Onset	Late Onset	Control	p value
Endocan (ng/mL)	37.29 (5.9–263.1)	37.29 (5.9–138.6)	22 (5.09–139)	0.523

* Kruskal Wallis test, p value < 0.05

4. DISCUSSION

In socio-demographic characteristics of the study subjects, there was no significant difference between age, height, weight and BSA in the three study groups. Body weight and BSA, which are thought to have an effect on the incidence of preeclampsia, in this study did not appear to be significantly different in early onset preeclampsia compared to late onset, suggesting that obesity may not be a risk factor for early onset preeclampsia. The results of this study differed from the study conducted by Iacobelli et al., which stated that there was a significant difference between BMI in preeclampsia compared to normal controls, but there was no difference between early onset preeclampsia and late onset preeclampsia. Likewise with parity, there was no significant difference, indicating that parity is not a risk factor for preeclampsia. This study is also in line with Iacobelli's (Iacobelli et al, 2017). Significant differences were only seen at gestational age. By definition, early onset preeclampsia occurs at <34 weeks' gestation (31,37 $\pm$ 1,96). The results of this study refute the old theory that preeclampsia is influenced by body weight and parity.

Endocan is a soluble proteoglycan secreted by vascular endothelium. Proteoglycans (PG) are proteins that have glycosaminoglycan chains that are covalently related to their protein nuclei. Some PG is found in the extracellular matrix of connective tissue. Its main biological action is to provide structural support. Endocan is a type of PG that is secreted by vascular endothelium.[10] This protein has been found to be elevated in patients with sepsis, hepatocellular and renal malignancies, as well as in other inflammatory disorders, such as Behck's disease. The endocan may play a key role in blood vessels against specific organ inflammation and in endothelial dependent pathologies, and may also be a marker for endothelial cell dysfunction. Endocan secretion is regulated by cytokines and growth factors, such as Tumor Necrosis Factors-α (TNF-α) and Vascular Endothelial Growth Factor (VEGF) (Iacobelli et al, 2017; Yuksel et al, 2015; Kali, 2014). Endocan itself is a marker of Glycocalyx disorders (Bar et al, 2019).

Endocan was first discovered in 1996 by Lassalle et al. They succeeded in identifying a human endothelial cell-specific molecule (ESM) that was cloned from human umbilical vein endothelial cells. They also suspect the protein is related to cell biology in blood vessels, inflammation and lung physiology. Because of its very limited distribution in vascular endothelial cells, this molecule was originally named ESM-1. Subsequent research indicated that this ESM-1 belonged to the proteoglycan group, and was later called Endocan (Bar et al, 2019; Zhang et al, 2012).

Endocan can play a role in molecular interactions with broad biological activities that are important for various biological processes such as adhesion, migration, proliferation and neovascularization of

cells (Kali et al, 2014). In acute infection, vascular endothelium and leukocytes will express adhesion molecules that play an important role in the migration of leukocytes out of blood vessels. towards the site of infection. Tumor Necrosis Factor (TNF) and interleukin-1 (IL-1) secreted by macrophages stimulate the expression of selectin E and P, as well as other ligand integrins such as Intercellular Adhesion Molecule-1 (ICAM-1) in the endothelium of small blood vessels adjacent to the inflamed areas. During the activation process of the infection response, leukocytes need ligands to bind to E, P and integrin selectins, namely Lymphocyte Function-Associated Antigen-1 (LFA-1). As a result of the binding of the selectin with the ligand, circulating leukocytes begin to attach to the endothelial surface and the LFA-1 bond with ICAM 1 will initiate the process of leukocyte transmigration to the site of infection in peripheral tissues. Endocan core protein binding with LFA-1 has a negative effect on the interaction of LFA-1 with endothelial ICAM-1. As a result, Endocan will inhibit leukocyte migration Kali et al, 2014).

Although Endocan was first described as a soluble circulating dermatan sulfate proteoglycan, which is secreted by human umbilical vein endothelium, various in vitro studies have shown that various human endothelial cells also express Endocan. Endothelial that is activated due to inflammatory processes or due to tumor progression will show several-fold increase in Endocan mRNA Zhang et al, 2012). Endothelial dysfunction, atherosclerosis and hypertension are related and since Endocan is also associated with endothelial dysfunction and inflammation, it is thought that Endocan is also associated with hypertension (Demirkol et al, 2014; Afsar et al, 2014).

In the pilot study, it was reported that the concentration of circulating endocan (ESM-1) could be used. as a new marker of essential hypertension (Demirkol et al, 2014). From this study, it was found that in 18 patients diagnosed with new-onset hypertension, serum Endocan concentrations were significantly associated with carotid artery tunica media thickness and were associated with high C reactive protein (CRP) levels when compared to controls. Other studies have shown that Endocan is an endothelial cell-specific molecule whose expression is regulated by cytokines and VEGF (than et al, 2008). Zhang et al. study also showed that Endocan is expressed in actively proliferating cells and tissues, one of which is endothelium (Zhang et al, 2012). Endocan is not seen in tissue cells that are in a silent or resting state, such as the splenic endothelium and large arteries (Ünal et al, 2017; Demirkol et al, 2014). Research Balta et al stated that circulating concentrations of Endocan could be used as a new marker for hypertension and other cardiovascular diseases (Balta et al, 2015).

The endothelium plays an important role in regulating vascular tone and maintaining cardiovascular homeostasis by releasing various vasoactive factors. In addition, endothelial function is also affected by systemic inflammation. Since hypertension and endothelial dysfunction are thought to be associated, Endocan is thought to be a new marker that may be associated with cardiovascular disease and its consequences (Demircioglu et al, 2018).

So far there have been two studies that have examined Endocan levels in preeclampsia. The study measured Endocan levels in 49 pregnant women with preeclampsia and 32 normotensive pregnancies (Yuksel et al, 2015). Measurement of Endocan levels was carried out using an Enzyme Linked Immunosorbent Assay (ELISA) kit. The study showed no difference in Endocan levels between the two study groups (10.7 ± 4.5 vs. 10.3 ± 3.2 ng / mL, p =0.763). Other studies have shown that the median plasma concentrations of Endocan were

higher in the preeclampsia group. compared to the normotension group (p = 0.004), but in the preeclampsia group itself, Endocan plasma levels did not differ significantly based on the degree of severity.

Endothelial dysfunction is associated with sepsis and inflammatory events, ranging from vasodilation, edema to coagulopathy, ischemia and organ failure. Because inflammatory mediators such as IL-1 and TNF-α will induce Endocan expression, the levels of these proteoglycans in the blood will increase, and are thought to reflect the severity of the inflammation that occurs (kali et al, 2014). This is the rationale that serum Endocan levels can be used one of the predictor markers of preeclampsia. Endocan is mainly secreted by inflamed vascular endothelial cells. Serum endocan is said to be associated with the severity of the abnormality that occurs (Lee et al, 2014). In this study, endocan levels in maternal serum were relatively the same in early onset preeclampsia [37.29 (5.9–263.1)] and late onset preeclampsia [37.29 (5.9–138.6)], but their value was relatively higher than that of the normotensive pregnancy group [22 (5.09–1390], but this difference was not statistically significant. This is in line with a study conducted by Adekola et al which showed that the concentration of Endocan in maternal plasma was higher in preeclampsia than in normotensive pregnancies (p = 0.004) (Adekola et al, 2015). In addition, this study also stated that there was no significant difference in the concentration of maternal endocan in preeclampsia that occurred before 34 weeks of gestation and or after 34 weeks. others also showed that serum endocan concentrations were significantly increased in preeclamptic women compared to normotensive (20.04 (12.26) ng / mL vs. 15.55 (6.19) ng / mL, p <0.001) (Cakmak et al, 2016).

5. CONCLUSION

The results of this study indicate that endocan is a promising diagnostic marker in preeclampsia, but of course it still requires further research with a larger study sample and continuous testing starting early in pregnancy.

DISCLOSURE STATEMENT

The authors report no conflict of interest.

REFERENCES

[1]. Roberts JM, Cooper D. Pathogenesis and genetics of preeclampsia. Lancet. 2001;357:53–6.
[2]. Walker JJ. Pre-eclampsia. Lancet. 2000;356:1260–5.
[3]. Huppertz B. Placental Origins of Preeclampsia. Hypertension. 2008;51(4):970–5.
[4]. Petrák O, Widimský J, Zelinka T,. Biochemical markers of endothelial dysfunction in patients with endocrine and essential hypertension. Physiol Res. 2006;55(6):597–602.
[5]. Widlansky ME, Gokce N, Keaney JF, Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol. 2003;42(7):1149–60.
[6]. Ganz P, Vita JA. Testing Endothelial Vasomotor Function: Nitric Oxide, a Multipotent Molecule. Circulation. 2003;108(17):2049–53.
[7]. Possomato-vieira JS, Khalil RA. Mechanisms of Endothelial Dysfunction in Hypertensive Pregnancy and Preeclampsia. Adv Pharmacol. 2016;77: 361–431.
[8]. Yuksel MA, Tuten A, Oncul M, Serdar A, Yuksel Iikbak, Toprak MS

et.al. Serum endocan concentration in women with pre-eclampsia. Arch Gynecol Obstet. 2015;292(1):69–73.

[9]. Iacobelli S, Bonsante F, Robillard P. Comparison of risk factors and perinatal outcomes in early onset and late onset preeclampsia : A cohort based study in Reunion Island Comparison of risk factors and perinatal outcomes in early onset and late onset preeclampsia : A cohort based study in Reunion Island. J Repro Immun. 2017;123:12–6.

[10]. Kali A, Shetty KSR. Endocan: A novel circulating proteoglycan. Indian J Pharmacol. 2014;46(6):579–83.

[11]. Bar A, Targosz-korecka M, Suraj J, Chclopicki S, Proniewski B, Sternak M, et al. Degradation of Glycocalyx and Multiple Manifestations of Endothelial. J Am Heart Assoc. 2019; 8(6):1–18.

[12]. Zhang SM, Zuo L, Zhou Q,. Expression and distribution of endocan in human tissues. Biotech Histochem. 2012;87(3):172–8.

[13]. Demirkol S, Kurtoglu E, Demir M,Balta S, Mikhailidis DP, Ozturk C, et. al.. Endocan—A Novel Inflammatory Indicator in Newly Diagnosed Patients With Hypertension. Angiology. 2014;65(9):773–7.

[14]. Afsar B, Takir M, Kostek O, Covic A, Kanbay M. Endocan: A New Molecule Playing a Role in the Development of Hypertension and Chronic Kidney Disease? J Clin Hypertens. 2014;16(12):914–6.

[15]. Than NG, Abdul Rahman O, Magenheim R, Nagy B, Fule T, Hargital B, et al. Placental protein 13 (galectin-13) has decreased placental expression but increased shedding and maternal serum concentrations in patients presenting with preterm pre-eclampsia and HELLP syndrome. Virchows Arch. 2008;453(4):387–400.

[16]. Ünal S, Açar B, Ertem AG, Sen F. Endocan in Hypertension and Cardiovascular Diseases. Angiology. 2017;68(1):85.

[17]. Balta S, Mikhailidis DP, Demirkol S, Ozturk C, Celik T, Iyisoy A. Endocan: A novel inflammatory indicator in cardiovascular disease? Atherosclerosis. 2015;243(1):339–43.

[18]. Demircioglu K, Yalcinkaya E, Aciksari G, Kavas M, Atici A, Erman H, et al. Endocan Levels and Endothelial Dysfunction in Patients With Sarcoidosis. Angiology. 2018;69(10):878–83.

[19]. Lee HG, Choi HY, Bae JS. Endocan as a potential diagnostic or prognostic biomarker for chronic kidney disease. Kidney Int. 2014;86(6):1079–81.

[20]. Adekola H, Romero R, Chaemsaithong P. Endocan, a putative endothelial cell marker, is elevated in preeclampsia, decreased in acute pyelonephritis, and unchanged in other obstetrical syndromes. J Matern Neonatal Med. 2015;28(14):1621–32.

[21]. Cakmak M, Yilmaz H, Baılar E, Darcin E, Inan O, Celik T, Ozdemir O, et al. Serum levels of endocan correlate with the presence and severity of pre-eclampsia. Clin Exp Hypertens. 2016;38(2):137–42.