CEOG Clinical and Experimental Obstetrics & Gynecology

Alteration of serum endocan in normal pregnancy and preeclampsia

H.Z. Wang[1], Y. Jin[1], P. Wang[1], C. Han[1], Z.P. Wang[1], M.Y. Dong[1,2,3]

[1] Women's Hospital, School of Medicine, Zhejiang University, Hangzhou
[2] Key Laboratory of Reproductive Genetics, Ministry of Education, Hangzhou
[3] Key Laboratory of Women's Reproductive Health of Zhejiang Province, Hangzhou (China)

Summary

Endocan is a novel marker for inflammation but its significance in preeclampsia remains unknown. *Objective:* The aims of this study were to delineate the changes of maternal endocan in normal pregnancy and preeclampsia and to explore its possible role in the pathogenesis of preeclampsia. *Materials and Methods:* Blood samples were taken from 42 non-pregnant women, from 43 normal pregnant women at third trimester, and from 41 preeclamptic women. Serum endocan levels were determined with enzyme-linked immunosorbent assay (ELISA) and compared among the groups. *Results:* There were no significant differences in serum endocan among non-pregnant women, normal pregnant women, and women with preeclampsia ($\chi^2 = 2.207$, $p = 0.137$) and there was no significant difference in serum endocan between women with mild and severe preeclampsia ($Z = 0.368$, $p = 0.713$). The authors concluded that serum endocan did not change in pregnancy and preeclampsia, indicating endocan may not be involved in the pathogenesis of preeclampsia.

Key Words: Endocan; Pregnancy; Preeclampsia; Vascular endothelium.

Introduction

Preeclampsia is a pregnancy-specific complication characterized by newly onset of hypertension and proteinuria in the second half of pregnancy [1-3]. It complicates 2% to 8% of pregnancies and increases the mortality and morbidity in both mother and child [1-3]. The outcomes of preeclampsia have been improved in the past decades, but the etiology of the disease remains largely unknown [1-3]. However, it is well accepted that normal pregnancy is a condition of low grade inflammation, and exaggerated inflammation and vascular endothelial dysfunction are the important pathogenesis and play critical roles in the development of preeclampsia [1-3].

Very recently, it was described that serum endocan levels were significantly higher in newly diagnosed patients with essential hypertension compared with normotensive controls [4]. Endocan, previously named endothelial cell-specific molecule 1 (ESM-1), is a novel human endothelial cell-specific molecule and is expressed by the vascular endothelium and secreted into the blood stream in healthy individuals [5]. The levels of endocan in circulation are increased in acute and severe inflammation [4, 6-8]. Accumulating evidences indicate that endocan is a novel marker of inflammation and endothelial dysfunction [6, 9]. On the other hand, it has been shown that the expression of endocan is upregulated by vascular endothelium growth factor (VEGF), interleukin (IL)-1, and tumor necrosis factor (TNF)-α [10, 11) while the blood levels of VEGF, IL-1, and TNF-α were increased in patients of preeclampsia [12-14].

On the basis of these findings, the present authors hypothesized that circulating endocan level might be altered in normal pregnancy and/ or preeclampsia. To verify their hypothesis, they determined serum concentrations of endocan in non-pregnant, normal pregnant, and preeclamptic women.

Materials and Methods

Subjects

Forty-two healthy non-pregnant women, 43 normal pregnant women, and 41 women with preeclampsia (23 mild and 18 severe) were recruited in Women's Hospital, School of Medicine, Zhejiang University.

Pregnancy was diagnosed upon positive human chorionic gonadotropin test after missed menstruation. Gestational age was calculated by menstrual dating. Ultrasound was performed to confirm pregnancy and gestational age. Preeclampsia was diagnosed and classified according to the criteria recommended by American College of Obstetrics and Gynecologist (ACOG): a systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher on two occasions at least six hours apart occurring after 20 weeks of gestation in a pregnant woman with previously normal blood pressure and detectable urinary protein (≥ 1 + by dipstick or 0.3 g/24 hours and more) [15]. Severe preeclampsia was defined as a blood pressure greater than or equal to 160/110 mm Hg with either a urine dipstick showing 3+ or 4+ in a random urine sample or greater than 5.0 grams of proteinuria over 24 hours [15]. Other evidence of severe disease included elevated serum creatinine, eclampsia, pulmonary edema, oliguria

Revised manuscript accepted for publication November 10, 2015

(less than 500 ml per 24 hours), fetal growth restriction, oligohydramnios, and symptoms suggesting significant end-organ involvement (headache, visual disturbance, or epigastric or right upper quadrant pain). Women who met criteria of preeclampsia but not severe preeclampsia were diagnosed mild preeclampsia.

Exclusion criteria were multiple gestation, diabetes mellitus, chronic hypertension, infectious diseases recognized in pregnancy, premature rupture of membrane, active labor, polyhydramnios, and signs of other concurrent medical complication. The control women had no sign of gestational complications and fetal distress and gave birth to healthy neonates of appropriate size for gestational age.

Clinical data and demographic data were collected according to the medical records. The approval of the current study was obtained from Institutional Ethical committee of Women's Hospital, School of Medicine, Zhejiang University, and all the participants provided their informed consents.

Sample collection and assay

Blood samples were taken from pregnant women after admission before medical treatment or from non-pregnant women at their healthy examination and centrifuged after standing at room temperature for at least 30 minutes. Serum was separated and aliquoted in -80 C until assayed. Endocan concentrations were measured with a commercially available human endocan/ESM-1 enzyme-linked immunosorbent assay (ELISA) kits.

Statistic analysis

Data were tested by Kolmogorov-Smirnov test for their distribution. Normally distributed data were presented in mean ± standard deviation (SD) and compared by Student's *t*-test or one-way ANOVA, while data with skew distribution were presented in median and quartiles and compared with Mann-Whitney test or Kruskal-Wallis test. Category data were compared with chi-square (χ^2) test and the correlation was analyzed with regression analysis. Statistic package SPSS was used for data analysis. A $p < 0.05$ was considered to be statistically significant.

Results

Table 1 shows the clinical data. There were significant differences in maternal age among healthy non-pregnant women, normal pregnant women, and women with preeclampsia (F = 5.532, $p = 0.005$). The maternal age was not significantly different between non-pregnant and normal pregnant women ($P = 0.239$), but women with preeclampsia were significantly older than non-pregnant women ($p = 0.001$) and normal pregnant women ($p = 0.037$). As expected, there were significant differences in gestational age at sampling ($t = 8.096$, $p < 0.001$), gestational age at delivery ($t = 7.804$, $p < 0.001$), and neonatal birth weight ($t = 7.708$, $p < 0.001$) between normal pregnant women and women with preeclampsia. There was no significant difference in fetal gender between groups ($\chi^2 = 1.248$, $p = 0.282$).

There were no significant differences in serum endocan among non-pregnant women, normal pregnant women and women with preeclampsia ($\chi^2 = 2.207$, $p = 0.137$; Figure 1) and there was no significant difference in serum endocan between women with mild and severe preeclampsia (Z = 0.368, $p = 0.713$; Figure 2).

Table 1. — *Clinical data.*

	Non-Pregnancy 42	Normal Pregnancy 43	Preeclampsia 41	*p*
Maternal age (y)	28.14±2.95	29.00±2.94	30.53±3.93	0.005
Gestational age sampling (w)	-	38.81±1.14	34.26±3.45	< 0.001
Gestational age at delivery (w)	-	38.88±1.09	34.61±3.41	< 0.001
Birth weight (g)	-	3370±394	2217±894	< 0.001
Fetal gender	-	Male: 21 Female: 22	Male: 16 Female: 25	0.282

Correlation analysis showed that serum endocan was not significantly correlated with maternal age in non-pregnant women (r = 0.035, $p = 0.826$), normal pregnant women (r = 0.107, $p = 0.501$), preeclamptic women (r = 0.131, $p = 0.413$), and all subjects (r = 0.060, $p = 0.507$). Similarly, there was no significant correlation between gestational age at delivery and endocan in normal pregnant women (r = 0.04, $p = 0.763$), preeclamptic women (r = 0.013, $p = 0.934$), and all subjects (r = -0.081, $p = 0.461$).

Discussion

In the current observation, the authors revealed for the first time that serum endocan, a novel marker of inflammation, did not significantly differ among healthy non-pregnant women, normal pregnant women, and preeclamptic women. Furthermore, correlation analysis which revealed no significant correlation between endocan level and maternal age or gestational age, excluded possible confounding effects on serum endocan by maternal age or gestational age. These findings indicate that endocan, an inflammatory mediator, might not be involved in the pathogenesis of preeclampsia.

Very recently, Balta *et al.* [4] reported that circulating endocan was significantly increased in patients with essential hypertension compared with normotensive subjects and serum endocan level was significantly correlated with carotid intima-media thickness (cIMT) and high-sensitivity C-reactive protein (hsCRP). In patients with psoriasis vulgaris, Behcet disease, and inflammatory bowel disease, all of which are inflammatory diseases, serum levels of endocan were significantly increased compared with controls [7, 8, 16]. Furthermore, serum endocan level was significantly correlated with psoriasis activity and severity index (cIMT and hsCRP) in psoriasis patients [7], and, CRP, erythrocyte sedimentation rate, and Behcet disease activity in patients with Behcet disease [8]. In addition, serum endocan level was dramatically increased in patients with septic shock [5]. These articles suggest that endocan is a marker for inflammation and endothelial dysfunction and has a functional role in the development of endothelium-dependant pathological disorders.

Normal pregnancy is a condition of low-grade systemic

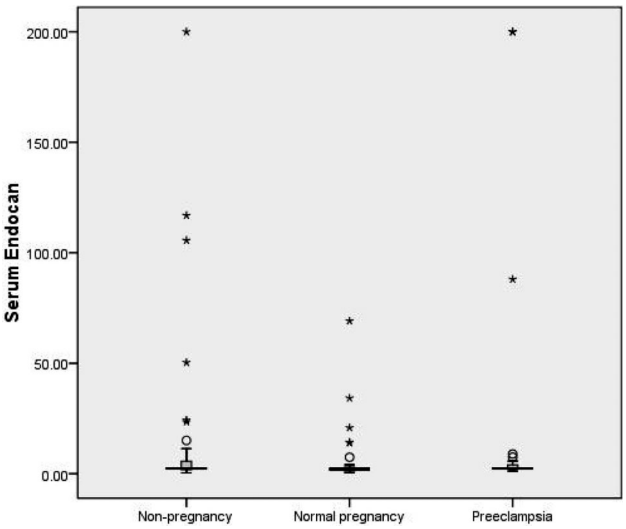

Figure 1. — Comparison of serum endocan among non-pregnant women, normal pregnant women, and women with preeclampsia. There were no significant differences among healthy non-pregnant women (non-pregnancy), normal pregnant women (normal pregnancy), and preeclamptic women (preclampsia) ($\chi^2 = 2.207$, $p = 0.137$). The bold line represents the median, the box represents the quartiles, and the ends of whiskers represent 97.5 and 2.5 percentiles. Circles and asterisks represent mild and extreme outliers.

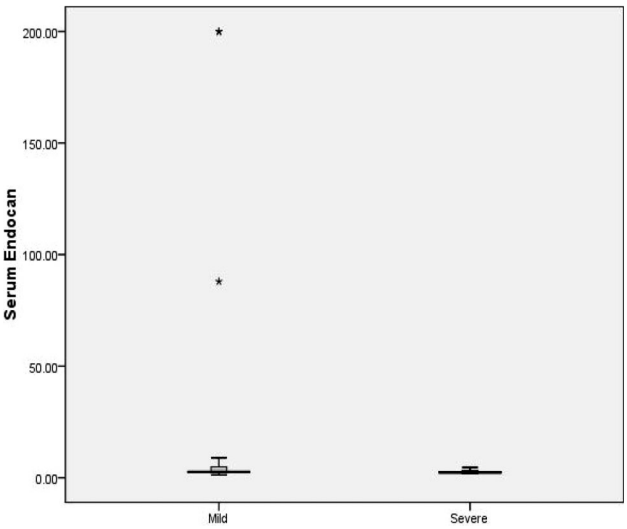

Figure 2. — Comparison of serum endocan between mild and severe preeclampsia. There was no significant difference in serum endocan between women with mild (mild) and severe (severe) preeclampsia ($Z = 0.368$, $p = 0.713$). The bold line represents the median, the box represents the quartiles, and the ends of whiskers represent 97.5 and 2.5 percentiles. Circles and asterisks represent mild and extreme outliers.

inflammation and preeclampsia is an excessive systemic inflammation response with dysfunction of endothelium. Previously, the present authors reported that serum levels of IL-15, IL-16, and IL-18 were significantly increased in women with preeclampsia compared with normal pregnant women [17, 18]. In a meta-analysis of 23 studies comprised of 1,015 preeclamptic women and 925 normotensive pregnant women, it was found that maternal circulating TNF-α was significantly higher in women with preeclampsia than normotensive pregnant women and women with severe preeclampsia, and women with mild preeclampsia both had significantly higher circulating TNF-α than normotensive pregnant women [13]. Furthermore, the levels of maternal IL-6 and IL-10 were altered in a similar pattern [13]. Maternal IL-1β increased significantly in women with preeclampsia compared with normal pregnant women [12]. On the other hand, dysfunction or activation of vascular epithelial cell is one of characteristics of preeclampsia and is the key pathology leading placental ischemia to systemic pathology [2]. Maternal biomarkers of endothelial injury including soluble intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin (E-selectin), thrombospondin, soluble fms-like tyrosine kinase-1 (sFlt-1), VEGF, and nitric oxide were significantly changed in preeclampsia (14, 19-20).

Endocan is a protein encoded by ESM-1 gene in humans, and is preferentially expressed in epithelium of neovasculatures [21, 22]. Some tumor cells at least including renal cell carcinoma [23], stomach cancer [24], and glioblastoma [25] also express endocan. The expression of endocan is regulated by a number of cytokines and growth factors. VEGF, fibroblast growth factor-2 (FGF-2, also named basic FGF or bFGF), TNF-α, and IL-1 upregulate endocan expression in vitro [10, 11, 22]. In addition to elevation of blood VEGF, IL-1, and TNF-α as summarized above, serum FGF-2 was also significantly increased in patients with mild preeclampsia [26].

Endocan is a biomarker for inflammation response and dysfunction of vascular endothelial cell, while preeclampsia is a condition of exaggerated systemic inflammation response accompanied by dysfunction of vascular endothelium. On the other hand, cytokines and growth factors upregulating endocan expression in vitro increases in patients with preeclampsia. As these two factors are considered, it is reasonable to propose that endocan level may be elevated in patients of preeclampsia. However, the present authors did not find any significant alteration in serum endocan in either normal pregnancy or preeclampsia. These findings imply that endocan is among the molecules involved in the pathogenesis of preeclampsia. Further studies are required to confirm these findings and explore possible roles of endocan in preeclampsia.

Conclusion

Serum level of endocan did not significantly differ among healthy non-pregnant women, pregnant women, and women with preeclampsia, and endocan may not play an important role in the pathogenesis of preeclampsia.

Acknowledgements

This work was financially supported by Natural Science Foundation of China (81170572 and 81370726).

References

[1] Uzan J., Carbonnel M., Piconne O., Asmar R., Ayoubi J.M.: "Pre-eclampsia: pathophysiology, diagnosis, and management". *Vasc. Health. Risk. Manag.*, 2011, *7*, 467.

[2] Steegers E.A., von Dadelszen P., Duvekot J.J., Pijnenborg R.: "Pre-eclampsia". *Lancet*, 2010, *376*, 631.

[3] Duley L., Gulmezoglu A.M., Henderson-Smart D.J., Chou D.: "Magnesium sulphate and other anticonvulsants for women with pre-eclampsia". *Cochrane Database. Syst. Rev.*, 2010, 11, CD000025.

[4] Balta S., Mikhailidis D.P., Demirkol S., Ozturk C., Kurtoglu E., Demir M., *et al.*: "Endocan--A novel inflammatory indicator in newly diagnosed patients with hypertension: a pilot study". *Angiology*, 2014, *65*, 773.

[5] Bechard D., Meignin V., Scherpereel A., Oudin S., Kervoaze G., Bertheau P., *et al.*: "Characterization of the secreted form of endothelial-cell-specific molecule 1 by specific monoclonal antibodies". *J. Vasc. Res.*, 2000, *37*, 417.

[6] Scherpereel A., Depontieu F., Grigoriu B., Cavestri B., Tsicopoulos A., Gentina T., *et al.*: "Endocan, a new endothelial marker in human sepsis". *Crit. Care. Med.*, 2006, *34*, 532.

[7] Balta I., Balta S., Demirkol S., Mikhailidis D.P., Celik T., Akhan M., *et al.*: "Elevated serum levels of endocan in patients with psoriasis vulgaris: correlations with cardiovascular risk and activity of disease". *Br. J. Dermatol.*, 2013, *169*, 1066.

[8] Balta I., Balta S., Koryurek O.M., Demirkol S., Mikhailidis D.P., Celik T., *et al.*: "Serum endocan levels as a marker of disease activity in patients with Behcet disease". *J. Am. Acad. Dermatol.*, 2014, *70*, 291.

[9] Lassalle P., Molet S., Janin A., Heyden J.V., Tavernier J., Fiers W., *et al.*: "ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines". *J. Biol. Chem.*, 1996, *271*, 20458.

[10] Delehedde M., Devenyns L., Maurage C.A., Vives R.R.: "Endocan in cancers: a lesson from a circulating dermatan sulfate proteoglycan". *Int. J. Cell. Biol.*, 2013, *2013*, 705027.

[11] Rennel E., Mellberg S., Dimberg A., Petersson L., Botling J., Ameur A., *et al.*: "Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer". *Exp. Cell. Res.*, 2007, *313*, 1285.

[12] Dong W., Yin L.. "Expression of lipoxin A, TNFalpha and IL-1beta in maternal peripheral blood, umbilical cord blood and placenta, and their significance in pre-eclampsia". *Hypertens. Pregnancy*, 2014, *24*, 1.

[13] Lau S.Y., Guild S.J., Barrett C.J., Chen Q., McCowan L., Jordan V., *et al.*: "Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: a systematic review and meta-analysis". *Am. J. Reprod. Immunol.*, 2013, *70*, 412.

[14] Celik H., Avci B., Isik Y.: "Vascular endothelial growth factor and endothelin-1 levels in normal pregnant women and pregnant women with pre-eclampsia". *J. Obstet. Gynaecol.*, 2013, *33*, 355.

[15] ACOG Committee on Obstetric Practice.: "ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists". *Int J Gynaecol Obstet.*, 2002, *77*, 67.

[16] Voiosu T., Balanescu P., Bengus A., Voiosu A., Baicus C.R., Barbu M., *et al.*: "Serum endocan levels are increased in patients with inflammatory bowel disease". *Clin. Lab.*, 2014, *60*, 505.

[17] Huang X., Huang H., Dong M., Yao Q., Wang H.: "Serum and placental interleukin-18 are elevated in preeclampsia". *J. Reprod. Immunol.*, 2005, *65*, 77.

[18] Hu W., Wang H., Wang Z., Huang H., Dong M.: "Elevated serum levels of interleukin-15 and interleukin-16 in preeclampsia". *J. Reprod. Immunol.*, 2007, *73*, 166.

[19] Masoura S., Kalogiannidis I., Makedou K., Theodoridis T., Koiou K., Gerou S., *et al.*: "Biomarkers of endothelial dysfunction in preeclampsia and neonatal morbidity: a case-control study". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2014, *175*, 119.

[20] Stenczer B., Molvarec A., Veresh Z., Gullai N., Nagy G.R., Walentin S., *et al.*: "Circulating levels of the anti-angiogenic thrombospondin 2 are elevated in pre-eclampsia". *Acta. Obstet. Gynecol. Scand.*, 2011, *90*, 1291.

[21] Zhang S.M., Zuo L., Zhou Q., Gui S.Y., Shi R., Wu Q., *et al.*: "Expression and distribution of endocan in human tissues". *Biotech. Histochem.*, 2012, *87*, 172.

[22] Abid M.R., Yi X., Yano K., Shih S.C., Aird W.C.: "Vascular endocan is preferentially expressed in tumor endothelium". *Microvasc. Res.*, 2006, *72*, 136.

[23] Leroy X., Aubert S., Zini L., Franquet H., Kervoaze G., Villers A., *et al.*: "Vascular endocan (ESM-1) is markedly overexpressed in clear cell renal cell carcinoma". *Histopathology*, 2010, *56*, 180.

[24] Zhang S., Zuo L., Gui S., Zhou Q., Wei W., Wang Y.: "Induction of cell differentiation and promotion of endocan gene expression in stomach cancer by melatonin". *Mol. Biol. Rep.*, 2012, *39*, 2843.

[25] Maurage C.A., Adam E., Mineo J.F., Sarrazin S., Debunne M., Siminski R.M., *et al.*: "Endocan expression and localization in human glioblastomas". *J. Neuropathol. Exp. Neurol.*, 2009, *68*, 633.

[26] Hohlagschwandtner M., Knofler M., Ploner M., Zeisler H., Joura E.A., Husslein P.: "Basic fibroblast growth factor and hypertensive disorders in pregnancy". *Hypertens. Pregnancy*, 2002, *21*, 235.

Corresponding Author:
M.Y. DONG, M.D., Ph.D.
Women's Hospital, School of Medicine
Zhejiang University
1 Xueshi Road, Hangzhou
Zhejiang Province, 310006 (China)
e-mail: mydong.cn@hotmail.com