Research Article

Soluble Endoglin Serum Level is Higher in Preeclampsia Compared to Molar and Normal Pregnancy

Kadar Soluble Endoglin Serum lebih Tinggi pada Preeklampsia dibanding Kehamilan Molar dan Hamil Normal

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Abstract

Objective: To analyze the differences of maternal serum soluble endoglin level in preeclampsia, molar pregnancy, and normal pregnancy, and to analyze the correlation between maternal serum soluble endoglin level with gestational age.

Method: This is a cross-sectional comparative analytic study involving 18 preeclampsia cases, 18 molar pregnancies, and 18 normal pregnancies. The sample were obtained from Dr. Hasan Sadikin hospital and six satellite hospitals from January until March 2013. The comparison of mean seng serum level of the preeclampsia, molar pregnancy, and normal pregnancy group was calculated using Kruskal Wallis, and the correlation were calculated using Rank Spearman.

Result: The mean level of seng serum in preeclampsia group was higher (168.79 ng/ml) than in molar pregnancy (43.47 ng/ml) and normal pregnancy (32.38 ng/ml). There is no significant difference of serum seng level between molar and normal pregnancy, with p value of 0.393 (p>0.05). There is significant differences of seng serum level between preeclampsia group and molar pregnancy (p=0.000), but no significant differences between molar and normal pregnancy, p value=0.393 (p>0.05). There is positive correlation between seng serum level of normal pregnancy with gestational age (rs=0.647; p<0.001).

Conclusion: Maternal serum seng level in preeclampsia is higher than the level of which in molar pregnancy and normal pregnancy.

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Keywords: molar pregnancy, normal pregnancy, preeclampsia, soluble endoglin level

Abstrak

***Tujuan**: Menganalisis perbedaan kadar seng serum ibu antara kehamilan preeklampsia, kehamilan mola dan kehamilan normal, serta menganalisis korelasi antara kadar seng serum ibu dengan usia kehamilan pada kehamilan preeklampsia, kehamilan mola dan hamil normal.*

***Metode**: Rancangan penelitian ini adalah uji potong silang terhadap 18 kasus preeklampsia, 18 kasus mola, dan 18 kehamilan normal yang memenuhi kriteria inklusi, yang datang ke RS Dr. Hasan Sadikin dan enam RS jejaring periode Januari-Maret 2013. Perbandingan rerata kadar seng serum preeklampsia, kehamilan mola dan hamil normal dihitung menggunakan uji Kruskal-Wallis, dan korelasi antara kadar seng serum dengan usia kehamilan dihitung menggunakan uji korelasi Rank Spearman.*

***Hasil**: Rerata kadar seng pada preeklampsia lebih tinggi (168,79 ng/ml) dibandingkan dengan kehamilan mola (43,47 ng/ml) dan hamil normal (32,38 ng/ml). Terdapat perbedaan yang bermakna antara kadar seng serum kehamilan preeklampsia dengan kehamilan mola dan hamil normal (p=0,000), namun tidak terdapat perbedaan kadar seng serum yang bermakna antara kehamilan mola dengan kehamilan normal, nilai p = 0,393 (p>0,05). Uji korelasi memperlihatkan adanya korelasi positif antara seng serum ibu kehamilan normal (rs=0,647; p<0,001) dengan usia kehamilan, dan didapatkan korelasi negatif antara kadar seng kehamilan preeklampsia dan kehamilan mola, (rs=-0,064; p=0,908 dan -0,029; p=0,908) dengan usia kehamilan.*

***Kesimpulan**: Tampak kadar seng serum ibu pada kehamilan preeklampsia lebih tinggi dibandingkan kelompok kehamilan mola dan kehamilan normal.*

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***Kata kunci**: hamil normal, kehamilan mola, preeklampsia, soluble endoglin*

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INTRODUCTION

In normal pregnancy, placenta undergoes vascularization to allow adequate circulation between mother and her fetus. This vascularization consists of extensive vasculogenesis and angiogenesis. Angiogenesis is a process of neovascularization out of the preexisting blood vessels, while vasculogenesis is a process of forming new vessels. Placental perfusion depends on the angiogenesis, which is responsible in increasing placental blood flow in pregnancy.[1] Disruption in normal placental formation due to disorder of trophoblastic invasion has been pointing to incidence of preeclampsia. On the other hand, the trophoblastic uncontrolled invasion and abnormal growth is associated with inci-

dences of hydatidiform mole and choriocarcinoma.[2]

Preeclampsia is still a main cause of maternal and perinatal morbidity and mortality. Also, preeclampsia is often found in molar pregnancy. The unclear etiology and pathophysiology of preeclampsia would affect in not optimal management of the disorder. Meanwhile, various researchs have been paying attention to theories of endothelial damage as the pathogenesis of preeclampsia, which is caused by disruption of angiogenesis. One of the substances involved in this process is soluble endoglin. This substance increases in endothelial damage. Therefore, the measurement of its level would show the extent of endothelial damage in the early stage of preeclampsia. This substance has been known to exert anti-angiogenic effect, thus disrupting endothelial differentiation in blood vessels.[3]

In molar pregnancy, the growth or proliferation of trophoblastic cells is excessive. In a former study about an anti-angiogenic factor sFlt-1 in molar pregnancy, Kanter, et al. reported a significant increase of protein sFlt-1 in molar pregnancy compared to the normal pregnancy. The imbalance of those anti-angiogenic factors is strongly believed to trigger abnormal growth and migration of trophoblasts.[2,4,5]

Soluble endoglin is a 65kDa truncated form of endoglin which does not have any trans membrane and domain cytoplasm. It is produced by placenta, and known to be increased in maternal serum of preeclamptic women. Seng inhibits the formation of capillary tubes in vitro, while induces vascular permeability and hypertension with focal glomerular endotheliosis in vivo. Therefore, endoglin and seng seems promising as a marker of pregnancy complications associated with inadequate placentation.[6]

Hydatidiform mole and preeclampsia are two disorders unique to pregnancy. Both have placental dysfunctions which are integrated to the process of each disease. Hydatidiform mole is a disorder in genetic formation, characterized by various extent of trophoblastic proliferation and hydrophic change of chorionic villi.[7]

Evidences have supported the important role of soluble endoglin in the pathophysiology of preeclampsia, upon its increased level in severe preeclampsia, early-onset preeclampsia, and fetal restriction.[8] Because of this involvement, the protein might be used as an early biomarker of preeclampsia and molar pregnancy. The increased level of soluble endoglin found in a case of partial mole with preeclampsia might reflect an imbalance of angiogenetic factor in molar pregnancy.[5]

METHOD

This is a cross-sectional comparative analytic study involving groups of molar pregnancy with preeclampsia, and molar pregnancy with normal pregnancy. Subjects were pregnant women with normal pregnancy, molar pregnancy, or preeclampsia, which is examined in Dr. Hasan Sadikin Hospital and its network hospitals (Cibabat Hospital, Ujung Be-rung Hospital, Majalaya Hospital, Slamet Garut Hospital, and Sumedang Hospital) within the study period, who fulfilled the inclusion criteria. There were 18 subjects in each group. From each subject, 5 cc of blood were withdrawn and measured for soluble endoglin level in Prodia laboratory with high sensitivity indirect sandwich Enzyme-Linked Immunosorbent Assay (ELISA) using human endoglin immunoassay kit.

Serum endoglin levels as numerical data were tested for normality with Kolmogorov-Smirnov/ Shaphiro Wilk test. Characteristics of the three groups would be compared with Anova test if the data were normally-distributed or non-parametric Kruskal-Wallis test if they were not. Upon finding of significant differences between the characteristics, the groups would then be analyzed with Post Hoc.

The difference of serum seng level between the group of preeclampsia and normal pregnancy, preeclampsia and molar pregnancy, and also molar and normal pregnancy, were analyzed by Independent T test if the data were normally-distributed, or Mann-Whitney test if they were not. P value of <0.05 reflects a significant result. SPSS version 18.0 for Windows was used in analyzing data.

Upon finding of significant difference of seng level and characteristics in each group, data could be further analyzed for correlation with Pearson correlation test if they were normally distributed, or Rank Spearman test if they were not. Then Guilford Empirical Rules was used to analyze the correlation strength of both variables.

RESULT

During the study period, 54 subjects fulfilled inclusion criteria, consisted of 18 subjects in each group of normal pregnancy, severe preeclampsia, and molar pregnancy. The characteristics such as maternal age, parity, and gestational age, were matched in order to be comparable.

Table 1 shows no significant difference of maternal age (p=0.730) and parity (p=0.249) between the preeclampsia, molar pregnancy, and normal pregnancy groups, while there is a significant difference of gestational age (p=0.000).

Kruskal-Wallis test shows that at least two groups have significantly different level of serum seng (p=0.000). Therefore, Post Hoc analysis would be done to analyze which groups had a significant difference of seng level.

Table 1. Comparative characteristics from the research group.

Characteristic	Group			p value
	Normal pregnancy (n=18)	Molar pregnancy (n=18)	Preeclampsia (n=18)	
Maternal age (years old)				0.730*
• <20	0	3	1	
• 20-24	5	1	5	
• 25-29	4	6	3	
• 30-35	9	8	9	
• Mean (± SD)	27.67 (4.3)	28.33 (6.6)	28.44 (5.7)	
Range	21-34	17-35	19-35	
Parity				0.249**
• 0	9	5	7	
• 1-3	9	11	11	
• ≥ 4	0	2	0	
• Mean (± SD)	1.50 (5.14)	1.83 (6.18)	1.61 (5.02)	
Range	1-2	1-3	1-2	
Gestational age (weeks)				0.000*
• < 20	6	14	0	
• 20-28	8	4	5	
• ≥ 29	4	0	13	
• Mean (± SD)	22.22 (9.52)	17.33 (2.79)	31.61 (4.4)	
Range	7-34	12-22	23-34	

Table 2. Post Hoc analysis of maternal serum seng level between three study groups.

Post Hoc Analysis of Seng Level (ng/ml)	p value*
Normal pregnancy (32.38 ± 19.13) vs molar pregnancy (43.47 ± 51.71)	0.393
Molar pregnancy (43.47 ± 51.71) vs pre-eclampsia (168.79 ± 48.58)	0.000
Normal pregnancy (32.38 ± 19.13) vs pre-eclampsia(168.79 ± 48.58)	0.000

*Note: * Mann-Whitney test*

Table 2 shows no significant difference of seng level between normal pregnancy and molar pregnancy (p=0.393). While there is significant difference of serum seng level between molar pregnancy and preeclampsia (p=0.000) and also normal pregnancy and preeclampsia (p=0.000). Seng level in preeclampsia group is significantly higher than the level of which in molar and normal pregnancy groups.

Table 3. Correlation between seng level and gestational age in normal pregnancy, molar pregnancy, and preeclampsia.

Group	Correlation between Serum Endoglin Level (ng/ml) and Gestational Age	
	r_s	p value
Normal pregnancy (n=18)	0.647	<0.001
Molar pregnancy (n=18)	-0.029	0.908
Preeclampsia (n=18)	-0.064	0.800

Table 3 shows that Spearman correlation test in 95% confidence interval results in a significant correlation between seng level and gestational age in normal pregnancy group, with r value of 0.647 (p<0.001).

DISCUSSION

The characteristics compared in this study were parity and gestational age, as they are anticipated to be confounding in the incidence of preeclampsia and molar pregnancy, and therefore the biggest bias in this study.

This study shows that serum seng level of preeclamptic women is higher than those of molar and normal pregnancy. Maternal serum seng level in preeclampsia is significantly higher than the level of which in normal pregnancy, with p value of <0.05. This is consistent with the previous studies. Levine, et al. reported an increase of seng level in women with early-onset preeclampsia, higher than control group (46.4 ng/ml vs 9.8 ng/ml, p<0.001). Another study by Salahuddin, et al. Results in significant increase of serum seng level in preeclamptic women (69.2 ± 42.5 ng/ml) compared to the control group (15.5 ± 6.9 ng/ml).

Endoglin is expressed in low level in normal endothelium, while being very high in vascular endothelium during embryogenesis, inflammatory tissue and wound healing, vascular trauma, and vascular tumor. Its expression is also found in some cells in cardiovascular system.[9-13]

Soluble endoglin, the soluble form of endoglin which circulates with blood, is produced by placenta. The level of seng in circulation is found elevated in the last two months of normal pregnancy in a longitudinal study, while an increase in concentration is observed earlier in pregnancy with recurrent preeclampsia.[8,14]

A case control study reported that concentration of seng in circulation increases in preeclamptic women. This elevation can be detected 2-3 months before the clinical manifestations of preeclampsia are observed. Meanwhile, sFlt-1, another antiangiogenic factor, is detected 5 weeks before the clinical onset. These allow seng to be an early marker of preeclampsia.[8,15,16] Thus, elevation of soluble endoglin level in normal pregnancy group may indicate risk of preeclampsia in the previously normal pregnancy.

On the other hand, another study found that incidence of preeclampsia is higher when seng elevation accompanies the elevation of other biomarkers, such as sFlt-1, VEGF, or PlGF. This needs to be understood, as early-onset preeclampsia causes neonatal morbidity and mortality due to iatrogenic prematurity, whereas definitive treatment for preeclampsia is still limited. Therefore, early detection would be very beneficial in observing and optimalization of the management.[17,18]

However, previous data shows that serum seng starts to elevate as gestational age advances in normal pregnancy population.[8,19] This is believed to cause bias to the study, as the control serum samples were withdrawn not only from the early trimester, but also the third trimester.

This study also shows a positive correlation between serum seng level with gestational age in normal pregnancy. Unlike the other two groups however, in which the correlation does not exist. The result is consistent with two previous studies showing positive correlation between serum seng level with the advancing gestational age in normal pregnancy.[8,18] It is, on the other hand, different from a study by Vaisbuch, et al. which resulted in no significant correlation between serum seng level with the advancing gestational age (Spearman rho 0.3, p=0.06).[20]

Another study reported that beside gestational age and history of chronic disease, tissue hypoxia can also affect the expression of soluble endoglin. It is proven by elevation soluble endoglin concentration following hypoxic condition of human umbilical vein endothelial cells (HUVEC). This is consistent with the pathophysiology of preeclampsia, in which hypoxic condition is observed. Meanwhile, elevation of seng level is also observed in molar pregnancy, although it is still lower than the level of which in preeclampsia, supporting the theory of placental hypoperfusion in hydatidiform mole.

The data distribution shows some extreme point in molar pregnancy group. Subject C10 have the highest soluble endoglin level, far above those of the other subjects'. The subject turns out to be a partial mole case with manifestations of preeclampsia. This is consistent with the previous literature which states that there was an increase of seng level in partial mole with preeclampsia as its complication.[2]

This study had some data deviate quite far. This can be explained by some improvement in the performance of the test done on the sample, which is probably different in its sensitivity. Therefore, the test results differently although the previous studies were also using the same kit. Moreover, the duration of sample storage can also affect the protein concentration measured.

CONCLUSION

There is a significant difference of serum soluble endoglin level between preeclampsia and molar pregnancy. The level is higher in preeclampsia than it is in molar pregnancy. There is a positive correlation between serum seng level and gestational age in normal pregnancy.

REFERENCES

1. Agarwal I, Karumanchi SA. Preeclampsia and The Anti-angiogenic State. Pregnancy Hypertens. 2011; 1(1): 17-21.
2. Kanter D, Lindheimer MD, Wang E, Borromeo RG, Bousfield E, Karumanchi SA, et al. Angiogenic Dysfunction In Molar Pregnancy. Am J Obstet Gynecol. 2010; 202(2): 184.e1-5.
3. Yelumalai S, Subramanian K, Omar SZ, Qvist R, Muniandty S. Angiogenic Factors In The Pathogenesis and Pathophysiology of Preeclampsia: A Mini review. Biomed Res. 2010; 21(3): 246-51.
4. Espinoza J, Uckele JE, Starr RA, Seubert DE, Espinoza AF, Berry SM. Angiogenic Imbalances: The Obstetric Perspective. Am J Obstet Gynecol. 2010; 203(1): 17.e1-8.
5. Espinoza J, Uckele JE, Starr RA, Lorenz RP, Bronsteen RA, Berry SM. Insights Into Angiogenic Imbalances During Pregnancy. Obstet Gynecol. 2009; 114(2): 437-40.
6. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble Endoglin Contributes to The Pathogenesis Of Preeclampsia. Nat Med. 2006; 12(6): 642-9.
7. Fraser HM, Dickson SE, Lunn SF, Wulff C, Morris KD, Carroll VA, et al. Suppression Of Luteal Angiogenesis in The Primate After Neutralization Of Vascular Endothelial Growth Factor. Endocrinol. 2000; 141(3): 995-1000.
8. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble Endoglin And Other Circulating Antiangiogenic Factors In Preeclampsia. N Engl J Med. 2006; 355(10): 992-1005.
9. Miller DW, Graulich W, Karges B, Stahl S, Ernst M, Ramaswamy A, et al. Elevated Expression Of Endoglin, A Component Of The TGF-β-Receptor Complex, Correlates With Proliferation Of Tumour Endothelial Cells. Int J Cancer 1999; 81: 568-72.
10. Bernabeu C, Lopez-Novoa JM, Quintanilla M. An Emerging Role Of TGF-β Co-Receptors In Cancer. Biochem Biophys Acta. 2009; 1792: 954-9.
11. Perlingueiro RC. Endoglin Is Required For Hemangioblast And Early Hematopoietic Development. 2007; 34: 3041-8.
12. Torsney E, Charlton R, Parums D, Collis M, Arthur HM. Inducible Expression Of Human Endoglin During Inflammation And Wound Healing In Vivo. Inflamm Res. 2002; 51: 464-70.
13. Szekanecz Z, Haines GK, Harlow LA, Shah MR, Fong TW, Fu R, et al. Increased Synovial Expression Of Transforming Growth Factor (TGF)-β Receptor Endoglin And TGF-β1 In Rheumatoid Arthritis: Possible Interactions In The Pathogenesis Of The Disease. Clin Immunol Immunopathol. 1995; 76: 187-94.
14. Chien PF, Arnott N, Gordon A, Owen P, Khan KS. How Useful Is Uterine Artery Doppler Flow Velocimetry In The Prediction Of Preeclampsia, Intrauterine Growth Retardation And Perinatal Death? An Overview. BJOG. 2000; 107: 196-208.
15. Baumann MU, Bersinger NA, Mohaupt MG, Raio L, Gerber S, Surbek DV. First-Trimester Serum Levels Of Soluble Endoglin And Soluble Fms-Like Tyrosine Kinase-1 As First-Trimester Markers For Late-Onset Preeclampsia. Am J Obstet Gynecol 2008; 199: 1-6.
16. Benedetto C, Valensise H, Marozio L, Giarolo M, Massobrio M, Romanini C. A Two-Stage Screening Test For Pregnancy-Induced Hypertension And Preeclampsia. Obstet Gynecol. 1998; 92: 1005-11.

17. Sibai B, Dekker G, Kupferminc M. Preeclampsia. Lancet. 2005: 785-99.
18. Papageorghiou AT, Yu CK, Cicero S, Bower S, Nicolaides KH. Second-Trimester Uterine Artery Doppler Screening In Unselected Populations: A Review. J Mat Fetal Neonatal Med. 2002; 12: 78-88.
19. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, et al. A Longitudinal Study Of Angiogenic (Placental Growth Factor) And Anti-Angiogenic (Soluble Endoglin And Soluble Vascular Endothelial Growth Factor Receptor-1) Factors In Normal Pregnancy And Patients Destined To Develop Preeclampsia And Deliver A Small For Gestational Age Neonate. J Mat Fetal Neonatal Med. 2008; 21(1): 9-23.
20. Vaisbuch E, Whitty JE, Hassan SS, Romero R, Kusanovic JP, Cotton BD, et al. Circulating Angiogenic And Antiangiogenic Factors In Women And Eclampsia. Am J Obstet Gynecol. 2011; 204: 1-9.