Research Article

High Expression of Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1) is Highly Correlated with Eclampsia

Ekspresi VEGFR-1 yang Tinggi Berkorelasi dengan Eklampsia

Efendi Lukas[1], Upik A Miskad[2], Miranty Firmansyah[1]

1Department of Obstetrics and Gynecology
[2]Department of Pathology
Faculty of Medicine University of Hasanuddin/
Dr. Wahidin Sudirohusodo Hospital
Makassar

Abstract

Objectives: To understand the expression of placental vascular endothelial growth factor receptor (VEGFR-1) in severe preeclampsia with complication (eclampsia and HELLP syndrome).

Methods: The study was an observational study with cross sectional design, performed at several hospitals of Department of Obstetrics and Gynecology Medical Faculty of University of Hasanuddin, Makassar. Subjects met to inclusion criteria were taken as samples. Placental tissue samples were taken from cord insertion site and fixated with formalin buffer solution. Immunohistochemical examination was performed at Center of Research University of Hasanuddin. Antibody used were primary antibody of Mouse monoclonal anti VEGFR-1 antibody (Santa Cruz) dissolved to 1:100 and link antibody (secondary antibody labeled with biotin) (Dakopatt). Placental tissues were examined in Streptavidin biotin peroxides and interpreted according to the intensity of trophoblast cytoplasmic dye.

Results: High VEGFR-1 expression was found in 100% of the placenta from patients with eclampsia , 42.9&% in patients with HELLP syndrome and 37.8% in patients with severe preeclampsia. High VEGFR-1 expression was correlated to incidence of eclampsia (p=.000) and not correlated to severe preeclampsia and HELLP syndrome (p=0.734).

Conclusion: High VEGFR-1 expression was correlated to eclampsia and not correlate to severe preeclampsia and HELLP syndrome.

[Indones J Obstet Gynecol 2012; 36-3:116-20]

Keywords: eclampsia, HELLP syndrome, severe preeclampsia, VEGFR-1 expression

Abstrak

***Tujuan**: Untuk mengetahui ekspresi vascular endothelial growth factor receptor-1 (VEGFR-1) plasenta pada penderita preeklampsia berat, eklampsia dan sindroma HELLP.*

***Metode**: Penelitian ini adalah penelitian observasional dengan desain studi potong lintang yang dilaksanakan pada beberapa rumah sakit pendidikan di Bagian Obstetri dan Ginekologi Fakultas Kedokteran Universitas Hasanuddin, Makassar. Sampel dari subjek yang memenuhi kriteria inklusi diambil dari plasenta pada tempat daerah insersi tali pusat, kemudian difiksasi dengan larutan formalin buffer. Pemeriksaan imunohistokimia plasenta dilakukan di Pusat Kegiatan Penelitian UNHAS. Antibodi yang digunakan adalah antibodi primer Mouse monoclonal anti VEGFR-1 antibody (Santa Cruz) yang dilarutkan 1:100 dan link antibody (antibodi sekunder yang dilabel dengan biotin) (Dakopatt). Sediaan plasenta diperiksa dengan Streptavidin biotin peroksidase, kemudian interpretasinya dinilai berdasarkan intensitas pewarnaan sitoplasma sel trofoblas.*

***Hasil**: Pada plasenta penderita eklampsia ditemukan 100% ekspresi kuat VEGFR-1 dibandingkan pada sindroma HELLP (42.9%) dan preeklampsia berat (37.8%). Ekspresi kuat VEGFR-1 berhubungan dengan kejadian eklampsia (p=.000) dan tidak berhubungan dengan preeklampsia berat dan sindroma HELLP (p=.734).*

***Kesimpulan**: Ekspresi kuat VEGFR-1 pada plasenta berhubungan dengan eklampsia dan tidak berhubungan dengan kejadian preeklampsia berat dan sindroma HELLP.*

[Maj Obstet Ginekol Indones 2012; 36-3:116-20]

***Kata kunci**: eklampsia, ekspresi VEGFR-1, preeklampsia berat, sindroma HELLP.*

Correspondence: Efendi Lukas. Department of Obstetrics and Gynecology. Faculty of Medicine University of Hasanuddin/Dr. Wahidin Sudiro Husodo Hospital, Makassar. Telephone: 08124122090; Facsimile: 0411-585688. Email: drefendispog@yahoo.com

INTRODUCTION

Preeclampsia and eclampsia are pregnancy complications that could cause maternal and perinatal death. Global burden of diseases 2000 estimated that the incidence of preeclampsia was around 2.8% of live births in developing countries and 0.4% in developed countries. Incidence of eclampsia was around 2.3% of preeclampsia in developing countries and 0.8% in developed countries.[1]

According to Godlin (1982), HELLP syndrome was an initial form of severe preeclampsia while Weinstein (1982) reported HELLP syndrome as a unique variety of preeclampsia.[2] HELLP syndrome incidence was around 2 - 20% of severe preeclampsia and around 0.2-0.6% of pregnancies.[2,3] Etiology and pathogenesis of HELLP syndrome are always associated to preeclampsia, although etiology and pathogenesis of preeclampsia itself are not fully understood to these days.

Preeclampsia is considered as an antiangiogenic condition.[4] Recent studies showed that ischemic trophoblast synthesized antiangiogenic factors.[5] Hypoxia stimulated some protein expression including endothelin, vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor-1 (VEGFR-1). VEGF is a potent mitogenic and angiogenic factor for endothelial cells. VEGF and placental growth factor (PlGF) bound to abundant VEGFR-1 may take place in pathogenesis of maternal symptoms in preeclampsia.[6] Specifically, Maynard et al (2003) proposed a hypothesis stated that in pregnant women with preeclampsia, there was an increase of sVEGFR-1 produced by placenta in the circulation, which then bound the free VEGF and PlGF. As a consequence, normal vascularization in the kidney, lungs, and other vital organs fell to/became dysfunction. Also, injection of sVEGFR-1 into animal induced hypertension, proteinuria and glomerular endotheliosis, symptoms classic to preeclampsia.[7] Hence, VEGFR-1 may be the missing link between implantation disorder and preeclampsia maternal symptoms.[8] This study would like to show the VEGFR-1 expression in the placenta.

The remaining question is whether VEGFR-1 plays an important role in pathogenesis of HELLP syndrome and eclampsia. To these days, there is no study compares the expression of placental VEGFR-1 between severe preeclampsia and eclampsia or HELLP syndrome.

The aim of the study was to understand the expression of placental vascular endothelial growth factor receptor (VEGFR-1) in severe preeclampsia with complication (eclampsia and HELLP syndrome).

METHODS

This study was performed from November 2010 to January 2011 at several hospitals of Department of Obstetrics and Gynecology Faculty of Medicine University of Hasanuddin, Makassar.

Subjects were pregnant women with severe preeclampsia who met the inclusion criteria and taken by sonsecutive sampling.

Placental tissue samples were taken from cord insertion site and fixated with formalin buffer solution. Immunohistochemical examination was performed at Center of Research University of Hasanuddin. Antibody used were primary antibody of Mouse monoclonal anti VEGFR-1 antibody (Santa Cruz) dissolved to 1:100 and link antibody (secondary antibody labeled with biotin) (Dakopatt). Placental tissues were examined in Streptavidin biotin peroxides and interpreted according to intensity of trophoblast cytoplasmic dye.

Score 0 (negative) means that no dyed cell was found. Score +1 (low) means that <10% dyed trophoblast with weak/moderate intensity was found. Score +2 (low) means that 10-50% dyed trophoblast with weak/moderate intensity, or >50% with weak intensity was found. And score +3 (high) means that >50% dyed trophoblast with moderate/strong intensity was found.

The data analysis was performed using Kruskal Wallis test, Mann Whitney test and Chi-square test to compare between two unpaired variable groups.

RESULTS

The total of samples obtained were 61 samples, devided into 37 samples of severe preeclampsia, 14 samples of HELLP syndrome, and 10 samples of eclampsia.

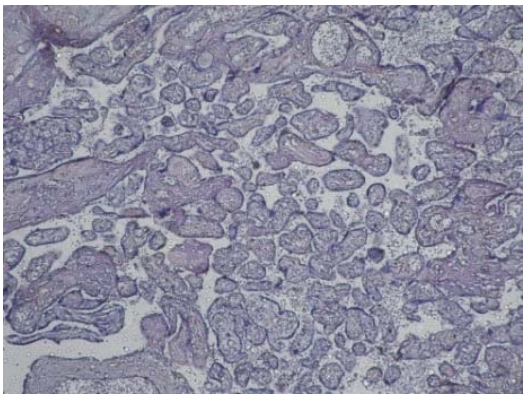

Figure 1a. Immunohistochemical dyeing of placental VEGFR-1 expression (score 0).

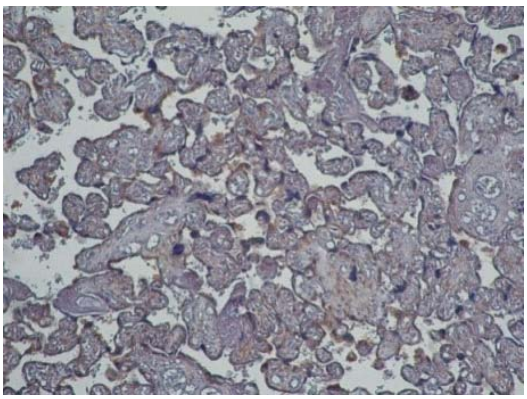

Figure 1b. Immunohistochemical dyeing of placental VEGFR-1 expression (score +1).

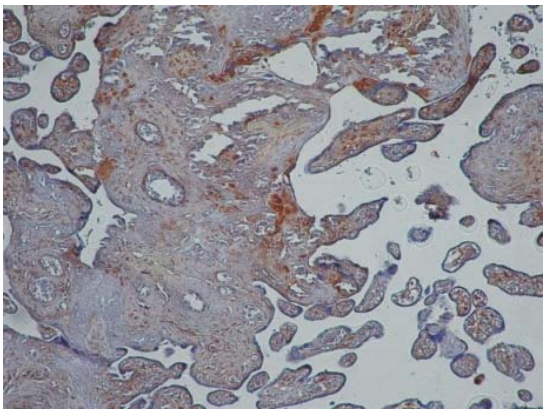

Figure 1c. Immunohistochemical dyeing of placental VEGFR-1 expression (score +2).

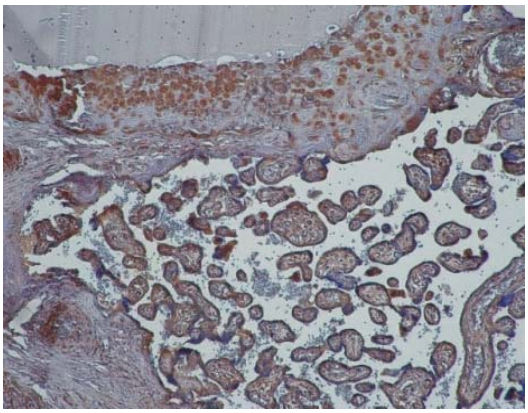

Figure 1d. Immunohistochemical dyeing of placental VEGFR-1 expression (score +3).

There was no significant difference in the subject characteristics of maternal age, parity, systolic blood pressure, diastolic blood pressure, and proteinuria.

Table 1. Characteristic distribution of samples

Variable	Group*			p
	Severe Pre-eclampsia	Eclampsia	HELLP syndrome	
Age	28.3±7.3	24.2±6.4	28.6±6.7	0.215
Parity	1.8±0.9	1.3±0.5	1.7±0.9	0.309
Systolic BP	168.9±20.5	176±23.2	171.4±12.3	0.475
Diastolic BP	108.1±13.1	116±11.7	112.1± 8.9	0.113
Proteinuria	3.0±0.7	3.4±0.5	3.2±0.4	0.197

** Mean*

Using Kruskal Wallis test, we found significant difference ($p<0.05$) of VEGFR-1 expression between severe preeclampsia, HELLP syndrome and eclampsia group. Percentage of score +3 in eclampsia group (100%) was higher than others.

Since there was significant difference found among the three groups, we performed further analysis test between two groups by Mann Whitney test. Significant difference were found between severe preeclampsia and eclampsia groups ($p=0.007$) and between eclampsia and HELLP syndrome groups ($p=0.014$). There was no significant difference between severe preeclampsia and HELLP syndrome groups ($p=0.745$).

Table 2. Expression of placental VEGFR-1 in severe preeclampsia, eclampsia and HELLP syndrome

VEGFR-1 expression	Severe preeclampsia		Eclampsia		HELLP syndrome		Total	
	n	%	n	%	n	%	n	%
Score	3	8.1	0	0	0	0	3	4.9
Score +1	12	32.4	0	0	5	35.7	17	27.9
Score +2	8	21.6	0	0	3	21.4	11	18
Score +3	14	37.8	10	100	6	42.9	30	49.2
Total	37	100	10	100	14	100	61	100

Chi Square Test, p=0.032
Group severe preeclampsia and eclampsia, p=0.007
Group severe preeclampsia and HELLP syndrome, p=0.745
Group eclampsia and HELLP syndrome, p=0.014

We looked for correlation between high VEGFR-1 expression (score +3) and complication (combined groups of eclampsia and HELLP syndrome). The percentage of sample with score +3 was higher in severe preeclampsia with complication (66.7%) than without complication (37.8%). There was significant difference ($p=0.028$) in the strength of VEGFR-1 expression between the group with complication of severe preeclampsia (3.3 times risk) and the group with no complication.

Table 3. Strength of VEGFR-1 Expression Based on Complication of Severe Preeclampsia

VEGFR-1 expression	Severe preeclampsia		Eclampsia+ HELLP syndrome		Total	
	n	%	n	%	n	%
High expression	14	37.8	16	66.7	30	49.2
Low expression	23	62.2	8	33.3	31	50.8
Total	37	100	24	100	61	100

Chi-Square test, p=0.028, OR 3.286 (95% CI 1.118-9.654)

We also looked for correlation between high VEFGR-1 expression (score +3) and eclampsia. The percentage of samples with score +3 was higher in patients with eclampsia (100%) than in combined

groups of HELLP syndrome and severe preeclampsia (39.2%). There was significant correlation (p=0.000) in the strength of VEGFR-1 correlation between eclampsia and combined groups of HELLP syndrome and severe preeclampsia. We could not estimate the risk since there was no low expression in eclampsia. However, it is a very significant expression because all eclampsia samples showed high expression of VEGFR-1.

Table 4. Strength of VEGFR-1 Expression on Eclampsia Compared to HELLP Syndrome and Severe Preeclampsia

VEGFR-1 expression	Severe preeclampsia HELLP syndrome		Eclampsia		Total	
	n	%	n	%	n	%
High expression	20	39.2	10	100	30	49.2
Low expression	31	60.8	0	0	31	50.8
Total	51	100	10	100	61	100

Chi-Square test, p=0.000, OR 0.667 (95% CI 0.518-0.859)

DISCUSSION

We found that the expression of VEGFR-1 in the placenta of severe preeclampsia, eclampsia and HELLP syndrome, varied from score 0 (4.9%), score +1 (27.9%), score +2 (18%) to score +3 (49.2%). It was in accordance with previous studies, which stated that in preeclamptic condition, expression of VEGFR-1 in syncitiotrophoblast was very high and the soluble level in maternal serum was also increased.[8,9] Helske et al found expression of VEGFR-1 in 50% syncitiotrophoblast of severe preeclampsia. The only 50% expression was explained by individual variation among the patients based on gestational age and severity of preeclampsia.[8] In this study, we found that VEGFR-1 expression in 95.1% of total samples or 91.9% of severe preeclampsia samples. This difference might be caused by homogenity in our sample characteristics and inclusion criteria of term pregnancy.

We found significant difference in VEGFR-1 expression among severe preeclampsia, eclampsia and HELLP syndrome. The higher the VEGFR-1 expression in the placenta, the more severe the complication of preeclampsia. This was seen as 100% of the samples with eclampsia had VEGFR-1 expression score +3, while only 37.8% of samples with severe eclampsia had VEGFR-1 expression score +3. The more severe the hypoxia, the higher the VEGFR-1 synthesis that will worsen the severe preeclampsia.[10] In eclampsia, there are cerebral edema and neurological complications that do not relate to the grade of hypertension (found also in normotension) and are reversible. It showed that edema might relate to the diminished autoregulation of cerebral blood flow, caused by endothelial dysfunction, and not because of the autoregulatory breakdown of cerebral blood flow. Findings in the head CT scan and MRI of eclampsia were similar with that of posterior reversible encephalopathy syndrome (PRES), with vasogenic cerebral edema and infarct. Findings with these characteristics were correlated with acute encephalopathy hypertension in conjunction with renal disease and immunosuppressive condition, for examples like in treatment of antiangiogenic agent for cancer therapy. This correlation supported the involvement of antiangiogenic factors in pathophysiology of eclampsia.[11,12] Blood pressure characteristics, both systole and diastole, in severe preeclampsia and eclampsia were homogen and thus supported the statement above.

VEGFR-1 expression was not significantly different between severe preeclampsia and HELLP syndrome. We performed further analysis to HELLP syndrome group. First test was performed to correlate the high VEGFR-1 expression to severe preeclampsia group (37.8%) versus combined groups of HELLP syndrome and eclampsia (66.7%). Second test was performed to correlate the high VEGFR-1 expression to combined groups of severe preeclampsia and HELLP syndrome (39.2%) versus eclampsia group (100%). The results showed that HELLP syndrome might not be a complication with similar pathogenesis with eclampsia. This can be interpreted as whether HELLP syndrome is a variant of severe preeclampsia or HELLP syndrome has causative factors other than VEGFR-1.[13,14]

HELLP syndrome may have a pathogenesis that different from severe preeclampsia. Some studies found endoglin (Eng) expression, an anti-angiogenicprotein, in abundance in preeclamptic placenta. Extracellular domain of Eng can be detached and found in serum as soluble endoglin (sEng). sEng regulates vascular tonus by interaction with endothelial nitric oxide synthase. sEng will induce a milder form of proteinuria and hypertension than VEGFR-1. sEng and VEGFR-1 induce endothelial dysfunction through different mechanism. But when sEng were expressed concurrently with VEGFR-1, the developed preeclampsia will be accompanied with thrombocytopenia and elevated

liver enzyme as seen in HELLP syndrome.[3,15] sEng is also elevated in maternal serum several months before the onset of preeclampsia, thus sEng can be used as a predictor for patients who may undergo a severe variant of preeclampsia.[15]

From this study, it was concluded that the high expression of placental VEGFR-1 was correlated with eclampsia and there was no difference in placental VEGFR-1 expression between severe preeclampsia and HELLP syndrome.

REFERENCES

1. Dolea C, AbouZahr C. Global burden of hypertensive disorders of pregnancy in the year 2000. Geneva: Evidence and Information for Policy (EIP), World Health Organization; 2003.
2. Roeshadi RH. Sindroma HELLP. In: Hariadi R, editor. Ilmu Kedokteran Fetomaternal. Surabaya: Himpunan Kedokteran Fetomaternal POGI; 2004. 500-5.
3. Hawfield A, Freedman BI. Pre-eclampsia: the pivotal role of the placenta in its pathophysiology and markers for early detection. Ther Adv Cardiovasc Dis. 2009; 3(1):65-73.
4. Bujold E, Romero R, Chaiworapongsa T, Kim YM, Kim GJ, Kim MR, et al. Evidence supporting that the excess of the sVEGFR-1 concentration in maternal plasma in preeclampsia has a uterine origin. J Matern Fetal Neonatal Med. 2005; 18(1):9-16.
5. Berkane N, Lefevre G, Hertig A. Angiogenic factors in preeclampsia: so complex, so simple? Nephrol Dial Transplant. 2007; 22:2753-6.
6. Lam C, Lim KH, Karumanchi SA. Circulating Angiogenic Factors in the Pathogenesis and Prediction of Preeclampsia. Hypertension. 2005; 46:1077-85.
7. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase (sFlt 1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003; 111:649-58.
8. Helske S, Vuorela P, Carpen O, Hornig C, Weich H, Halmesmaki E. Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. Mol Hum Reprod. 2001; 7(2):205-10.
9. Gu Y, Lewis DF, Wang Y. Placental Productions and Expressions of Soluble Endoglin, Soluble fms-Like Tyrosine Kinase Receptor-1, and Placental Growth Factor in Normal and Preeclamptic Pregnancies. J Clin Endocrinol Metab. 2008; 93:260-66.
10. Ahmad S, Ahmed A. Elevated Placental Soluble Vascular Endothelial Growth Factor Receptor-1 Inhibits Angiogenesis in Preeclampsia. Circ Res. 2004; 95:884-91.
11. Cipolla MJ. Cerebrovascular function in pregnancy and eclampsia. Hypertension. 2007; 50:14-24.
12. Wang A, Rana S, Karumanchi SA. Preeclampsia: the role of angiogenic factors in its pathogenesis. Physiology. 2009; 24:147-58.
13. Sibai BM. Diagnosis, Controversies, and Management of the Syndrome of Hemolysis, Elevated Liver Enzymes, and Low Platelet Count. ACOG. 2004; 103(5):981-91.
14. Järvenpää J. Placental angiogenesis and angiogenesis related risk factors in severe pre-eclampsia [Academic Dissertation]. Oulu: University of Oulu; 2008.
15. Mutter WP, Karumanchi SA. Molecular Mechanism of Preeclampsia. Microvasc Res. 2008; 75(1):1-8.