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Placental Growth Factor as a Diagnostic Glycoprotein in Preeclampsia

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Abstract

Angiogenic factor imbalance, such as that caused by placental growth factor (PLGF), is a feature of preeclampsia. However, the connection amony PLGF, clinical, laboratory indicators and the severity of preeclampsia is not entirely understood. Ninety subjects were incorporated in this study (The age range 17-40years),(30) were healthy pregnant with mean age (26.57±1.07). (30) Mild Preeclampsia cases with mean age (30.42±0.78) and (30) sever PE with mean age (33.32±1.09) wherever all women were more than 20 weeks of pregnancy. Mean blood pressure and level of proteinuria were used as indicators of the severity of the disease. ELISA was used to measure the levels of PLGF in the serum. Serum lipid profile were measured by enzymatic methods. The qualitative dip-stick technique was used to assess the amount of urine protein. A substantial difference between the PE group's serum PLGF concentration and that of the healthy subjects (p= 0.001) could be seen. Serum PLGF concentrations of patients with mild PE and those with severe PE differed significantly. Additionally, there were strong positive relationships between the serum PLGF concentration and the systolic BP and proteinuria serum PLGF levels, biochemical indicators, and other factors, however, did not significantly correlate. The diagnostic accuracy in distinguishing PE from the healthy patient group was good thanks to the excellent area under the curve (AUC = 0.83, p = 0.0001) In conclusion, Levels of PLGF in area of PE patients were decreaed. PLGF was significantly correlated with PE patients.

Key word: placental growth factor, preeclampsia, proteinuria, blood pressure.

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عامل نمو المشيمة كتشخيص البروتين السكري في تسمم الحمل

شيماء شلال الكيلان و إخلاص عبد الله حسن

قسم الكيمياء ، كلية العلوم ، جامعة ديالى

الخلاصة

يعد عدم توازن العامل الوعائي,سمة من سمات تسمم الحمل ,مثل ذلك الناتج عن عامل النمو المشيمي PLGF فتبين ان العلاقة بين PLGF,المؤشرات السريرية, ,المخبرية,وشدة تسمم الحمل غير مفهومة... تم تضمين تسعين شخصًا في هذه الدراسة ، كان 30 منهم حاملاً بصحة جيدة بمتوسط عمر (35.6) سنة. (30) حالة خفيفة من تسمم الحمل مع متوسط العمر (32.65) سنة تسمم الحمل ، و (30) حالة PE شديدة بمتوسط العمر (32.65) حيثما كانت جميع النساء أكثر من 20 أسبوعًا من الحمل. تم استخدام متوسط ضغط الدم ومستوى البيلة البروتينية كمؤشرات على شدة المرض. تم استخدام ELISA لقياس مستويات PLGF في المصل. تم قياس مستوى الدهون في الدم بالطرق الأنزيمية. تم استخدام تقنية الغمس النوعي)لتقييم كمية بروتين البول. يمكن رؤية اختلاف جوهري بين تركيز PLGF في مصل مجموعة PE) وتلك الخاصة بالأشخاص الأصحاء p 0.001). اختلفت تركيزات PLGF في الدم للمرضى الذين يعانون من PE المعتدل وأولئك الذين يعانون من PE الشديد بشكل كبير. بالإضافة إلى ذلك ، كانت هناك علاقات إيجابية قوية بين تركيز PLGF في الدم و SBP والبروتينية. ومع ذلك ، لم ترتبط مستويات PLGF في الدم والمؤشرات الكيميائية الحيوية وعوامل أخرى بشكل كبير. كانت الدقة التشخيصية في تمييز PE المعتدل من مجموعة المرضى الأصحاء جيدة بفضل المنطقة الممتازة تحت المنحنى AUC 0. =83، p <0.0001). في الختام ، تم خفض مستويات PLGF في(منطقة مرضى PE. ارتبط PLGF بشكل كبير مع مرضى PE.

الكلمة المفتاحية: عامل نمو المشيمة ، تسمم الحمل ، بروتينية ، ضغط الدم.

Introduction

Pre-eclampsia (PE) is a pregnancy-related multisystem disorder with unrecognized cause. The main cause of PE is currently being investigated. It is believed to take place in two phases, though. The decidual fetal trophoblastic invasion impairment and the placental localized hypoxia are included in the first stage [1,2]. The second stage include the release of molecules associated with placental blood into the mother's circulation as well as abnormal synthesis of pro-inflammatory, antiangiogenic, and angiogenic factors [3, 4]. Around week 20 of pregnancy, PE frequently shows clinical signs or later in the pregnancy, with the regression condition after

birth. Typical symptoms include proteinuria and elevated blood pressure [5]. Early-onset PE (which manifests before 34 weeks of gestation) and late-onset PE are the two main kinds of PE (which occurs beyond 34 weeks of gestation) [6,7]. Although the precise cause of this pregnancy-related disease is unknown, mounting evidence points to an imbalance between placental pro- and anti-angiogenic hormones that damages maternal vascular endothelium and causes preeclampsia, which then manifests clinically. Serum tests for pro- and anti-angiogenic factors, particularly placental growth factor, soluble vascular endothelial growth factor receptor-1, and soluble fms-like tyrosine kinase-1, have been used to diagnose this disorder, gauge its severity, and assess its therapeutic potential [8,9,10,11,12,13,14]. Placental growth factor (PLGF) is a transmembrane homodimeric glycoprotein that is extensively produced on the cellular membrane of endothelium. [15].The maternal circulation is responsible for the antiangiogenic effects seen in preeclampsia. It does this by adhering to circulating TGF-1 and preventing it from sending signals to endothelial cells (specifically, the endothelium's usual proangiogenic and vasodilator functions) [16]. These results suggest that PLGF may play a role in the preeclampsia's etiology and the disruption of the angiogenesis pathway may have a significant influence on the emergence of the clinical conditions and laboratory symptoms as well as its outcome. The imbalance of angiogenic factors plays a crucial role in the pathogenesis of PE [17] vascular- endothelial growth factor (VEGF) is necessary for glomerular capillary repair and in maintaining the health of the endothelium [15] particularly in the kidney, liver, and brain [17] PLGF is a potent angiogenic growth factor with structural homology to VEGF-A and is thought to amplify VEGF signaling [18].Placental Growth Factor is expressed early in pregnancy by the placenta. In men and non-pregnant individuals, low levels are produced by the heart, lung, thyroid, skeletal muscle, and adipose tissue [19]. Vascular Endothelial Growth Factor and PLacental Growth Factor have a common receptor, VEGF receptor-1, also called Flt-1 in endothelial cells.The link between blood clinical and test results, as well as clinical PLGF levels and test results, as well as PLGF levels in preeclampsia patients is poorly understood, despite the wealth of studies examining the roles of soluble Fm- like Tyrosine Kinase-1 (sFlt-1) and PLGF in the condition. This study examined whether blood PLGF levels in preeclampsia patients might going through severe sickness and poor maternal outcomes.

Materials and Methods

AL-batool Educational Hospital's Obstetrics and Gynecology Department conducted this cross-sectional, case-control study from Sep. 2022 to March 2023. The Diyala Sciences College's Ethical Committee granted its permission for this study's ethical conduct. The research included 30 pregnant women (≥20 gestational weeks) with severe PE and 30 with mild PE at admission. Thirty, and excepting to continue in good health.

• Proteinuria was < 2+ on urine dipstick on at least two random specimens collected more than 4 hours apart after the 20th week of pregnancy, and mild PE was systolic BP 140 mmHg or diastolic BP 90 mmHg validated by 6 or more hours apart.

• Systolic PE was identified by having systolly of at least 160 mmHg, diastolly of at least 110 mmHg, proteinuria > 2+ on a urine dipstick, as well as headache, visual disturbances, upper abdominal pain, and oliguria. The systolic preeclamptic group's whole female population experienced both proteinuria and hypertension.

• Healthy pregnant: Normotensive pregnant women with gestational ages of more than 20 weeks, no chronic medical conditions, and no signs of labour. They had healthy blood pressure and were normotensive during pregnancy. Patients were not having a history of diabetes, kidney illness, hypertension, other cardiovascular issues, or infectious disease with symptoms were not allowed to participate.

Sample collection

Blood samples were taken from the patients' antecubital veins while they were lying supine after a minimum of eight hours of fasting and one hour of rest. The serum was then collected and stored at -20 C until use; whereas the urine that was still in the sterile container was examined with a Dipstick.

Urine sample: First morning urine sample was taken in a clean container free from any detergent. A mid-stream sample was taken, and it was put in a sterile container. The sample was then moved to an all-purpose container, and the urine or sample still present in the sterile

container was analyzed using a Dipstick. The qualitative dip-stick technique was used to assess the protein content of urine.

Anthropometries Measurements

Age, weight, and height were established using anthropometric measurements. Calculating one's body mass index (BMI) involves employing a method that is based on the fundamental formula of weight divided by height squared. Using a stethoscope, sphygmomanometer, which consists of a blood pressure cuff and a mercury column pressure gauge, and auscultation, which involves listening to sounds, blood pressure was indirectly measured using 1902 by Russian physician N.S. Korotkoff. Systolic and diastolic blood pressure were both measured in millimeters of mercury, and for each individual, the average of the previous two readings was taken.

Urine analysis

A mid-stream sample was taken, and it was put in a sterile container. The sample was then moved to an all-purpose container, and the urine still present in the sterile container was analyzed using a Dipstick. The qualitative dip-stick technique was used to assess the amount of urine protein (CYBOWTM DFI Co Ltd, Republic of Korea). It was determined in accordance with the manufacturer's description. The test's underlying principle is the protein "error of indicators." The presence of protein causes indicator dyes to release H^+ ions, which cause them to turn greenish yellow to greenish blue whenever a buffer keeps the pH stable. A visual inspection is an assay. A new strip was briefly dipped into urine that had been gathered early in the morning and stored in clean, dry plastic containers. In less than two seconds, the Up to the test region, the strip was inserted. To eliminate extra urine, the strip's edge was drawn around the brim of the vessel to ensure the test area did not contact it. Since too much urine on the strip might produce chemical interactions between neighboring pads that would lead to inaccurate findings, To remove any remaining urine, the strip was turned over and tapped against a piece of absorbent paper. In well-lit situations, both the test result that was displayed on the strip that was held horizontally and the colour scheme that was included on the bottle label were contrasted.

Study of groups in clinical laboratories

The concentrations of PLGF were measured using an ELISA plate reader (ca Inc., San Diego, CA, USA). An automated enzymatic technique was used to evaluate the serum lipid profile testing.

Statistical analysis

Using statistical package for social and sciences (SPSS), the statistical analysis was completed (version 25). For numerical variables having normally distributed data, the data were converted into the average and the variation from the mean, respectively, and for categorical variables, into frequency/percentage. While a t-test performed independently and an ANOVA test were employed to see whether there was a significant difference between the typically numerical variables.

Result

The mean ages of the normal participant group (26.57±1.07) and the T2DM patients (31.42±0.78) are shown in Table 1 with p≤ 0.05, respectively. Between the healthy subject group's (27.63±0.73 kg/m2) and T2DM patients' (30.68±1.51 kg/m2) mean BMIs, there was no significant difference (p > 0.05). The findings from the subgroups, are given in table 2.

Table 1: Anthropometries and biochemical markers of healthy individuals and patient populations with PE

VARIABLES	PE PREGNANT PATIENTS (N = 60)	HEALTHY PREGNANT (N = 30)	P- VALUE
Age mean ± SE	31.42 ±0.78	26.57 ±1.07	0.061
BMI mean ± SE	30.68 ±1.51	27.63 ±0.73	0.0491*
Week of pregnancy	29.94 ±0.9	29.73 ±0.76	0.6402
Number of children	2.92 ±0.265	2.33 ±0.25	0.073
Systolic BP	14.33 ±0.22	11.43 ±015	0.0001**
Diastolic BP	9.75 ±0.09	6.73 ±0.14	0.0001**
Cholesterol	249.87 ±10.735	210.30 ±14.62	0.0423*
Triglyceride	214.704 ±10.89	170.80 ±14.61	0.0001**
HDL-C	58.52 ±2.89	63.73 ±3.72	0.305
LDL-C	145.92 ±10.28	115.67 ±14.64	0.043*
VLDL-C	40.27 ±2.32	34.60 ±2.96 b	0.0297*

*Significant 0.05 (P≤0.05), **highly significant, 0.01 (P≤0.01), NS is not significant.

Table 2: The subgroups of PE patients and healthy pregnant women's anthropometry and biochemical markers.

VARIABLES	HEALTHY PREGNANT (N = 30)	MILD PE PREGNANT PATIENTS (N = 38)	SEVER PE PREGNANT PATIENTS (N = 22)	LSD	P-VALUE
Age mean ± SE	26.57 ±1.07	30.42 ±0.78	33.23 ±1.09	2.798 **	0.0001
BMI mean ± SE	27.63 ±0.73	29.88 ±0.82	30.80 ±1.51	2.791 *	0.0491
Week of pregnancy	29.73 ±0.76	31.47 ±0.71	27.76 ±1.70	2.835 *	0.0402
Number of children	2.33 ±0.25	2.37 ±0.18	3.47 ±0.35	0722 **	0.0057
Systolic BP	11.43 ±015	14.00 ±0.12	14.67 ±0.31	0.519 **	0.0001
Diastolic BP	6.73 ±0.14 b	9.50 ±0.09	9.67 ±0.10	0.344 **	0.0001
Cholesterol	210.30 ±14.62	249.97 ±11.27	249.76 ±10.20	36.638 *	0.0423
Triglyceride	170.80 ±14.61	178.37 ±10.31	251.04 ±11.46	35.824 **	0.0001
HDL-C	63.73 ±3.72	60.84 ±2.43	56.19 ±3.36	9.092	0.305
LDL-C	115.67 ±14.64	152.26 ±10.31	139.57 ±10.06	35.267 *	0.043
VLDL-C	34.60 ±2.96	35.97 ±2.03	44.57 ±2.62	7.322 *	0.0297
*Significant 0.05 (P≤0.05), **Highly significant, 0.01 (P≤0.01) , NS is not significant					

A healthy pregnant woman's serum PLGF in PE groups

PLGF is abnormally low in women with preeclampsia as compared with their gestational age-matched controls. The decrease in PLGF level is evident as early as the beginning of the second trimester of pregnancy, before the development of signs and symptoms of the disease.The level of serum PLGF was significantly lower in pregnant patients with mild and sever PE. These findings are depicted in Figure1.

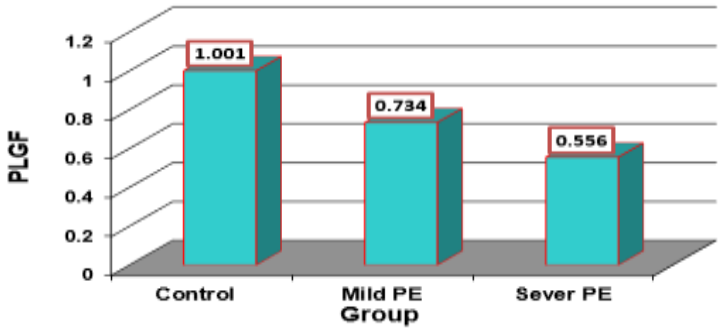

Figure 1: Comparison between difference groups in PLGF

A correlation between the severity of the illness and the serum PLGF level.

As shown in Table 3, there was a significant positive association between the serum PLGF concentration and proteinuria in the mild PE group (r = 0.47, p< 0.05) as well as systolic BP (r

= 0.36, p< 0.05). Additionally, In the group with severe PE, Serum PLGF concentration correlated substantially with proteinuria (r = 0.74, p <0.001) and systolic BP (r = 0.54, p< 0.001). A significant inverse association between the serum PLGF levels and the weeks of pregnancy at the time of sampling, however, also exists. Serum PLGF did not significantly correlate with any biochemical variable, including the lipid profile (p > 0.05).

Table 3 : Correlation coefficient-r between PLGF and other parameters

PARAMETERS	CORRELATION COEFFICIENT-R WITH SERUM PLGF		
	Control	Mild PE	Sever PE
Age	-0.19 NS	0.20 NS	-0.22 NS
BMI	0.24 NS	0.18 NS	-0.13 NS
PW	0.21 NS	-0.44 *	-0.52 *
No Child	-0.15 NS	-0.04 NS	0.30 NS
Systole P	0.06 NS	0. 36*	0.54 **
Diastole P	0.08 NS	0.04 NS	0.30 NS
Cholesterol	-0.02 NS	0.13 NS	0.29 NS
Triglyceride	0.31 NS	0.008 NS	0.13 NS
HDL	0.37 *	-0.07 NS	-0.28 NS
LDL	-0.21 NS	0.16 NS	0.21 NS
VLDL	0.27 NS	-0.02 NS	-0.06 NS
proteinuria	0.07 NS	0.47 *	0.74 **
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

Receiver operating characteristic (ROC) curve analysis Test

In PLGF analysis, area under the ROC curve (AUC) is (0.83). The best cut-off point derived from the ROC curve shows a sensitivity of (80 %) and specificity of (97%). It is found to be 0.889ng/ml accordingly, test value above 0.8 ng/ml is considered abnormal case whereas below this value represents the healthy condition as shown in table (4) Figure (2).

Table 4: Area Under the Curve of PLGF

AREA	STD. ERROR[A]	ASYMPTOTIC SIG.[B]	ASYMPTOTIC 95% CONFIDENCE INTERVAL	
			Lower Bound	Upper Bound
0.889	0.045	0.000	0.802	0.976
The test result variable(s): PLGF has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.				
b. Null hypothesis: true area = 0.5				

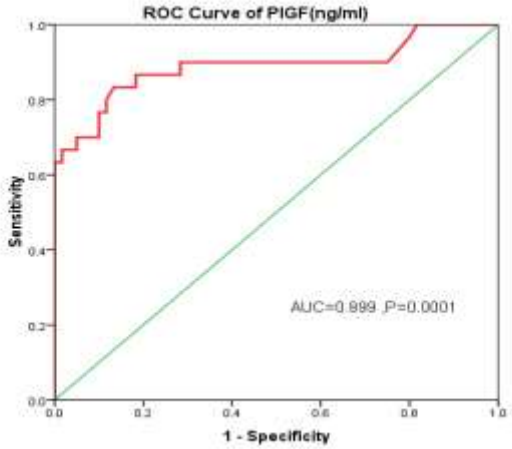

Figure 2: The Roc curve of PLGF (ng/ml)

Discussion

This study evaluated PLGF levels in women with mild and severe PE and healthy pregnant women. Angiogenic imbalance may be implicated in PE pathophysiology [20]. Anti,and pro-angiogenic drug, inhibits hypoxia endothelial cell death and regulates vascular tone via nitric oxide [21,22]. An anti-angiogenic protein called Placental growth factor prevents TGF-b1 from attaching to its receptors and suppresses downstream signaling, which includes endothelial Nitric Oxide Synyhase (eNOS) activation and vasodilation [23].

Preeclamptic patients have greater serum PLGF levels than typical pregnant women. We also found that PLGF levels rose with preeclampsia severity, suggesting that they accurately signify the deterioration of the systemic vascular endothelium. Worse preeclampsia symptoms were associated with higher blood PLGF levels, including proteinuria exceeding 2g/day. Our findings support the idea that this particular antiangiogenic factor is altered in women with preeclampsia and that these biochemical abnormalities become more prominent as the condition progresses, especially when preterm. [24,25].

Additionally,this study found that the serum PLGF levels and the number of weeks pregnant had a substantial negative correlation. A significant inverse relationship between the blood PLGF levels and gestational age at sampling has been shown by prior studies1 [26,27].

Troisi et al. (2008) linked PLGF to pregnancy-related elevated blood pressure [28]. In one PE animal model, PLGF-encoding adenovirus induced hypertension, proteinuria, and endothelial

dysfunction, which were exacerbated by PLGF and sFlt1 administration and brought about the help syndrome and prenatal growth restriction [29]. This cross sectional study results are supported by the positive correlation between systolic blood pressure and PLGF levels in preeclamptic patient. Since glomeruli separate the blood and urine compartments, angiogenesis is crucial to renal homeostasis. Genetic studies in mice show that VEGF and PlGF are crucial to renal development and vascular health [30]. sFlt-1 and PLGF, anti-angiogenic factors, are linked to glomerular injury and proteinuria. Venkatesha et al. [26] discovered nephrotic-range proteinuria in PLGF-treated rats and severe proteinuria in sFlt-1-treated rats. Masuyama et al. [31] found higher proteinuria in high-sEng patients. Serum PLGF has a high AUC of 0.95 and more than 90% sensitivity and specificity for PE diagnosis in normal pregnant women. Based on our findings, PLGF is a serum marker with excellent accuracy in identifying PE. Its higher sensitivity and specificity in severe and early-onset PE patients makes it more accurate. De Vivo et al. [32] found that PLGF may predict PE early with over 80% sensitivity and specificity in blood samples from 24–28 weeks of gestation. Lim et al.[33] found PLGF PE prediction AUC to be 0.83. Baumann et al. [34] found an AUC of 0.62 for PLGF in predicting late-onset PE in first trimester serum. Our ROC curve study shows that PLGF is better at diagnosing PE in normal pregnant women than severe and early-onset cases.

References

1. T. H. Hung, J. N. Skepper, D. S. Charnock-Jones, Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia, Circ Res., 90(12):1274–81(2002)
2. N. Soleymanlou, I. Jurisica, O. Nevo, Molecular evidence of placental hypoxia in preeclampsia, J Clin Endocrinol Metab., 90(7),4299–308(2005)
3. C. A. Turpin, S. A. Sakyi, W. K. Owiredu, Association between adverse pregnancy outcome and imbalance in angiogenic regulators and oxidative stress biomarkers in gestational hypertension and preeclampsia, BMC Pregnancy Childbirth, 15(1),189(2015)

4. S. Y. Kim, H. M. Ryu, J. H. Yang, Increased sFlt-1 to PlGF ratio in women who subsequently develop preeclampsia, J Korean Med Sci., 22(5),873–7(2007)
5. K. O. Osungbade, O. K. Ige, Public Health Perspectives of Preeclampsia in Developing Countries: Implication for Health System Strengthening, Journal of Pregnancy, 2011,4810956(2011)
6. J. Wilkinson, G. Cole, Preeclampsia knowledge among women in Utah, Hypertens Pregnancy, 37(1),18–24(2018)
7. D. Raymond, E. Peterson, A critical review of early-onset and late-onset preeclampsia, Obstet Gynecol Surv, 66(8),497–506(2011)
8. R. J. Levine, S. E. Maynard, C. Qian, K. H. Lim, L. J. England, K. F. Yu, E. F. Schisterman, R. Thadhani, B. P. Sachs, F. H. Epstein, B. M. Sibai, V. P. Sukhatme, S. A. Karumanchi, Circulating angiogenic factors and the risk of preeclampsia, N Engl J Med., 350,672–683(2004)
9. R. J. Levine, C. Lam, C. Qian, K. F. Yu, S. E. Maynard, B. P. Sachs, B. M. Sibai, F. H. Epstein, R. Romero, R. Thadhani, S. A. Karumanchi, CPEP Study Group, Soluble endoglin and other circulating antiangiogenic factors in preeclampsia, N Engl J Med., 355,992–1005(2006)
10. A. Leaños-Miranda, I. Campos-Galicia, I. Isordia-Salas, R. Rivera-Leaños, J. F. Romero-Arauz, J. A. Ayala-Méndez, A. Ulloa-Aguirre, Changes in circulating concentrations of soluble fms-like tyrosine kinase-1 and placental growth factor measured by automated electrochemiluminescence immunoassays methods are predictors of preeclampsia, J Hypertens, 30,2173–2181(2012)
11. S. Rana, C. E. Powe, S. Salahuddin, S. Verlohren, F. H. Perschel, R. J. Levine, K. H. Lim, J. B. Wenger, R. Thadhani, S. A. Karumanchi, Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia, Circulation, 125,911–919(2012)
12. A. Leaños-Miranda, I. Campos-Galicia, K. L. Ramírez-Valenzuela, Z. L. Chinolla-Arellano, I. Isordia-Salas, Circulating angiogenic factors and urinary prolactin as

predictors of adverse outcomes in women with preeclampsia, Hypertension, 61,1118–1125(2013)

13. A. Leaños-Miranda, F. Méndez-Aguilar, K. L. Ramírez-Valenzuela, Circulating angiogenic factors are related to the severity of gestational hypertension and preeclampsia, and their adverse outcomes, Medicine (Baltimore), 96:e6005e (2017)
14. H. Zeisler, E. Llurba, F. Chantraine, M. Vatish, AC. Staff, M. Sennström, M. Olovsson, S. P. Brennecke, H. Stepan, D. Allegranza, P. Dilba, M. Schoedl ,M. Hund, S. Verlohren, Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia, N Engl J Med., 374,13–22(2016)
15. B. C. Young, R. J. Levine, S. A. Karumanchi, Pathogenesis of preeclampsia, Annual Review of Pathology, 5,173-192(2010)
16. B. Sibai, G. Dekker, M. Kupferminc, Pre-eclampsia, Lancet Feb 26-,365(9461),785-799(2005)
17. S. E. Maynard, J. Y. Min, J. Merchan, K. H. Lim, J. Li, S. Mondal, T. A. Libermann, J. P. Morgan, F. W. Sellke, I. E. Stillman, F. H. Epstein, V. P. Sukhatme, S. A. Karumanchi, Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia, J Clin Invest, Mar,111(5),649-658(2003)
18. M. Autiero, A. Luttun, M. Tjwa, P. Carmeliet, Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders, J Thromb Haemost, 1(7),1356-1370(2003)
19. S. De Falco, The discovery of placenta growth factor and its biological activity, Exp Mol Med, Jan 31,44(1),1-9(2012)
20. C. J. Robinson, D. D. Johnson, Soluble endoglin as a second-trimester marker for preeclampsia, Am J Obstet Gynecol, 197, 174.e171–175(2007)
21. C. Li, R. Issa, P. Kumar, I. N. Hampson, J. M. Lopez-Novoa, CD105 prevents apoptosis in hypoxic endothelial cells, J Cell Sci, 116, 2677–2685(2003)

22. M. Jerkic, J. V. Rivas-Elena, M. Prieto, R. Carro´n, F. Sanz-Rodrı ´guez, Endoglin regulates nitric oxide-dependent vasodilatation, FASEB J,18, 609–611(2004)
23. S. Venkatesha, M. Toporsian, C. Lam, J. Hanai, T. Mammoto, Soluble endoglin contributes to the pathogenesis of preeclampsia, Nat Med, 12, 642–649(2006)
24. A. Leaños-Miranda, I. Campos-Galicia, K. L. Ramírez-Valenzuela, Z. L. Chinolla-Arellano, I. Isordia-Salas, Circulating angiogenic factors and urinary prolactin as predictors of adverse outcomes in women with preeclampsia, Hypertension, 61,1118–1125(2013)
25. A. Leaños-Miranda, F. Méndez-Aguilar, K. L. Ramírez-Valenzuela, Circulating angiogenic factors are related to the severity of gestational hypertension and preeclampsia, and their adverse outcomes, Medicine (Baltimore), 96:e6005e(2017)
26. L. O. Perucci, K. B. Gomes, L. G. Freitas, L. C. Godoi, P. N. Alpoim, M. B. Pinheiro, A. S. Miranda, A. L. Teixeira, L. M. Dusse, L. P.Sousa, Soluble endoglin, transforming growth factor-Beta 1 and soluble tumor necrosis factor alpha receptors in different clinical manifestations of preeclampsia, PLoS One, 9,e97632(2014)
27. S. Rana, A. S. Cerdeira, J. Wenger, S. Salahuddin, K. H. Lim, S. J. Ralston, R. I. Thadhani, S. A. Karumanchi, Plasma concentrations of soluble endoglin versus standard evaluation in patients with suspected preeclampsia, PLoS One, 7,e48259(2012)
28. R. Troisi, K. Braekke, N. K. Harsem, M. Hyer, R. N. Hoover, Blood pressure augmentation and maternal circulating concentrations of angiogenic factors at delivery in preeclamptic and uncomplicated pregnancies, Am J Obstet Gynecol, 199, 653,e651–610(2008)
29. S. Venkatesha, M. Toporsian, C. Lam, J. Hanai, T. Mammoto, Soluble endoglin contributes to the pathogenesis of preeclampsia, Nat Med, 12, 642–649(2006)
30. M. Tjwa, A. Luttun, M. Autiero, P. Carmeliet, VEGF and PlGF: two pleiotropic growth factors with distinct roles in development and homeostasis, Cell Tissue Res, 314, 5–14(2003)

31. H. Masuyama, H. Nakatsukasa, N. Takamoto, Y. Hiramatsu, Correlation between soluble endoglin, vascular endothelial growth factor receptor-1, and adipocytokines in preeclampsia, J Clin Endocrinol Metab, 92, 2672–2679(2007)
32. A. De Vivo, G. Baviera, D. Giordano, G. Todarello, F. Corrado, R. D'anna, Endoglin, PlGF and sFlt-1 as markers for predicting pre-eclampsia, Acta obstetricia et gynecologica scandinavica, 87(8), 837-842(2008)
33. J. H. Lim, S. Y. Kim, S. Y. Park, J. H. Yang, M. Y. Kim, H. M. Ryu, Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors, Obstetrics and gynecology, 111(6), 1403-1409(2008)
34. M. U. Baumann, N. A. Bersinger, M. G. Mohaupt, L. Raio, S. Gerber, D. V. Surbek, Firs-trimester serum levels of soluble endoglin and soluble fms-like tyrosine kinase-1 ats first-trimester markers for late-onset preeclampsia, American journal of obstetrics and gynecology 199(3), 266, e1-e6(2008)