# ANGIOGENIC FACTORS SERUM LEVEL VARIATIONS IN PREGNANCY WITH CHRONIC ARTERIAL HYPERTENSION

UDC 576.385.7:618.2+616.12–008.331.1–002.2
Received 28.03.2012

© **E.V. Volkova,** PhD, the Department of Obstetrics and Gynecology[1]; Scientific Consultant[2];
**L.S. Djokhadze,** Postgraduate, the Department of Obstetrics and Gynecology[1]; Research Associate[2];
**E.Yu. Lyssuk,** PhD, Senior Research Associate[2, 3]

[1]The Russian National Research Medical University named after N.I. Pirogov, Ostrovitianov St., 1, Moscow, Russian Federation, 117997;
[2]Closed Joint Stock Company "Proteinsynthesis", 3rd Kadashevsky per., 6, build. 2, Moscow, Russian Federation, 115035;
[3] Institute of Gene Biology of Russian Academy of Sciences, Vavilova St., 34/5, Moscow, Russian Federation, 119334

The variation of angiogenic proteins level in normotensive pregnancy and pregnancy with chronic arterial hypertension (CAH) was investigated in this study.

**Materials and Methods.** The detailed clinical analysis of gestational course of 69 pregnant women with CAH in and 49 women with normotensive pregnancy was carried out. All patients were studied for the serum level of pro-angiogenic protein PlGF and anti-angiogenic biomarkers sFlt-1 and endoglin at gestational age 22–24, 28–34 and more than 37 weeks.

**Results.** In pregnancy with CAH hypertension the level of pro- and anti-angiogenic proteins at 22–24 weeks of gestation was the same as in normotensive pregnancy. At the gestational age 28–34 weeks the increase of sFlt-1 (5573±774 pg/ml vs. 3299±544 pg/ml in control group, $p<0.001$) and endoglin concentration (7.1±0.8 ng/ml vs. 5.4±0.4 ng/ml in control, $p<0.001$) in the group with complicated pregnancy was detected. At the same time the level of pro-angiogenic PlGF in this group decreased (155.8±30.4 pg/ml vs. 238.5±66.1 pg/ml, $p<0.05$ in control). At the gestational age more than 37 weeks the sFlt-1 level increased both in complicated and normotensive pregnancy (13334±909 pg/ml and 13298±740 pg/ml, respectively), and PlGF level dropped up to 144.4±81.4 pg/ml and 94.5±78.2 pg/ml, respectively ($p<0.01$).

**Conclusion.** In the third trimester of pregnancy there is the variation of angiogenic proteins level: in pregnancy with CAH the serum concentration of anti-angiogenis sFlt-1 was higher and the concentration of pro-angiogenic PlGF was lower than that in normotensive pregnancy. It may indicate the placentation abnormalities.

**Key words:** PlGF; sFlt-1; endoglin; chronic arterial hypertension; pregnancy complications; gestational hypertension.

The problem of the elevated blood pressure (BP) in pregnancy is considered to be one of the most complex aspects of arterial hypertension, both in clinical epidemiology, diagnostics, classification, and treatment and prevention of complications [1]. The pathology has a significant affect on the pregnancy course and outcome, and is the leading cause of both maternal and perinatal morbidity and mortality. [2–4]. Arterial hypertension can result in many critical conditions including: abruption of normal placenta, retinal detachment, eclampsia with cerebrovascular disease and multiple organ failure, severe forms of disseminated intravascular coagulopathy (DIC). The complications of arterial hypertension are progressive placental insufficiency and intrauterine growth restriction (IUGR), and in serious cases — fetal asphyxia and death [5].

In recent years a lot of investigations concerning primary hypertension (idiopathic hypertensia) are focused on angiogenic protein factors because of increased frequency of different vascular abnormalities, including the process of vessel formation (angiogenesis) [6–8]. The reduced microvessel density in arterial hypertension has been demonstrated experimentally and clinically so the cause-effect relations of this phenomenon is the point of many discussions [9–11].

In normal angiogenesis there is the balance between pro- and antiangiogenic factors [12]. The variations in the pro- and antiangiogenic proteins level, particularly PlGF (placental growth factor) and soluble vascular endothelial growth factor receptor sFlt-1, result in imbalance between them. As a concequens of this state increase the possibility of such complications as preeclampsia, IUGR, chronic placental insufficiency, and preterm delivery [13–19]. Besides sFlt-1 and PlGF a lot of investigations study the effect of another antiangiogenic protein — endoglin, which is the component of transforming growth factor (TGF) receptor complex. In particular it was demonstrated that the blood level of soluble endoglin increased in preeclampsia patients [20].

Unfortunately, there is a very few information about variation of angiogenic proteins level in pregnancy with chronic arterial hypertension (CAH).

For contacts: Lyssuk Elena Yurievna, phone: 8(499)135-99-70, +7 916-646-99-80; e-mail: elenlys@gmail.com

**The aim of the investigation** was to study the variation of serum level of proangiogenic biomarker PlGF and antiangiogenic proteins endoglin and sFlt-1 in physiological pregnancy, and pregnancy with chronic arterial hypertension.

**Materials and Methods.**

***Patients.*** A total of 118 patients were enrolled in this study: 49 normotensive pregnant women and 69 with I grade CAH (without target lesions), the term of gestation was between 22 and 40 weeks. All patients were recruited from Russian National Research Medical University. Mean age for normotensive patients (control group) was 29.6±1.3 years (from 21 to 43) and 30.3±1.5 years (from 18 to 46) for group with CAH.

***Material.*** 10 ml of venous blood was collected and allowed to clot for 30–40 min. Serum was separated by centrifugation and stored at –20$^0$C in sigle-use aliquots.

***Immunoassay (ELISA).*** The levels of sFlt-1, PlGF and endoglin in the blood serum were detected by standardized sandwich enzyme-linked immunosorbent assay (Quantikine ELISA, R&D Systems, UK) in duplicate according to the manufacturer's protocol.

***Data analysis.*** The average of duplicate assays represented the value of individual samples in the stasistical analysis. All values are expressed as mean±SD. and analysed by Statistica 7.0 (StatSoft) and MS Excel 2010 (Microsoft) software. Comparision between unpaired groups were performed using two-tail Student t-test, $p<0.05$ was considered statistically significant.

**Results.**

***The study of clinical characteristics.*** We studied various clinical characteristics to estimate the role of different factors in CAH pregnancy pathogenesis as well as determine CAH effect on pregnancy outcome.

The study of body mass-height indices demonstrate statistically significant difference of body mass index (BMI) in groups before pregnancy ($p<0.05$): in control group we detected norman BMI (22.4±0.8 kg/m$^2$), at the same time the index in CAH patients was 27.0±1.4 kg/m$^2$ indicating overweight. In CAH group the most women had impaired fat metabolism before pregnancy — 48% (33/69) versus 8% (4/49) in control group ($p<0.05$). In complicated pregnancy total gestation weight gain was 15.8±2.3 kg, in control group — 12.7±1.2 kg ($p<0.05$). All these data indicate the association between the impaired fat metabolism and pregnancy with CAH.

All patients with complicated pregnancy indicated the stable increased BP before pregnancy, two of them took antihypertensive drugs; patients from control group had no elevated BP. Across pregnancy the level of maximum systolic BP in complicated pregnancy was 144.7±3.3 mm Hg (in control group — 107.9±4.3), diastolic BP — 90.3±2.2 mm Hg (vs. 70.2±1.7 in control).

CAH patients during gestation more frequently had threatening miscarriage (77% (53/69) versus 47% (23/49) in control); in this group placental abruption was diagnosed in 13% women (9/69), 13% patients had a preeclampsia overlay. No pathology was found in the control group.

In CAH group 28% patients (19/69) had indications to premature delivery, 37% of them (7/19) — due to the deterioration of functional fetal status, 47% (9/19) — due to preeclampsia impairment, and in 16% — due to progressing of placental abruption. There were total 33% of preterm deliveries (23/69) in CAH group whereas all control patients had term birth. Average duration of gestational in CAH group was 37.1±0.5 weeks versus 39.6±0.2 weeks in normotensive pregnancy ($p<0.05$).

33% of neonates (23/69) in CAH group were preterm infants; mean weight of newborns in this group was 2854±180 g and mean body height — 48.2±0.9 cm (versus 3501±82 g and 51.6±0.5 cm in control group, respectively). In complicated pregnancy the IUGR syndrome was detected in 27.54% (17/69) cases, in control group the syndrome was not found.

Thus, CAH pregnant patients are characterized by overweight and stable elevated BP before pregnancy. Even moderately elevated BP across pregnancy increased the risk of development of such serious pathologies as abruption of placenta and preeclampsia. IUGR was detected more frequently in CAH patients, and more neonates in this group were preterm infants.

Since all above mentioned pathologies are possible to be related to the placentation process abnormalities, and the proper angiogenesis is needed for normal placentation, blood serum of all patients were tested for the content of some pro- and antiangiogenic protein factors. The investigations were carried out in 22–24, 28–32 and after 37 weeks of gestation (before the delivery).

***sFlt-1 protein level in CAH pregnancy.*** We found that the average sFlt-1 level in blood serum in 22–24 weeks of gestation is higher in patients with CAH comparably with normotensive ones, though the difference was not statistically significant (2472±558 versus 1859±536 pg/ml, respectively) (Fig. 1).

In the third trimester of pregnancy (28–34 weeks) we detected statistically significant increase of sFlt-1 concentration in blood of the patients of both groups compared to the period of 22–24 weeks: 5573±774 pg/ml — in complicated pregnancy ($p=3{,}0\cdot10^{-8}$) and 3299±544 pg/ml — in control group ($p=0.0006$). At the gestational age of 28–34 weeks sFlt-1 level in GH patients was significantly higher compared to the normotensive pregnancy ($p=8.0\cdot10^{-6}$).

In the period of 37-week gestation, sFlt-1 level in the serum of all pregnant women increased, though the difference of its content almost leveled out: 13334±909 pg/ml in CAH group versus 13298±740 pg/ml in control (in comparison with the gestational age of 28–34 weeks $p=3.0\cdot10^{-14}$ and $2.0\cdot10^{-19}$ respectively).

Thus, sFlt-1 level in pregnancy with CAH was significantly higher than that in the control group at 28–34 weeks of gestation.

***Endoglin level in pregnancy with CAH.*** We detected the comparable concentration of endoglin in blood serum of the patients of both groups at gestational age of 22–24 weeks: 4.4±0.6 ng/ml — in pregnant patients with CAH, and 4.3±0.3 ng/ml — normotensive pregnancy (Fig. 2).

At 28–34 week of gestation the endoglin level was found to increase and significantly differed between the groups ($p=0.0005$): in complicated pregnancy —

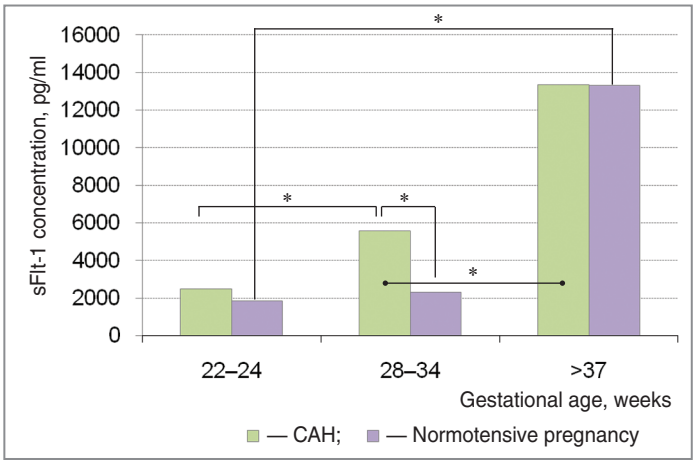


**Fig. 1.** sFlt-1 level in blood serum in normotensive pregnancy and in pregnancy with CAH. At the gestational age 22–24 weeks and more than 37 weeks the protein concentration in both groups is comparable, at 28–34 weeks of gestation the sFlt-1 level in CAH group is significantly higher; * — p<0.0011

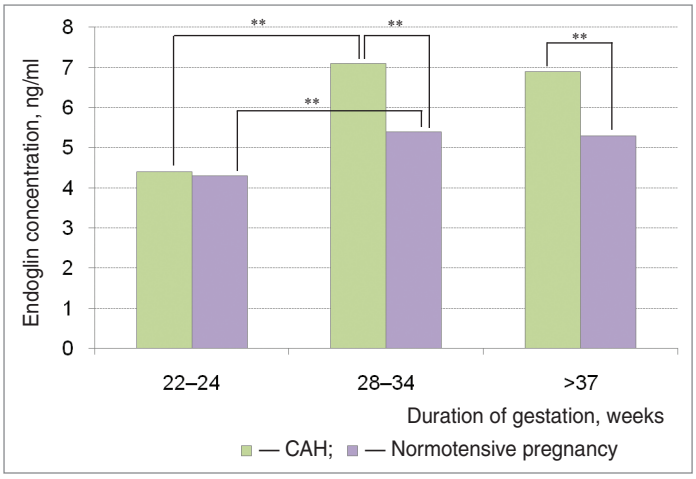


**Fig. 2.** Endoglin level in blood serum of pregnant women with CAH and in normotensive pregnancy. At the gestational age 22–24 weeks the protein concentration is comparable in both groups, in terms 28–34 week and after 37 weeks the endoglin level in patients with complicated pregnancy is significantly higher; * — p<0.05; ** — p<0.001

7.1±0.8 ng/ml of protein (between the terms of 22–24 and 28–34 weeks $p=1.0 \cdot 10^{-6}$), in control group — 5.4±0.4 ng/ml. After 37 weeks of gestation the endoglin level was the same compared to 28–34-week gestation: 6.9±1.3 and 5.3±0.5 ng/ml respectively (p=0.046 between the groups).

Thus, in both groups in the second trimester the endoglin level was the same compared to the control, but in late pregnancy the protein content was higher in pregnant patients with CAH.

***PlGF level in pregnancy with CAH.*** Since the angiogenesis is regulated by the balance of angiogenic protein factors which are activate or inhibit this process, we also studied the level of proangiogenic protein PlGF in the serum of patients from both groups. Similarly to endoglin, PlGF level at the gestational age of 22–24 weeks in both groups did not significantly differ: we detected 196.0±85.8 pg/ml of protein in the blood of pregnant women with CAH and 171.7±73.4 pg/ml — in normal pregnancy (Fig. 3).

At 28–34 weeks of gestation protein concentration in CAH pregnancy slightly decreased up to 155.8±30.4 pg/ml, and at the same time in normotensive pregnancy its level increased up to 238.5±66.1 pg/ml. In this period PlGF level in the group with complicated pregnancy was on average 1.5 times lower than that in control (p=0.03).

After 37 weeks of gestation compared to 28–34-week gestation period we found out the protein level reduction in both groups: up to 144.4±81.4 pg/ml in CAH patients, and up to 94.5±78.2 pg/ml in control group. That is at the gestation age more than 37 weeks PlGF level in CAH

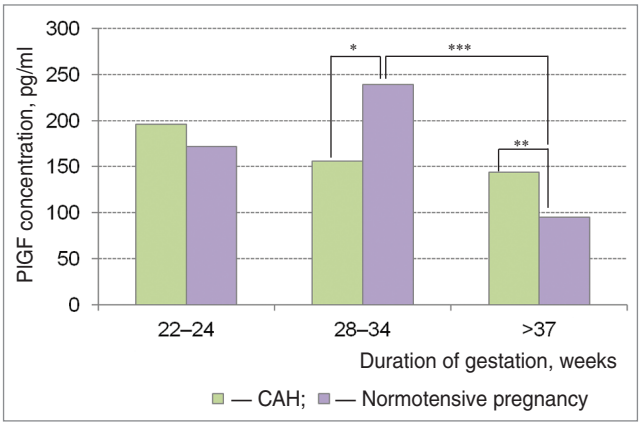


**Fig. 3.** PlGF level in blood serum in pregnant women with CAH and in normotensive pregnancy. At the gestational age 22–24 weeks the protein concentration is comparable in both groups, in term 28–34 weeks the PlGF level is significantly lower in patients with complicated pregnancy, and in term more than 37 weeks its concentration is significantly higher than in healthy pregnant women; * — p<0.05; ** — p<0.01; *** — p<0.001

pregnancy was in 1,5 times higher compared to the normal pregnancy (p=0.009).

Thus, PlGF level in pregnant women with CAH had the tendency to decrease over a studied period of pregnancy, and compared to the normotensive pregnancy, its concentration at the gestational age of 22–24 weeks was higher than the control level, and after a 37-week period it was lower.

**Discussion.** Increased frequency of hypertension syndrome in pregnancy determines the necessity to find out the risk factors which enable to detect a group of patients with high risk of serious complications onset. Our results show that even slightly impaired fat metabolism is directly associated with chronic arterial hypertension, which in its turn complicates the gestation course. These findings confirm other studies performed with pregnant women of other nationalities [21, 22]. Moreover, the presence of primary AH was proved to result in stable BP increase in following pregnancy and associated pathologies [23]. In addition, even moderately increased BP in pregnancy was shown to be associated with an increased risk of developing complications including those related to placentation process abnormality.

Normal placental development is possible only under normal regulation of angiogenic processes; on the other hand, hypertensive processes are frequently associated with vascular disorders [9, 11]. We found that at late second trimester of pregnancy there were no obvious changes of the angiogenic proteins level in pregnancy with CAH. However, by the middle of the third trimester, i.e. at the gestational age of 28–34 weeks, we detected the imbalance of the pro- and antiangiogenic protein factors level in the blood of patients with complicated pregnancy compared to the normotensive ones.

Late gestation period was marked by similar changes in the levels of angiogenic factors in pregnancy both normotensive and CAH complicates. In the gestational age closed to a full-term pregnancy we detected a sharp increase of antiangiogenic protein (sFlt-1) concentration in the blood of patients from both groups as well as the decrease of proangiogenic factor (PlGF) concentration. The PlGF serum concentration differed between groups only in the middle of the third trimester: in normal pregnancy the protein level decreased significantly by the end of gestation after the increase of its concentration in the middle of the third trimester, and in CAH pregnancy there was the tendency for its slowly decrease throughout the gestation period under study.

S. Verlohren et al. [24] determined sFlt-1/PlGF ratio after the 34$^{th}$ week of pregnancy in different hypertensive pathologies and demonstrated that the ratio increased both in normotensive and CAH pregnancy compared to early gestation periods. Though the ratio gain is possible in case of sFlt-1 concentration increase, when PlGF level decreases concurrently, that is in agreement with our findings. The similarity of protein level changes in physiological pregnancy and in pregnancy with hypertensive complications indicate that the increase of antiangiogenic sFlt-1 and the decrease of proangiogenic PlGF in the gestational age closed to a full-term pregnancy may serve as one of the delivery initiating mechanisms.

**Conclusion.** At the late second trimester of pregnancy there were no significant changes in angiogenic factors in pregnant women with chronic arterial hypertension. At the early third thrimester of pregnancy with CAH the abnormal angiogenesis regulation expressed in the increased level of antiangiogenic sFlt-1 and endoglin, and reduced proangiogenic PlGF concentration is observed. By the end of gestation, the detected sFlt-1 serum level in CAH patients is higher than in the healthy ones; at the same time the PlGF protein level fall down, though it occurs both in normotensive and CAH pregnancy.

The imbalance of angiogenic proteins in CAH pregnancy in 28–34 weeks of gestation may indicate the abnormality of placentation processes.

### References


**1.** Sibai B.M. Diagnosis, prevention, and management of eclampsia. *Obstet Gynecol* 2005; 105(2): 402–410.

**2.** Tokova Z.Z. Statisticheskie aspekty gipertenzivnykh rasstroystv vo vremya beremennosti, rodov i v poslerodovom periode. V kn.: *Materialy VI Rossiyskogo foruma «Mat' i ditya»* [Statistical aspects of hypertensive disorders in pregnancy, labors, and puerperal period. In: Proceedings of VI Russian Forum "Mother and Child"]. Moscow; 2004; p. 645.

**3.** Duley L. Pre-eclampsia and hypertension. *Clin Evid* 2004; 12: 2016–2034.

**4.** Zhang J., Meikle S., Trumble A. Severe maternal morbidity associated with hypertensive disorders in pregnancy in the United States. *Hypertens Pregnancy* 2003; 22(2): 203–212.

**5.** Sukhikh G.T., Murashko L.E. *Preeklampsiya* [Preeclampsy]. Moscow: GEOTAR-Media; 2010; 576 p.

**6.** Kiefer F.N., Neysari S., Humar R., Li W., Munk V.C., Battegay E.J. Hypertension and angiogenesis. *Curr Pharm Des* 2003; 9(21): 1733–1744.

**7.** Feihl F., Liaudet L., Waeber B., Levy B.I. Hypertension: a disease of the microcirculation? *Hypertension* 2006; 48(6): 1012–1017.

**8.** Loomans C.J., Dao H.H., van Zonneveld A.J., Rabelink T.J. Is endothelial progenitor cell dysfunction involved in altered angiogenic processes in patients with hypertension? *Curr Hypertens Rep* 2004; 6(1): 51–54.

**9.** Debbabi H., Uzan L., Mourad J.J., Safar M., Levy B.I., Tibirica E., Increased skin capillary density in treated essential hypertensive patients. *Am J Hypertens* 2006; 19(5): 477–483.

**10.** Levy B.I., Ambrosio G., Pries A.R., Struijker-Boudier H.A. Microcirculation in hypertension: a new target for treatment? *Circulation* 2001; 104(6): 735–740.

**11.** Serne E.H., Gans R.O., ter Maaten J.C., Tangelder G.J., Donker A.J., Stehouwer C.D. Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. *Hypertension* 2001; 38(2): 238–242.

**12.** Roodink I., Leenders W.P. Targeted therapies of cancer: angiogenesis inhibition seems not enough. *Cancer Lett* 2010; 299(1): 1–10.

**13.** Solomon C.G., Seely E.W. Preeclampsia — searching for the cause. *N Engl J Med* 2004; 350(7): 641–642.

**14.** Makris A., Thornton C., Thompson J., Thomson S., Martin R., Ogle R., Waugh R., McKenzie P., Kirwan P., Hennessy A. Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. *Kidney Int* 2007; 71(10): 977–984.

**15.** Redecha P., van Rooijen N., Torry D., Girardi G. Pravastatin prevents miscarriages in mice: role of tissue factor in placental and fetal injury. *Blood* 2009; 113(17): 4101–4109.

**16.** Wallner W., Sengenberger R., Strick R., Strissel P.L., Meurer B., Beckmann M.W., Schlembach D. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clin Sci (Lond)* 2007; 112(1): 51–57.

**17.** Andraweera P.H., Dekker G.A., Roberts C.T. The vascular

endothelial growth factor family in adverse pregnancy outcomes. *Hum Reprod Update* 2012; 18(4): 436–457.

**18.** Aggarwal P.K., Chandel N., Jain V., Jha V. The relationship between circulating endothelin-1, soluble fms-like tyrosine kinase-1 and soluble endoglin in preeclampsia. *J Hum Hypertens* 2012; 26(4): 236–241.

**19.** Verlohren S., Stepan H., Dechend R. Angiogenic growth factors in the diagnosis and prediction of pre-eclampsia. *Clin Sci (Lond)* 2012; 122(2): 43–52.

**20.** Masuyama H., Segawa T., Sumida Y., Masumoto A., Inoue S., Akahori Y., Hiramatsu Y. Different profiles of circulating angiogenic factors and adipocytokines between early- and late-onset pre-eclampsia. *BJOG* 2010; 117(3): 314–320.

**21.** Baeten J.M., Bukusi E.A., Lambe M. Pregnancy complications and outcomes among overweight and obese nulliparous women. *Am J Public Health* 2001; 91(3): 436–440.

**22.** Fortner R.T., Pekow P., Solomon C.G., Markenson G., Chasan-Taber L. Prepregnancy body mass index, gestational weight gain, and risk of hypertensive pregnancy among Latina women. *Am J Obstet Gynecol* 2009; 200(2): 167.e1-7. doi: 10.1016/j.ajog.2008.08.021.

**23.** Brown M.A., Lindheimer M.D., de Swiet M., Van Assche A., Moutquin J.M. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; 20(1): IX–XIV.

**24.** Verlohren S., Herraiz I., Lapaire O., Schlembach D., Moertl M., Zeisler H., Calda P., Holzgreve W., Galindo A., Engels T., Denk B., Stepan H., The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am J Obstet Gynecol* 2012; 206(1): 58.e1-8. doi: 10.1016/j.ajog.2011.07.037.