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[1]Chair and Department of Obstetrics and Perinatology, [2]Chair and Department of Gastroenterology
Medical University of Lublin

MARZENA LASKOWSKA[1], BOŻENA LESZCZYŃSKA-GORZELAK[1],
KATARZYNA LASKOWSKA[2], JAN OLESZCZUK[1]

# *Analysis of the sFlt-1 in pregnancies complicated by preeclampsia with and without IUGR*

Preeclampsia, a syndrome affecting 5% of pregnancies, causes substantial maternal and fetal morbidity and mortality (1). It is a specific hypertensive pregnancy disorder with proteinuria, is characteristic only of human pregnancy. It has been suggested that endothelial cell activation is the primary event in the multisystem disorder of preeclampsia (2).

In preeclamptic pregnant women there is no adequate invasion of trophoblast and physiologic remodelling of spiral arteries, characteristic of normal pregnancy. Pathological adaptive changes in spiral arteries result in the increased sensitivity to vasoactive factors and decreased uteroplacental blood flow and may result in placental insufficiency with intrauterine fetal growth retardation.

Roberts and Lain proposed that the pathophysiology of preeclampsia consists of two stages: utero-placental insufficiency, which is followed by generalized endothelial cell dysfunction (3). In preeclampsia glomerular endotheliosis, a loss of endothelial cell integrity, with the consequent increase in vascular permeability is observed. There is much evidence that the endothelial alterations in preeclampsia result from one or more circulating factors.

Angiogenesis is a critical process for growth and development, it is reflected in IUGR. VEGF is essential for these processes (4). Extensive angiogenesis and invasion of the maternal decidua by trophoblast are essential for the development and function of the placenta. Vascular endothelial growth factors (VEGF), placenta growth factor (PlGF) and their receptors VEGFR-1 / Flt-1, VEGFR-2/KDR and VEGFR-3 / Flt-4 have important roles in vasculogenesis and angiogenesis (5). Clark et al. proposed that sVEGFR-1 was produced by human placenta and released to maternal circulation (6).

The soluble form of VEGFR-1 (sFlt-1) is an antagonist of PlGF and VEGF, which is a well-known angiogenic factor that promotes vascular endothelial proliferation and differentiation and could be one of several factors linking these two pathological processes in preeclampsia. Furthermore, it was demonstrated in pregnant rats that sFlt-1 treatment induced hypertension, prioteinuria and glomerular endotheliosis (1).

Moreover, administration of sVEGFR-1 to pregnant animals can induce the clinical manifestations of preeclampsia including hypertension ansd proteinuria (1, 7) and these animals develop glomerular endotheliosis, a pathologic change observed (8) in preeclampsia.

VEGFR1 and VEGFR2 consist of 1338 and 1356 amino acids in humans, respectively, and can be separated into 4 regions: the extracellular ligand-binding domain, transmembrane domain, tyrosine kinase domain, and downstream carboxy terminal region (9).

Soluble VEGFR1 may function as a natural VEGF-A inhibitor. This unique characteristic of VEGFR1 suggests that it acts as both a negative regulator via its ligand- binding domain and a positive regulator via its tyrosine kinase (9). Soluble VEGFR1 appears to form a barrier between the fetal and maternal sides, and regulates the placenta (9).

The aim of this study was to evaluate the soluble Vascular Endothelial Growth Factor Receptor type 1 (sFlt-1) in maternal serum in preeclamptic pregnancies with appropriate-for-gestational-age weight infants and in patients with preclampsia complicated by intrauterine growth retardation (IUGR).

## MATERIAL AND METHODS

The study was carried out on 39 preeclamptic patients with severe preeclampsia (group PRE). In group PRE, there were 20 patients with preeclampsia complicated by intrauterine growth retardation (group PI) and 19 preeclamptic patients with appropriate-for-gestational-age weight infants (group P).

Preeclampsia was determined by increased blood pressure >140 mm Hg systolic and >90 mm Hg diastolic in women who were normotensive before 20 weeks' gestation accompanied by proteinuria defined as the urinary excretion of more than 0.3 g protein in 24- hour specimen. Severe preeclampsia was defined as blood pressure >160/110 mmHg on at least two occasions 6 hours apart with proteinuria >2 g in a 24-hour urinary protein excretion.

The infant birth weight below the $10^{th}$ percentile for gestational age was classified as intrauterine growth retardation (10).

The control group consisted of 14 healthy normotensive pregnant patients with singleton uncomplicated pregnancies, without any renal, heart and vascular diseases and with normal laboratory tests (group C). All arterial blood pressure measurements in the control group were normal and did not exceed 135/85 mmHg. None of the patients from this group suffered from proteinuria. All patients in the study were non-smokers.

Five mililiters of blood were taken by venipuncture from each preeclamptic patient and from each woman from the control group and collected in sterile tubes. They were centrifuged for 15 min at 500xg immediately after sampling. Each obtained serum was frozen until assayed.

Maternal serum soluble receptor VEGFR1 (sFlt-1) concentrations were estimated using a sandwich ELISA assay according to the manufacturer's instructions (human VEGF-A and human sVEGF-R1 sandwich ELISA kit Bender MedSystems Vienna, Austria).

Data were expressed as mean +/- SD and were statistically analyzed with the computer program "Statistica 5.0" using the Shapiro-Wilk test for the normal distribution of data, and the equality of variance by the Levene test and, subsequently one-tailed Student's t-tests, or (in unequal variance) the Cochran-Cox test, (absence of normal distribution and non-parametric data) the Mann-Whitney U test and ANOVA Kruskal-Wallis test. The level of statistical significance was established as $p<0.05$.

## RESULTS

No statistically significant differences in gravidity and parity were found in patient profiles between groups. Creatinine and urea levels were normal in all patients. None of the patients from the control group suffered from proteinuria.

The mean maternal age was 26.346 +/- 7.406 years in the group of preeclamptic pregnant patients without IUGR and 27.412 +/- 4.492 years in pregnant women with preeclampsia complicated by IUGR versus 26.671 +/- 3.841 years in the control group.

There was lower gestation age in both preeclamptic groups in comparison with the healthy controls. But these differences were not statistically significant. The mean gestation age was 36.445 +/- 2.348 weeks in group P and 34.608 +/- 2.473 weeks in group PI vs 37.837 +/- 1.315 weeks in the control group. In all our patients from PI group asymetric IUGR were observed.

Systolic and diastolic blood pressure and mean arterial blood pressure were higher in the study group in comparison with the control group. These differences were statistically significant ($p<0.001$). The mean systolic blood pressure values were 160.75 +/- 12.07 mmHg in the group of preeclamptic pregnant patients and 101.47 +/- 6.81 mmHg in the control group. The mean diastolic blood pressure values were 109.61 +/- 8.95 mmHg in women with pregnancy complicated by preeclampsia and 68.53 +/- 9.67 mmHg in the healthy controls.

The preeclamptic patients (group PRE) revealed higher maternal serum levels of sVEGF-R1 concentrations in comparison with the controls. The mean values were 63.62 +/- 116.56 ng/mL (range from 0.273 to 478.98 ng/mL) in the group PRE compared with 11.17 +/- 28.33 ng/mL (ranging from 0.423 to 106.62 ng/mL) in the control group. This difference was statistically significant ($p=0.0126$) (Fig. 1).

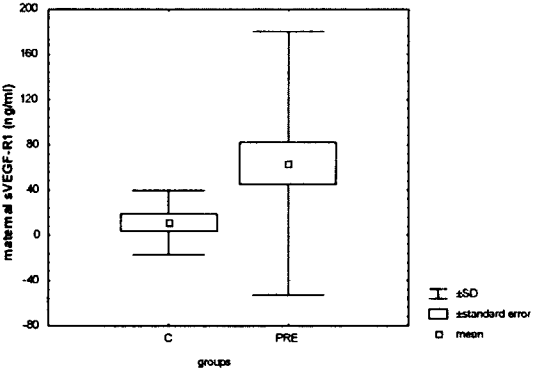


Fig. 1. Maternal soluble VEGF-R1 in preeclamptic patients and healthy controls

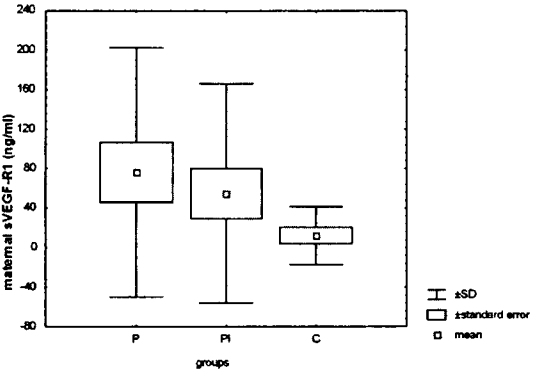


Fig. 2. Maternal soluble VEGF-R1 in studied groups of patients

When the preeclamptic women were further divided into preeclampsia with normal intrauterine fetal growth (group P) and preeclampsia complicated by intrauterine growth retardation (group PI), the levels of maternal serum sVEGF-R1 were higher in both study subgroups patients with preeclampsia in comparison with the healthy controls (p=0.1012 for group PI and p=0.0056 for group P respectively)

In the group of preeclamptic women with appropriate intrauterine fetal growth these levels were additionally higher compared with the patients with preeclampsia complicated by IUGR. But the difference between both groups of preeclamptic patients was not statistically significant (p=0.3353)

The mean values of maternal serum sVEGF-R1 were 72.30 +/- 124.11 ng/mL (range from 0.273 to 478.98 ng/mL) in group P vs. 54.93 +/- 111.21 ng/mL (range from 0.45 to 478.98 ng/mL) in group PI, and 11.17 +/- 28.33 ng/ml (range from 0.42 to 106.62 ng/ml) in the healthy controls (Fig. 2).

## DISCUSSION

Our study revealed elevated concentration of the soluble receptor type 1 (sFlt-1) in both groups of studied preeclamptic women, with and without IUGR, compared with the healthy controls. The highest level of the sFlt-1 was found in the group of patients with preeclampsia without IUGR. Soluble Flt-1 level was significantly higher in women with preeclampsia with appropriate intrauterine fetal growth compared with the control subjects and with the women with preeclampsia complicated by the intrauterine growth retardation.

Similar results were presented by Maynard et al. (1), who observed elevated concentrations of sFlt-1 and reduced levels of free VEGF in the maternal serum in the preeclamptic women compared with normotensive pregnancies.

Maynard et al. (1) demonstrated that increased circulating sFlt-1 in patients with preeclampsia is associated with the decreased levels of circulating free VEGF and PlGF, resulting in endothelial dysfunction *in vitro* that can be corrected by exogenous VEGF and PlGF. Additionally, VEGF and PlGF cause microvascular relaxation of renal arterioles *in vitro* in rats, that is blocked by sFlt-1, while administration of sFlt1 to pregnant rats induces hypertension, proteinuria, and glomerular endotheliosis, the classic conditions in preeclampsia (1). Maynard et al. (1) suggest that the excess of circulating sFlt1 contributes to the pathogenesis of preeclampsia.

Maternal serum sFlt-1 and VEGF are altered in pregnancies complicated by preeclampsia and IUGR but these changes seem to be the greatest at or near the time when the disorder becomes diagnosed (11).

Savvidou et al. (11) also demonstrated higher levels of sFlt-1 and decreased VEGF levels in pregnancy complicated by intrauterine growth retardation.

A possible explanation for lower levels of the VEGF observed in women with preeclampsia might be the enhanced production of soluble VEGF receptor (sFlt-1), acting as the antagonist of the vascular endothelial growth factor by binding to VEGF and inhibiting VEGF actions on endothelial cells and as a protecive physiological response in pregnancy to prevent vascular damage (12, 13).

Our study revealed elevated levels of sFlt-1 in pregnancies complicated by preeclampsia and in pregnancies complicated by intrauterine growth retardation in the course of preeclampsia. Similar results were presented by Vourela et al. (14), who concluded that preeclampsia is associated with the increased levels of soluble VEGFR-1, which are independent of erythopoietin, another hypoxia-inducing factor.

Also Chan-Wook Park et al. (15) showed that the median concentration of sFlt-1 in the maternal plasma, but not amniotic fluid in patients who developed preeclampsia was significantly higher

than in the control cases. The concentration of sFlt-1 in the plasma was higher in cases of severe preeclampsia than in those with mild preeclampsia without reaching statistical significance. These authors concluded that an elevated sFlt-1 concentration in the maternal plasma is a risk factor for the development of preeclampsia and severe preeclampsia.

Similarly Boutsikou et al. (4) observed higher sVEGFR-1 levels in pregnancies complicated by intrauterine growth retardation as compared to the normal fetal growth groups. They suggested that their results possibly reflected the predominance of antiangiogenic mechanisms present in IUGR.

Levine et al. (16) observed that increased levels of sFlt-1 and reduced levels of PlGF predict the subsequent development of preeclampsia. Changes in the levels of sFlt-1 and free PlGF were greater in women with the earlier onset of preeclampsia and in women in whom preeclampsia was associated with an infant small for the gestational age.

Rajakumar et al. (17) presented the evidence that mononuclear cells in the peripheral blood of preeclamptic women are capable of producing higher sFlt-1 than in normal pregnancy, either under normoxia or hypoxia. They suggested that significant extra-placental sources of circulating sFlt-1 may play a significant role in the pathogenesis of preeclampsia.

Furthermore, a study in mice by Hirashima et al. (18) suggested that trophoblast-derived sFlt-1 affected the maternal circulation in pregnancy, rather than local placental vasculature. These authors also speculated that sFlt-1 reducing strategy as a therapy for preeclampsia would have deleterious effect on the placenta itself.

Soluble VEGFR-1 binds VEGF and is the most potent regulator of the VEGF activity *in vivo* (19). By neutralizing VEGF, sVEGFR-1 may contribute to the inadequate placental vascularization. Thus elevated levels of the sFlt-1 may contribute to the pathogenesis of preeclampsia.

## CONCLUSIONS

According to data from the literature and our results increased concentrations of sFlt-1 in pregnancy complicated by preeclampsia with and without IUGR may suggest that elevated levels of the soluble Flt-1 are associated with preeclampsia and preeclampsia with intrauterine growth retardation.

## REFERENCES

1. Maynard S. E. et al.: Excess placental soluble fms-like tyrosine kinase 1 (sFLT1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J. Clin. Invest., 111, 649, 2003.
2. Hayman R. et al.: Preeclampsia: the endothelium, circulating factor(s) and vascular endothelial growth factor. J. Soc. Gynecol. Invest., 6 (1), 3, 1999.
3. Roberts J. M., Lain K. Y.: Recent insight into pathogenesis of preeclampsia. Placenta, 23 (5), 359, 2002.
4. Boutsikou T. et al.: Soluble vascular endothelial growth factor receptor-1 in intrauterine growth restricted fetuses and neonates. Early Hum. Dev., 82, 235, 2006.
5. Helske S. et al.: Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. Mol. Hum. Reprod., 7 (2), 205, 2001.
6. Clark D. E. et al.: A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. Biol. Reprod., 59, 1540, 1998.
7. Chaiworapongsa T. et al.: Plasma soluble vascular endothelial growth factor receptor-

1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. J. Matern. Fetal Neonatal Med., 17 (1), 3, 2005.
8. Kincaid-Smith P.: The renal lesion of preeclampsia revisited. Am. J. Kidney Dis., 17, 144, 1991.
9. Shibuya M.: Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. J. Biochem. Mol. Biol., 39 (5), 469, 2006.
10. Chauhan S. P. et al.: Suspicion of intrauterine growth restriction: Use of abdominal circumference alone or estimated fetal weight below 10%. J. Matern. Fetal Neonatal Med., 19 (9), 557, 2006.
11. Savvidou M. D. et al.: Maternal serum concentration of soluble fms-like tyrosine kinase 1 and vascular endothelial growth factor in women with abnormal uterine artery Doppler and in those with fetal growth restriction. Am. J. Obstet. Gynecol., 195, 1668, 2006.
12. Lyall F. et al.: Suppression of serum vascular endothelial growth factor immunoreactivity in normal pregnancy and in pre-eclampsia. Br. J. Obstet. Gynaecol., 104, 223, 1997.
13. Hefler L. et al.: Vascular endothelial growth factor serum levels in pregnancy and preeclampsia. Acta Obstet. Gynecol. Scand., 79, 77, 2000.
14. Vourela P et al.: Amniotic fluid-soluble vascular endothelial growth factor receptor-1 in preeclampsia. Obstet. Gynecol., 95, 353, 2000.
15. Park Ch. W. et al.: An elevated maternal plasma, but not amniotic fluid, soluble fms-like tyrosine kinase-1 (Flt-1) at the time of mid-trimester genetic amniocentesis is a risk factor for preeclampsia. Am. J. Obstet. Gynecol., 193, 984, 2005.
16. Levine R. J. et al.: Circulating angiogenic factors and the risk of preeclampsia. N. Engl. J. Med., 350 (7), 672, 2004.
17. Rajakumar A. et al.: Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women. Placenta, 26, 563, 2005.
18. Hirashima M. et al.: Trophoblast expression of Fms-like tyrosine kinase 1 is not required for the establishment of the maternal-fetal interface in the mouse placenta. Proc. Natl. Acad. Sci. USA, 100, 15637, 2003.
19. Wathén K. A. et al.: Maternal serum soluble vascular endothelial growth factor receptor-1 in early pregnancy ending in preeclampsia or intrauterine growth retardation. J. Clin. Endocrinol. Metab., 91, 180, 2006.

## SUMMARY

The aim of this study was to evaluate the soluble Vascular Endothelial Growth Factor Receptor type 1 (sFlt-1) in maternal serum in preeclamptic pregnancies with appropriate-for-gestational-age weight infants and in patients with preclampsia complicated by intrauterine growth retardation (IUGR). The study was carried out on 39 preeclamptic patients with severe preeclampsia (group PRE). In group PRE, there were 20 patients with preeclampsia complicated by intrauterine growth retardation (group PI) and 19 preeclamptic patients with appropriate-for-gestational-age weight infants (group P) and 14 healthy pregnant normotensive women. Maternal serum soluble receptor VEGFR1 (sFlt-1) concentrations were estimated using a sandwich ELISA assay. The preeclamptic patients revealed higher maternal serum levels of soluble VEGF receptor type 1 (sFlt-1) concentrations in comparison with the controls. The highest level of the sFlt-1 was found in the group of patients with preeclampsia without IUGR. Our findings may suggest the significant role of soluble Flt-1 in pathogenesis and sequelae of preeclampsia and intrauterine growth retardation.

Analiza sFlt-1 w ciąży powikłanej stanem przedrzucawkowym z i bez IUGR

Celem badania była ocena stężenia rozpuszczalnego receptora typu 1 VEGF (sFlt-1) w surowicy krwi matczynej w ciąży powikłanej stanem przedrzucawkowym z adekwatnym wzrostem płodu oraz u kobiet ciężarnych z zahamowaniem wewnątrzmacicznego wzrastania płodu w przebiegu stanu przedrzucawkowego. Badaniem objęto 39 kobiet z ciążą powikłaną ciężkim stanem przedrzucawkowym (grupa PRE). W tej grupie kobiet było 20 pacjentek z zahamowaniem wewnątrzmacicznego wzrastania płodu w przebiegu stanu przedrzucawkowego (grupa PI) oraz 19 ciężarnych z ciężkim stanem przedrzucawkowym i adekwatnym wzrostem wewnątrzmacicznym płodu (grupa P). Grupę kontrolną stanowiło 14 zdrowych kobiet ciężarnych z prawidłowym ciśnieniem tętniczym krwi i niepowikłanym przebiegiem ciąży. Ocenę sFlt-1 wykonano metodą ELISA. W grupie badanych kobiet z ciążą powikłaną ciężkim stanem przedrzucawkowym odnotowano podwyższone poziomy rozpuszczalnego receptora typu 1 VEGF (sFlt-1) w stosunku do kobiet z grupy kontrolnej. Najwyższe wartości sFlt-1 zaobserwowano w grupie kobiet z ciążą powikłaną stanem przedrzucawkowym i prawidłowym wzrostem płodu. Wyniki naszych badań sugerują istotną rolę sFlt-1 w patogenezie i następstwach stanu przedrzucawkowego i IUGR.