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aVEGF-A and its Soluble Receptor Type 1 (sVEGFR-1, sFlt-1) Concentrations in Pregnancies with Intrauterine Growth Restriction in the Presence or Absence of Preeclampsia.

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ABSTRACT

The aim of this study was to determine the maternal serum aVEGF-A and its soluble receptor type 1 (sVEGFR-1, sFlt-1) concentrations in pregnancies with intrauterine growth restriction (IUGR) in the presence or absence of preeclampsia. The study was performed on 65 normotensive pregnant patients with isolated IUGR, 64 preeclamptic women with IUGR and 51 preeclamptic patients with normal intrauterine foetal growth and 65 healthy normotensive pregnant women with singleton uncomplicated pregnancies. The maternal serum active VEGF and sVEGFR-1 concentrations were determined using a sandwich enzyme-linked immunosorbent assays. The study revealed increased levels of aVEGF-A in patients with pregnancies complicated by preeclampsia and/or IUGR. But these differences were not statistically significant. The levels of aVEGF-A were decreased in the patients with HELLP syndrome and in the subgroup of patients with pregnancy complicated by eclampsia. This study revealed the higher serum sFlt-1 levels in both groups of preeclamptic patients and similar levels of sVEGFR-1 in normotensive patients with isolated IUGR to those observed in the control group. Findings presented in this study confirm the importance of aVEGF-A for the physiological course of pregnancy. Increased levels of sVEGFR-1, appear to be an important limitation, which may have a significant impact on the pathogenesis of severe preeclampsia and IUGR in the course of preeclampsia. Importantly, IUGR without preeclampsia does not present significant differences relative to physiological control for sVEGFR-1 levels, suggesting different aetiopathogenetic mechanisms leading to “pure” IUGR.

Keywords: active Vascular Endothelial Growth Factor-A (aVEGF-A), soluble receptor type 1 for VEGF (sVEGR-1), intrauterine foetal growth restriction (IUGR), severe preeclampsia.

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INTRODUCTION

Pathophysiological processes underlying intrauterine fetal growth restriction and/or preeclampsia are complicated, multifactorial and unclear, although there seem to have a common pathology, possibly due to an impairment of the trophoblast invasion with insufficient endovascular remodelling and changes specific for normal pregnancy [1-4]. Vascular endothelial growth factor (VEGF) is a proangiogenic factor, which plays a key role in the vasculogenesis, proper angiogenesis and implantation, which is essential for a healthy pregnancy [5]. The biological activity of VEGF is mediated by specific receptors. Vascular endothelial growth factor receptor 1 (VEGFR-1), with a molecular weight of about 180kDa is mainly expressed in vascular endothelial cells, monocytes, macrophages, trophoblast and renal glomerular mesangial cells [5-6]. Soluble form of the type 1 receptor for VEGF (sVEGFR-1), a natural inhibitor of VEGF-A and most potent regulator of its activity in vivo, is one of the peptides actively involved in the development of preeclampsia [2, 6-8]. It was found in the blood serum of pregnant women, but is absent in the serum of men and nonpregnant women [6]. A rich source of sVEGFR-1 is the placenta, and its expression was demonstrated in villous trophoblast and endothelial cells of villous blood vessels [6]. The regulation of VEGF activity locally and systemically is essential to successful pregnancy [6]. sVEGFR-1 by means of neutralization of VEGF activity may contribute to the impaired implantation and to the placental vasculature disorders, leading to pregnancy complications such as preeclampsia or IUGR [2, 6-8]. Already published information regarding the role of VEGF-A and its soluble receptor sVEGFR-1 in normotensive pregnancies complicated by isolated fetal intrauterine growth restriction is limited.

Thus, the aim of this study was to find out how VEGF-A and sVEGFR-1 are different in the maternal serum in pregnancies complicated by fetal intrauterine growth restriction in the course of preeclampsia, and to compare the results with normotensive pregnant women with isolated IUGR. The study was approved by ethical Committee of Lublin Medical University and all patients were intended of the study and provided their consent before participation in the study (*KE-0254/51/2010*).

PATIENTS AND METHODS

The study was carried out on 65 normotensive pregnant patients with pregnancy complicated by isolated intrauterine growth restricted fetuses (the iugr group) and 64 patients with IUGR in the course of preeclampsia (the PI group) and 51 preeclamptic patients with appropriate-for-gestational-age weight infants (the P group). Amongst preeclamptic patients there were 10 patients with HELLP syndrome (9 in the PI group and 1 in the P group) and 6 patients with eclampsia (5 in the P group and 1 in the PI group). The control group consisted of 65 healthy normotensive pregnant patients with singleton uncomplicated pregnancies (the C group).

Fetal biometry was based on non-invasive ultrasound method and included the estimation of gestational age in early gestation and diagnosis of fetal intrauterine growth restriction by monitoring fetal growth later in the second or third trimester of pregnancy [6,20,21]. Intrauterine foetal growth restriction was defined as failure of the foetus to achieve its genetically determined growth potential and IUGR foetuses were classified as such according to ultrasonographic measurement when the weight of the fetus was lower than expected in relation to the gestational age, as determined by the standard curves characteristic of the Polish population, when the fetus was below the 10th centile for gestational age [3,9]. Additionally, IUGR pregnancies were characterized by at least one disturbed placental function and abnormal ultrasonographic examination. The diagnosis was confirmed by the infant's weight at birth [3,9].

Severe preeclampsia was defined as blood pressure >160/110mmHg on at least 2 occasions 6h apart with proteinuria >5g in a 24-hour urinary protein excretion, and when hypertension and proteinuria were associated with one or more of the following clinical manifestations: renal abnormalities, haematological abnormalities (thrombocytopenia and microangiopathic hemolysis) or HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count and right-upper quadrant pain), or neurological symptoms (headache, visual disturbances and seizures). None of the pregnant patients with preeclampsia were affected by chronic hypertension, renal disorders and/or proteinuria before pregnancy and all were normotensive before 20th week of pregnancy. All patients in the study were non-smokers. An informed consent from all studied patients was obtained for peripheral blood sampling.

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Active VEGF-A and soluble VEGFR-1 determination

Active VEGF concentrations were measured in the maternal serum samples using commercially available enzyme-linked immunosorbent assay (human VEGF-A kit BioLISA - Bender MedSystem, Vienna, Austria). The soluble Vascular Endothelial Growth Receptor 1 (sVEGFR-1) concentrations in maternal serum were evaluated using a sandwich ELISA assay (human sVEGFR-1 sandwich ELISA kit Bender MedSystem, Vienna, Austria). Assay procedures were followed according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean +/- SD. All calculations were performed on the basis of the computer programming Statistica v.8 (StatSoft, Poland). Analysis of variance (ANOVA) tests were used to test differences between four independent groups. A statistically significant effect in ANOVA was followed up with follow-up post hoc test in order to assess which group is different from which other groups. A *p*-value of less than 0.05 was considered to be significant.

RESULTS

There were no statistically significant differences in gravidity, parity, maternal age and height in patient profiles between groups. Creatinine and urea levels were normal in all patients. Maternal weight and BMI were lower in the group of patients with pregnancy complicated by intrauterine fetal growth restriction than in the control group, and also in comparison with both groups of preeclamptic patients. The values of maternal weight and BMI were higher in group of preeclamptic patients without IUGR. Systolic and diastolic blood pressure and mean arterial blood pressure were significantly higher in the study groups of preeclamptic pregnant women than in the control group and in the pregnant patients with isolated growth restricted fetus. These differences were statistically significant ($p<0.00001$). Lower gestational age and birth weight of infants were observed in the both groups of preeclamptic patients and in the group of normotensive women with pregnancy complicated by intrauterine growth restricted fetuses in comparison with the control subjects. The birth weight of infants was the lowest in the PI group. However, the birth weight of infants in the group of patients with pregnancy complicated by isolated IUGR was also lower than in the P group in spite of a higher age of gestation in the IUGR group.

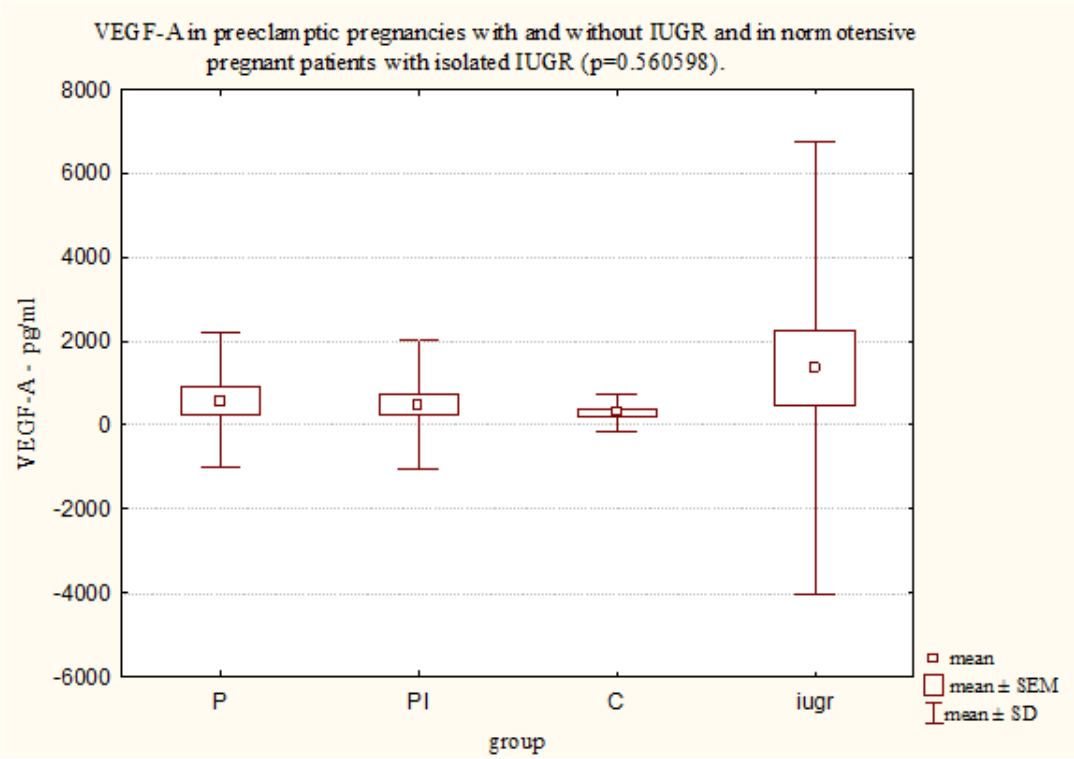

Figure 1: Analysis of VEGF-A in studied groups of pregnant women with pregnancy complicated by preeclampsia and/or IUGR.

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The pregnant normotensive patients with isolated IUGR revealed higher levels of serum aVEGF-A in comparison with the control subjects and both preeclamptic groups. The levels of aVEGF-A were slightly higher in both groups of preeclamptic patients in comparison with control subjects. The highest levels of serum VEGF-A were found in normotensive patients with pregnancy complicated by isolated IUGR. However, the differences between all studied groups of pregnant patients were not statistically significant. The mean serum VEGF-A levels were 1356.38 ± 5404.13pg/ml in the iugr group and 580.89 ± 1607.1pg/ml in the P group, 499.235 ± 1544.92pg/ml in the PI group and 302.26 ± 443,06pg/ml in the control group.

The levels of VEGF-A were decreased in the patients with HELLP syndrome and in the subgroup of patients with pregnancy complicated by eclampsia. The mean values were 134.63 ± 51.30pg/ml (range from 32.53 to 210.54pg/ml) in patients with pregnancy complicated by HELLP syndrome and 209.54 ± 124.80pg/ml (range from 118.06 to 481.48pg/ml) in women with eclampsia.

The preeclamptic women revealed higher levels of sVEGFR-1 than healthy controls and women with isolated growth restricted fetuses without preeclampsia. The serum levels of sVEGFR-1 in normotensive patients with isolated IUGR were similar to values observed in the control group. The highest levels of sVEGFR-1 were found in preeclamptic patients with appropriate-for-gestational-age fetuses (the P group) but the difference between both preeclamptic groups of pregnant patients was not statistically significant. The mean values of serum sVEGFR-1 levels were 46.18 ± 148.63ng/ml in the P group, 26.02 ± 71.36ng/ml in the PI group, 2.67 +/- 7.62ng/ml in the iugr group and 4.46 +/- 12.51ng/ml in the healthy controls.

Observed findings lead to the conclusion that the increase in sVEGFR-1 is definitely related to preeclampsia**.** The levels of sVEGFR-1 were also higher in the patients with HELLP syndrome, but unchanged in patients with eclampsia when compared with healthy controls. However, the levels observed in these women were lower than those reported in other preeclamptic patients from the P and PI groups. The mean values of serum sVEGFR-1 were 11.88±13,99ng/ml (range from 0.376 to 40.275ng/ml) in patients with pregnancy complicated by HELLP syndrome and 4.61±5.20ng/ml (range from 0.688 to 16.0ng/ml) in women with eclampsia.

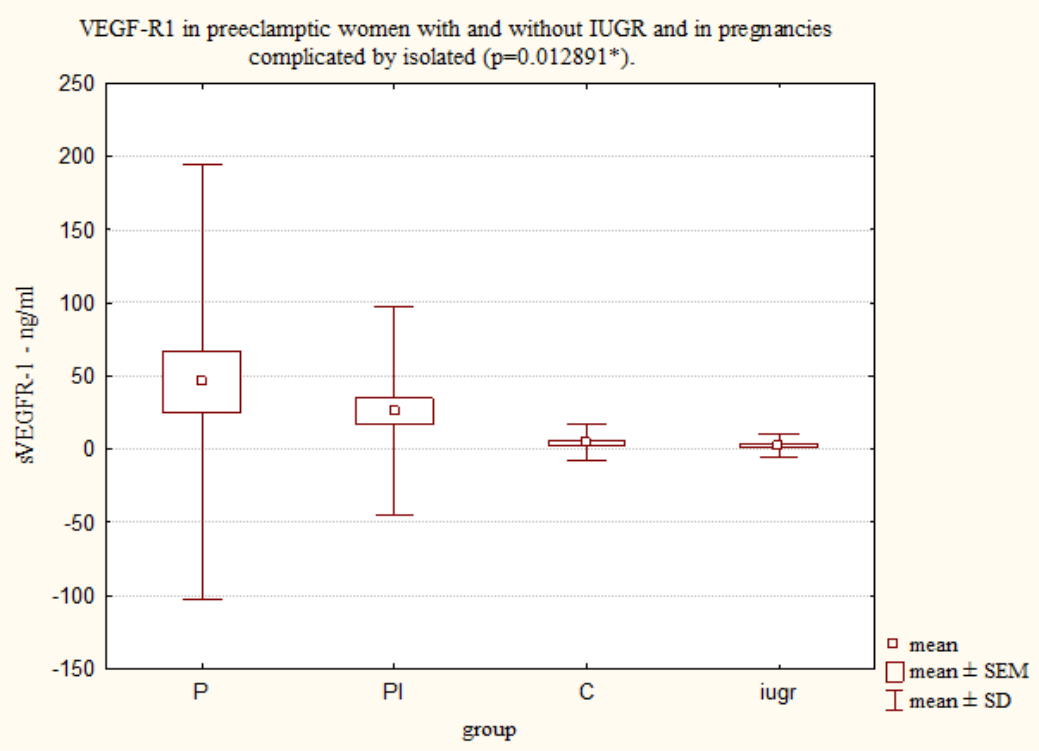

Figure 2: Analysis of sVEGFR-1 levels in serum of pregnant women with pregnancy complicated by severe preeclampsia and/or IUGR

There were no statistically significant correlations of VEGF-A or sVEGFR-1 neither with gravidity, parity, patients' weight, height, BMI nor with age of gestation and birth weight of infants in any studied groups of pregnant women.

The study revealed a positive correlation between serum sVEGFR-1 levels and diastolic blood pressure in the group of patients with pregnancy complicated by IUGR in the course of preeclampsia (correlation coefficient R=0.267258, p=0.042548). However, this correlation was not statistically significant in other groups of studied patients.

DISCUSSION

In present study serum levels of VEGF-A were higher in patients with pregnancy complicated by preeclampsia and/or IUGR in relation to healthy controls. But these differences were not statistically significant. The value of VEGF-A in women with pregnancies complicated by HELLP syndrome or eclampsia were lower.

Also, Anim-Nyame et al. [10] and Shaarawy et al. [11] showed higher levels of VEGF in preeclamptic women. Hunter et al. [12] found significantly elevated levels of VEGF in preeclamptic pregnancies compared to women with normal blood pressure, but also compared to women with pregnancy complicated by gestational hypertension.

Kupferminc et al. [13] observed higher levels of VEGF in patients with preeclampsia and its correlation between the severities of the disorder.

Hayman et al. [14] reported elevated circulating levels of VEGF-A in patients with pregnancy complicated by preeclampsia and its stimulatory effect on the endogenous prostacyclin production in vascular endothelial cells of small blood vessels. Similar observations have been obtained during the incubation of small uterine resistance vessels with VEGF-A. This response was similar to that observed after incubation with the serum of patients with preeclampsia.

Bosio et al. reported higher levels of VEGF in women with preeclampsia even before the clinical onset of preeclampsia, which were further elevated during the vasoconstricted state observed in this pregnancy disorder [15]. These authors speculated that hyperdynamic circulation characteristic of the latent phase of preeclampsia causes vascular shear stress, which in turn increases the levels of circulating VEGF and that its increase may represent an unsuccessful vascular rescue response [15]. It is possible that VEGF plays a cytoprotective role through a stimulating influence on vascular endothelial nitric oxide and prostacyclin production at elevated peripheral vascular resistance that occurs in preeclampsia [16,17].

It was also presented that VEGF inhibits apoptosis and leads to the increase of NO synthesis in vascular endothelial cells [18]. In an experimental model of renal disease in animals eightfold increase was found in excretion of stable NO metabolites in the urine and a doubling of eNOS expression within the glomeruli after administration of VEGF [18]. Plasma of patients with the uremic syndrome causes both reduction of nitric oxide synthase activity and abnormal L-arginine transport in vascular endothelial cell cultures, which results in reduced nitric oxide production [20]. According to these observations, elevated VEGF-A values obtained in present study may suggest an attempt to increase the production of nitric oxide and prostacyclin and to reverse or compensate for vascular endothelial cell dysfunction and severe vasoconstriction observed in preeclampsia and IUGR. Because VEGF normally acts as vasodilator, and the greatest stimulus for its release is hypoxia, its increase may be due to hypoxia, but also, taking into account the clinical condition and signs of preeclampsia, may indicate a failed attempt to rescue.

Different results were presented by Reuvekamp et al., who observed significantly lower levels of VEGF in preeclamptic pregnancies [21]. They suggested that these results may partially explain too superficial implantation of trophoblast in preeclampsia. Lower levels of VEGF in preeclampsia were presented also by Lyall et al., Maynard et al. and Livingston et al. [2,22,23].

The results similar to observed in present study in preeclamptic pregnancies, but different in normotensive pregnant women with isolated IUGR were presented by Bartha et al. [24]. In their studies, the values of VEGF in patients with isolated IUGR were similar to those observed in the control group. However,

they observed elevated levels of VEGF in the subgroup of 12 women with placental insufficiency. Lash et al. suggested that impaired fetal growth in women with normal blood pressure values are not associated with altered expression of vascular endothelial growth factor in the placenta [25].

The results presented in this study seem to confirm the significant role of VEGF-A in normal course of pregnancy. However, the findings of increased levels of sVEGFR-1, appear to be an important limitation, which may have a significant impact on the pathogenesis of severe preeclampsia and IUGR in the course of preeclampsia.

Similar results of increased sVEGFR-1 levels in preeclampsia and unchanged levels of sVEGFR-1 in pregnancy complicated by isolated IUGR were presented by other researches. [26,27]. But Diab et al. and Tsatsaris et al. reported elevated levels of sVEGFR-1 also in patients with pregnancy complicated by IUGR without preeclampsia [27,28]. Similar results were presented by Kupferminc and Sharkey [21,29].

It was suggested that sVEGFR-1 is a strong predictor of hypertensive disorders during pregnancy and that excess sVEGFR-1 associated with endothelial dysfunction is one of the main factors responsible for the pathogenesis of preeclampsia and the development of renal complications, glomerular endotheliosis and proteinuria, which occur in preeclampsia [29,30]. However, our findings elevated levels of VEGF-A and unchanged values of sVEGFR-1 in normotensive women with isolated IUGR may suggest a different pathomechanism leading to intrauterine fetal growth restriction in preeclamptic patients and in women with pregnancy complicated by IUGR without preeclampsia.

Taking into account the results of my previous study elevated levels of ADMA, an endogenous nitric oxide synthase inhibitor in patients with normotensive pregnancies complicated by isolated IUGR and the findings presented in this study may suggest that this may be an important restriction which causes inefficient NO synthase stimulation and it is one of the most important reasons for the lack of compensation of arising disturbances and possibly the failure of the increased production of VEGF, which does not bring the expected rescue [32,33].

In preeclamptic women both with and without IUGR significantly elevated levels of sVEGFR-1 can be regarded as one of the main causes leading to ineffective rescue response made by VEGF-A. However, it also cannot be excluded as one of the main existing pathomechanism in preeclamptic pregnancies, which involves the elevated serum asymmetric dimethylarginine. Moreover, since VEGF is a potent vasodilator and angiogenesis mediator, its reduced levels in pregnancy complicated by HELLP syndrome or eclampsia seem to confirm vascular endothelial dysfunction and abnormalities in maternal and fetal circulation, leading to organ failure. The observed reduced levels of VEGF-A in patients with HELLP syndrome or eclampsia seizure seem to be also responsible for additional stronger vasoconstriction of arterioles and weakened vasodilatation. It seems possible that in patients with HELLP or eclampsia endothelial dysfunction was so extremely severe that the mechanism causing the increased synthesis of VEGF has been exhausted.

Apart from the basic angiogenic activity the VEGF has neurotrophic and neuroprotective activity both in the central and peripheral nervous systems [34]. VEGF stimulates the growing maturation of new neurons in the hypoxic area; however, large doses of VEGF have primarily proangiogenic activity that can lead to swelling of the area covered by hypoxia [34]. Perhaps these disturbances are crucial in eclampsia seizures.

CONCLUSION

These findings suggest the importance of normal VEGF-A levels for the physiological course of pregnancy and confirm the causal role for sVEGFR-1 in the etiopathogenesis preeclampsia. Increased sVEGFR-1 levels in pregnancies complicated by severe preeclampsia may reflect the angiogenic disturbances in this pregnancy disorder and appear to be an important limitation, which may have a significant impact on the pathogenesis of severe preeclampsia and IUGR in the course of preeclampsia, but not in normotensive pregnancies complicated by IUGR. In conclusion, presented results define IUGR in the course of preeclampsia and “pure” IUGR as different pathologies.

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