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Evaluation of the vascular endothelial growth factor (VEGF-A) in preeclamptic pregnancies with intrauterine normal and growth retarded fetus

Preeclampsia complicates 5–10% of all pregnancies and is a major cause of maternal and fetal mortality and morbidity (1, 2). Pregnant patients with preeclampsia, apart from increased vascular tone, hypertension, proteinuria, enhanced platelet aggregation and decreased intravascular volume due to increased endothelial permeability, reveal pathological changes due to decreased perfusion in many organs (uterus, kidney, brain, placenta) (1, 2). Disturbances in vascular endothelium and in placental and uteroplacental vascular system development might be the primary cause of preeclampsia.

VEGF is a key survival factor for the vascular endothelium and it is important for maintenance of homeostasis in the vascular endothelial cells (1). VEGF increases vascular permeability, inhibits apoptosis, and mediates endothelium dependent vasodilatation as it exerts a long term stimulatory effect on endothelial NO generation by increasing endothelial NO synthase (3). Angiogenesis is a critical process for growth and development; it is reflected in IUGR. VEGF is essential for these processes (3–7).

It has been speculated that vascular endothelial growth factor (VEGF), that causes endothelial cell alteration is involved in the natural history of preeclampsia (4–6).

VEGF, a 45-kDa disulfide-linked homodimeric glycoprotein, induces endothelial cell growth *in vitro* and angiogenesis *in vivo*, and is a potent factor in increasing microvascular permeability (7). VEGF promotes neovascularization, reduces blood pressure, and is crucial in the formation and maintenance of the glomerular filtration barrier. VEGF-A is a major regulator for angiogenesis, stimulates inflammation, tumor growth and metastasis at least partly in a macrophage-dependent manner.

VEGF is required to support the proliferation and repair endometrium during the menstrual cycle, and provides a richly vascularized, receptive endomerium for implantation and placentation. VEGF is produced in several organs including the ovaries, uterus and placenta (7). It seems that decidualization might contribute to the induction of VEGF, and thus provide neovascularization of the feto-placental structure.

Angiogenesis is vital for the trophoblast invasion into spiral arteries, a key process in the normal placental development. Potent angiogenic growth factors, i.e. vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are probably responsible for normal trophoblast proliferation, migration, and invasion, and low levels of VEGF and PlGF, or antagonists to VEGF and PlGF, are plausible mediators of preeclampsia.

The aim of this study was to evaluate the maternal Vascular Endothelial Growth Factor A (VEGF-A) serum levels in pregnancies complicated by preeclampsia with and without intrauterine growth retardation (IUGR) and in normotensive pregnancies.

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 2 occasions 6 hours apart with proteinuria >2 g in a 24-hour urinary protein excretion.

The infant birth weight below the 10th percentile for gestational age was classified as intrauterine growth restriction. In all our patients from PI group asymmetric IUGR were observed.

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 VEGF-A concentrations were estimated using a sandwich ELISA assay according to the manufacturer's instructions (human VEGF-A 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 normal distribution of data, and equality of variance by 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

There were no statistically significant differences in gravidity, parity and maternal age 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 vs 26.671 +/- 3.841 years in the control group.

There were lower gestation ages in both preeclamptic groups in comparison with the healthy controls. But these diffecrences 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 asymmetric 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.

Pregnant women with severe preeclampsia had lower maternal serum VEGF-A concentrations than the normotensive controls. The mean values of maternal VEGF-A were 190.26 +/- 107.46 pg/mL (range from 118.26 to 695.48 pg/mL) in group PRE compared with 219.64 +/- 93.08 pg/mL (range from 136.44 to 487.24 pg/mL) in the control group respectively. This difference was not statistically significant (p=0.073).

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 concentrations of maternal serum VEGF-A were lower in both study groups in comparison with the control group. But these differences were not statistically significant (p=0.1012 and p=0.1236 respectively for the group P and PI versus healthy controls). The VEGF-A concentrations were lower in preeclamptic patients with normal intrauterine fetal growth in comparison with pregnant women with preeclampsia complicated by IUGR. But this difference was not statistically significant (p=0.9552). The mean values of maternal serum VEGF-A were 175.02 +/- 50.50 pg/mL (range from 123.10 to 315.52 pg/mL) in group P compared with 204.73 +/-142.22 pg/mL (range from 118.06 to 695.48 pg/mL) in group PI Fig. 1.

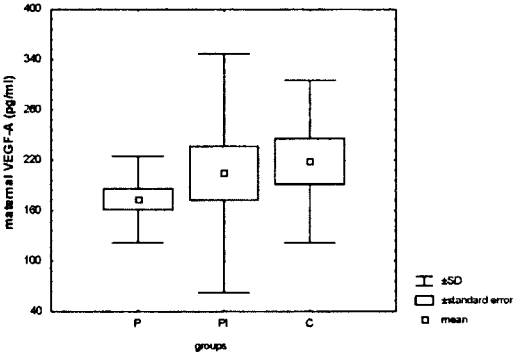

Fig. 1. Maternal VEGF-A in the studied groups of patients

DISCUSSION

VEGF concentrations have been measured in the maternal circulation by different investigators during normal and preeclamptic pregnancies (8). Conflicting results were presented. Several authors revealed elevated concentrations of the vascular endothelial growth factor in plasma of women with preeclampsia, while other presented decreased VEGF concentrations.

Our study revealed decreased maternal VEGF concentrations in both groups of studied preeclamptic women, with and without IUGR, compared with the healthy controls. The lowest value of VEGF-A was found in the group of patients with preeclampsia without IUGR.

Also Reuvekamp et al. (9) found significantly decreased serum concentrations of VEGF and PlGF in preeclamptic pregnancies compared with the pregnant controls. They suggest that this

selective deficit of angiogenic growth factors might partly explain the shallow placentation found in this pregnancy complication. Suppressed VEGF concentrations in preeclamptic pregnancies were also shown by Lyall et al. (10).

Similar results were presented by Maynard et al. (1), who observed reduced concentrations of free VEGF in the maternal serum in the preeclamptic women compared with normotensive pregnancies. VEGF and PlGF cause microvascular relaxation of renal arterioles *in vitro* in rats, which is blocked by sFlt-1 (1). Also Livingstone observed decreased maternal serum concentrations of the vascular endothelial growth factor in patients with severe preeclampsia (11).

Different results were presented by Bartha et al. (12) who observed significantly higher concentrations of maternal VEGF in the preeclamptic patients than in the healthy pregnant women and in normotensive pregnant women with fetal growth retardation, although the VEGF concentrations were increased in women with placental insufficiency defined as a pulsatility index in the umbilical artery greater than the 99th percentile for gestation. They concluded that the evidence is not strong enough to suggest any role of VEGF in IUGR. Similar suggestion that abnormal fetal growth is not associated with the altered expression of vascular endothelial growth factor in placenta was presented by Lash et al. (13). But it should be mentioned that in Bartha's study the women with pregnancies complicated by IUGR were normotensive (12).

Elevated concentrations of VEGF in preeclamptic women were presented by Shaarawy et al. (14). They suggested that elevated concentrations of VEGF in maternal serum observed in their studies, could confirm the existence of vascular reactivity and endothelial disturbance in preeclampsia and that the measurement of angiogenic factors such as VEGF and angiogenin might be a marker of severity of this disease and fetal outcome (14).

Also Hunter (8) observed significantly elevated concentrations of VEGF in preeclamptic pregnancies compared with the normotensive group and with the gestational hypertension and these elevated concentrations decreased within 24 hours after the delivery. Hayman et al. (5) found elevated concentrations of circulating VEGF in women with preeclampsia, and that VEGF increases the production of microvascular endothelial cell prostacyclin a dose-dependent manner, which is analogous to the acute effects of plasma from patients with preeclampsia. Similarily, in myographic studies, when myometrial resistance arteries are incubated with VEGF, their behaviour is similar to that found after incubation with plasma from patients with preeclampsia.

According to these observations decreased concentrations of VEGF in our study might be partly responsible for the lower prostacyclin, endothelial cell dysfunction and enhanced vasoconstrictive changes found in preeclamptic pregnancies.

Sharkey et al. (15) suggested that VEGF as a potent regulator of endothelial cell function and its increased concentration in women with preeclampsia indicated that VEGF might be involved in the maternal endothelial cell dysfunction associated with this condition. The increase in VEGF which is a potent regulator of microvascular permeability, may also contribute to the extravasation of plasma proteins and the subsequent development of proteinuria, both characteristic features of preeclampsia.

Anim-Nyame et al. (16) also observed higher concentrations of VEGF in preeclamptic patients compared with normotensive pregnant women and non-pregnant subjects. Baker et al. (4) suggested that elevated concentrations of the VEGF in patients with preeclampsia in their studies might suggest its role in the endothelial cell activation which occurred in this disease.

Bosio et al. (17) observed the increased concentration of VEGF in plasma before the clinical onset of preeclampsia and the elevated one during the vasoconstricted state in this disorder and they speculated that the hyperdynamic circulation characteristic of the latent phase of preeclampsia causes vascular shear stress, which in turn increases the concentrations of circulating VEGF. Because VEGF

normally acts as a vasodilator, its increase may represent an unsuccessful rescue response.

Bosio et al. (17) revealed the increased maternal vascular endothelial growth factor before the clinical onset of preeclampsia. Their concentration was further elevated during the vasoconstricted state observed in preeclampsia. But in early pregnancy concentrations of VEGF in women who had preeclampsia were not different from the concentrations found in other women during the first trimester of pregnancy (17). These authors suggested that the observed elevated concentration of VEGF, which is a potent angiogenesis growth factor, may therefore reflect the development of new blood vessles in the endometrium and trophoblast formation in response to the increased metabolic requirements (17).

Furthermore, the production of VEGF by vascular smooth muscle cells in the systemic circulation may be increased in response to estrogen (17). In this way VEGF may be involved in the initiation of the systemic vasodilatation perhaps due to the activity of nitric oxide (3). It is possible that VEGF may play a cytoprotective role through nitric oxide production (3) in the total peripheral resistance in preeclampsia.

On the other hand, Hefler et al. (6) reported no significant difference in serum VEGF concentrations between women with preeclampsia and healthy pregnant women.

Angiogenesis, which is a critical process for the growth and development, is altered in IUGR and VEGF is essential for these processes. Disturbances in the balance between vasoconstrictors and vasodilators and predominance of vasoconstrictors could be a cause of abnormal placental function and intrauterine growth retardation. Ahmed et al. suggested that the observed poor angiogenesis may explain the pathogenesis of IUGR (18). VEGF is required not only for proliferation, but also for the survival of endothelial cells and therefore it is important for angiogenesis (3).

According to the data from the literature and our results, decreased concentrations of VEGF in pregnancy complicated by preeclampsia with and without IUGR may suggest disturbances in the development of new blood vessels that result in the inadequate trophoblast proliferation into the maternal spiral arteries, endothelial dysfunction, disturbances in placental development and function, due to the inadequate fetal oxygenation and nutrition, which leads to IUGR.

Furthermore, as the vascular endothelial growth factor has a vasodilaltory effect on the resistance of isolated vessels, it seems that lower levels of VEGF may be responsible for lower vasodilatation and enhanced vasoconstriction of blood vessels and arterioles, which is characteristic of preeclampsia and intrauterine growth retardation.

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SUMMARY

The aim of this study was to evaluate the maternal Vascular Endothelial Growth Factor A (VEGF-A) serum concentrations in pregnancies complicated by preeclampsia with and without intrauterine growth retardation and in normotensive pregnancies. The study was carried out on 19 preeclamptic patients with severe preeclampsia (group P) and 20 patients with preeclampsia complicated by intrauterine growth retardation (group PI). The control group consisted of 14 healthy normotensive pregnant patients with singleton uncomplicated pregnancies (group C). Maternal serum VEGF-A concentrations were estimated using a sandwich ELISA assay. Our study revealed decreased maternal VEGF concentrations in both groups of studied preeclamptic women, with and without IUGR, compared with the healthy controls. The lowest value of VEGF-A was found in the group of patients with preeclampsia without IUGR. It seems that lower concentrations of VEGF may be involved in the pathophysiologic mechanism of intrauterine growth retardation and preeclampsia.

Ocena VEGF-A w ciąży powikłanej stanem przedrzucawkowym z adekwatnym wzrostem płodu i wewnątrzmacicznym zahamowaniem wzrostu płodu w przebiegu stanu przedrzucawkowego

Celem badań była ocena stężenia VEGF-A w surowicy krwi matczynej w ciąży powikłanej stanem przedrzucawkowym oraz w grupie pacjentek z wewnątrzmacicznym zahamowaniem wzrostu płodu w przebiegu stanu przedrzucawkowego oraz wśród kobiet ciężarnych z prawidłowym ciśnieniem tętniczym krwi. Badaniami objęto 19 kobiet ciężarnych z ciążą powikłaną ciężkim stanem przedrzucawkowym (grupa P) oraz 20 pacjentek z wewnątrzmacicznym zahamowaniem wzrostu płodu w przebiegu ciężkiego stanu przedrzucawkowego (grupa PI). Grupę kontrolną stanowiło 14 zdrowych kobiet ciężarnych z prawidłowym ciśnieniem tętniczym krwi i niepowikłanym przebiegiem ciąży (grupa C). Ocenę VEGF-A wykonano metodą ELISA. W naszych badaniach zaobserwowaliśmy obniżone stężenie VEGF-A w obu grupach kobiet z ciążą powikłaną ciężkim stanem przedrzucawkowym w odniesieniu do zdrowych kobiet ciężarnych. Najniższe wartości VEGF-A odnotowano wśród pacjentek z ciążą powikłaną stanem przedrzucawkowym z adekwatnym wzrostem płodu w stosunku do wieku ciążowego. Wyniki naszych badań sugerują, iż obniżone stężenia VEGF mogą mieć znaczenie w patofizjologii stanu przedrzucawkowego i wewnątrzmacicznego zahamowania wzrostu płodu.