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PLACENTAL GROWTH FACTOR IN MOTHER'S AND UMBILICAL CORD BLOOD IN PREGNANT WOMEN SUFFERING FROM TYPE-1 DIABETES MELLITUS

PLACENTNI ČIMBENIK RASTA U MAJČINOJ I UMBILIKALNOJ KRVI TRUDNICA OBOLJELIH OD DIJABETESA TIPA-1 I ZDRAVIH TRUDNICA

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Original paper

Key words: Placental growth factor, diabetic pregnancy

Summary. Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are key factors in physiological and pathological conditions of pregnancy. We investigated whether serum levels of PlGF in mother's and umbilical blood are different between healthy pregnant women and pregnant women suffering from type-1 diabetes mellitus. We performed a prospective study of 44 pregnant women with type 1 diabetes who did not have diabetic complications and of 34 healthy pregnant women of the adequate age and parity and the normal pregnancy course. Venous blood samples were collected from 8th weeks of pregnancy during the whole pregnancy, in distance from 4 weeks. Results are expressed as means±standard deviations. Statistical analysis was performed using ANOVA, Student t-test, linear regression, and non-parametrical Mann-Whitney U test. PlGF level in diabetic and healthy pregnant women from the 8th till the 15th week of pregnancy is comparatively low (23.16±4.94 pg/mL : 21.68 ±4.91 pg/mL), and after the 15th week of pregnancy it increases fast till the 31st week of pregnancy when the value is the highest (440.77±173.03 pg/ml : 390.41±138.07 pg/mL). After the 31st week of pregnancy there is a decrease of PlGF levels. Comparing PlGF values between the research groups in defined weeks of pregnancy no statistically significant difference was found. PlGF values in serum of healthy and diabetic pregnant women do not differ in same weeks of pregnancy. PlGF values in mother's and fetal serum immediately after the birth are a bit lower (but not statistically significant) in diabetic pregnant women in relation to a control group. A statistically significant correlation coefficient was found between PlGF level and a newborns weight and between PlGF and placenta weight. A statistically significant correlation coefficient was found between PlGF level of mother's blood and umbilical vein.

Izvorni članak

Ključne riječi: Placentarni čimbenik rasta, dijabetična trudnoća

Sažetak. Vaskularni endotelni čimbenik rasta (VEGF) i placentarni čimbenik rasta (PlGF) su ključni čimbenici u fiziološkim i patološkim trudnoćama. Cilj istraživanja je bio naći razliku razina PlGF-a u majčinoj i umbilikalnoj krvi između zdravih trudnica i onih koje boluju od dijabetsa tipa-1. Učinjeno je prospektivno istraživanje u 44 trudnice s dijabetesom tipa-1 i u 34 zdrave trudnice. Trudnice su bile istih dobnih skupina, pariteta i urednog tijeka trudnoće. Venska krv trudnica je skupljana od 8. tjedna trudnoće tijekom cijele trudnoće u vremenskim razmacima od 4 tjedna. Rezultati su prikazani srednjim vrijednostima ± standardne devijacije, a statistička analiza je učinjena sljedećim testovima: ANOVA, Student-t testom, linearnom regresijom i Mann-Whitneyevim U testom. Razina PlGF-a u dijabetičnih i zdravih trudnica je bila podjednako niska u vremenskom razdoblju od 8. do 15. tjedna trudnoće (23,16±4,94 pg/mL : 21,68±4,91 pg/mL), a nakon 15. tjedna dolazi do naglog povišenja razine sve do 31. tjedna trudnoće kada su vrijednosti bile i najviše (440,77±173,03 pg/ml : 390,41±138,07 pg/mL). Nakon 31. tjedna trudnoće dolazi do sniženja vrijednosti PlGF-a. Uspoređujući vrijednosti PlGF-a između istraživanih skupina u istim tjednima trudnoće nije nađena statistički znakovita razlika. Neposredno nakon poroda razina PlGF-a u majčinom i fetalnom serumu je bila nešto niža, ali ne i statistički znakovito niža u dijabetičnih trudnica u odnosu na zdrave trudnice. Nađene su statistički značajne korelacije između razina PlGF-a i porodne težine, između PlGF-a i težine placente i između razina PlGF-a seruma majke i seruma umbilikalne vene.

Introduction

The aim of a placenta is to ensure optimal conditions for fetal development. That is why normal development of placenta is important. The growth of placenta is regulated by a local production of growth factors which act by autocrine and paracrine mechanism in order to influence various cell functions.[1] A placenta is situated in such a way that it is influenced both by regulatory factors of mother and a fetus.[2]

The physiological process of placental development includes: (a) forming of blastocyst and trophoblast differentiation;[1] (b) blastocyst adhesion to decidua; (c) trophoblast invasion; (d) vasculogenesis and angiogenesis.

A trophoblast has to pass through the uterus epithelium in order to invade uterus, to reach blood vessels which will finally bath chorionic villi. The production of specific proteases and their inhibitors which is pre-

cisely regulated is important for controlled invasion of trophoblast in the uterus wall.[3,4]

On 21 day after ovulation, the major morphological features of placenta are developed (tertiary villi built of syncytiotrophoblast, cytotrophoblast, loose mesenchyme and differentiated blood vessels in it; anchored villi and peripheral cytotrophoblast covering).[3]

A cytotrophoblast which migrates into a decidual part of spiral arteries structurally changes these vessels and turns them into uteroplacental arteries. A cytotrophoblast enters a spiral artery in the myometrium area, and it can also affect distal parts of radial artery (*Fig. 1*). Morphological changes of spiral artery tunica media lead to: a) increased flow of mother's blood, which is the reason of dilation of these vessels in uteroplacental arteries; b) loss of muscular-elastic component, so uteroplacental arteries can no longer respond to changes within the autonomous nervous system.

Trophoblast stoppers close a bigger part of uteroplacental artery lumen till the end of the 11th week of development.[5] Intervillous area is filled by a transparent liquid, similar to mother's blood plasma, while fetal erythrocytes are in blood vessels of placental villi. In the 12th week of development, arteries are completely open, the increased amount of mother's blood enters the intervillous area, and so mother's erythrocytes appear together with blood plasma. From the 12th week, the pulsation flow of mother's blood starts, synchronous with heart beats.[6]

A placenta in its early stages of pregnancy secrets numerous angiogenic factors. However, a trophoblast in its early stages of development synthesizes big quantities of VEGFR-1 (Flt-1) receptor which inhibits angiogenic factors.[7,8] Turning of spiral arteries into uteroplacental blood vessels is completed in the middle of pregnancy, while the angiogenesis in placenta and in a fetus continues during the whole pregnancy. Numerous vascular endothelial growth factors (VEGF) participate in placental angiogenesis which are mostly synthesized by cytotrophoblast. Hildebrandt et al.[9] proved receptors for these factors on the endothelium of blood vessels and their number depends on the estrogens level.

Placental growth factor (PlGF) appears in syncytiotrophoblast and/or cytotrophoblast, in endothelium and in middle of blood vessel villi and in decidual cells. Hypoxia causes the VEGF concentration increase and decreased excretion of PIGF.[10,11]

Diabetes in pregnancy causes many problems both for a mother and a child. Thanks to good metabolic control of pregnant diabetics, antenatal and neonatal care, monitoring the fetal condition by cardiotocography and ultrasound, amniotic fluid analysis for proving a fetal lung maturity and more liberal attitude to the birth completion by caesarean section, the perinatal mortality has significantly decreased and is insignificantly higher in comparison to non-diabetic population.[12]

The purpose of this study was to examine the level of PlGF, in systemic and umbilical bloodstream and their progress during pregnancy in healthy pregnant women and pregnant women with type-1 diabetes and to determine differences between healthy pregnant women and pregnant women suffering from type-1 diabetes mellitus.

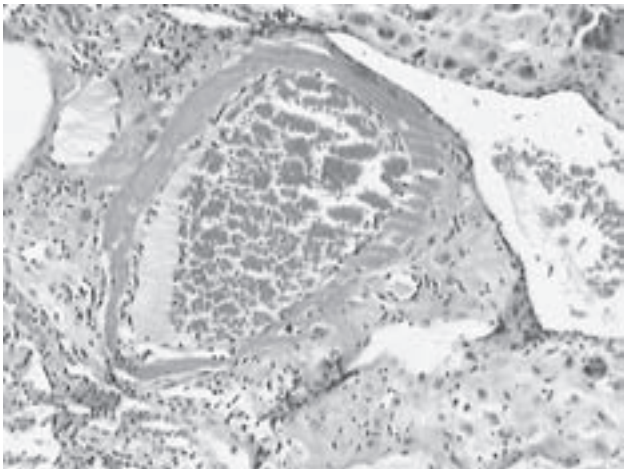

Figure 1. Utero-placental vessel in the myometrium showing adequate physiological change (H-E × 160)

Slika 1. Utero placentarna krvna žila u miometriju pokazuje adekvatnu fiziološku promjenu (H-E × 160)

Research design and methods

The research was prospective. The research group consisted of 44 pregnant women with type 1 diabetes who did not have diabetes complications and the control group of 34 healthy pregnant women of the adequate age and parity and the normal pregnancy course. The course of pregnancy was followed by usual antenatal care measures (regular obstetrical examinations, laboratory tests, ultrasound examinations, CTG). The pregnant women with type-1 diabetes treatment included advice about diet, adequate insulin therapy to achieve normoglycemia, ophthalmologist's and nephrologist's examination. All pregnant women with type-1 diabetes recieve two injections of medium-acting insulin every 12 hours for keeping basal insulin value in blood and also three injections of short-acting insulin immediately before main meals by using a syringe in the form of a pencil.

Pregnant women in diabetic and control group who developed some complication during pregnancy (hypertension/preeclampsia) were exempted from the sample. The gestation age is calculated from the first day of the last period, with the correction to the ultrasound age – the results of transvaginal ultrasound examination (within the routine examination of a pregnant woman). Venous blood samples were collected from 8th week of pregnancy during the whole pregnancy, in periods from 4 weeks. Immediately after the birth, the blood sample was taken from the umbilical vein and a placenta was weighed and sent to pathohistological analysis.

Serum was separated from the vein blood by the usual laboratory procedure (centrifugation at 4000 rpm

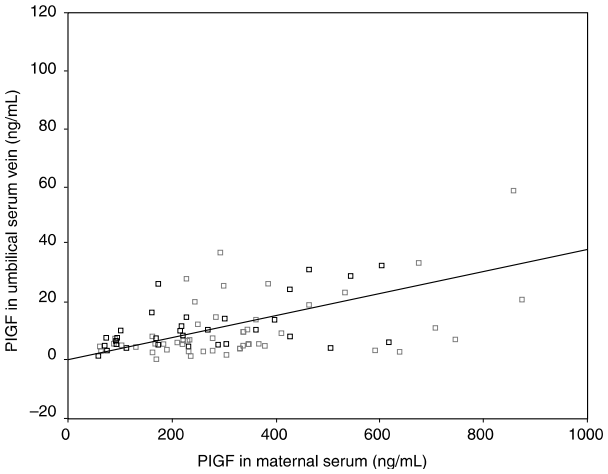

Figure 2. Linear correlation between PlGF levels of umbilical vein plasma and PlGF of mother's plasma (r=0.4; p<0.05)
Grafikon 2. Linearna korelacija između PlGF u plazmi umbilikalne vene i PlGF u plazme majke (r=0,4; p<0,05)

during 10 minutes, collected supersediment by pipette). Serum samples were stored at the temperature of –75°C till the moment of testing. Establishing PIGF values in samples was carried out by ELISA technique, commercial kit Quantikine (R&D Systems, Minneapolis, USA) for human PlGF. Umbilical vein blood samples (0.4 mL) were drawn into sterile syringes and analyzed for measurement pH, pO2 and base excess (BE) with an ABL5 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Statistical processing was done by a computer programme SPSS vers. 10.0, on a personal computer. Data are presented as the mean ± standard deviation. In the cross-sectional study, comparisons of clinical variables and immunoassay data in type-1 diabetes pregnant patient group were with the respective control group. Clinical variables were analyzed with the unpaired Student t test. Differences between two groups were estimated by Student t-test, by ANOVA with repeated measures and non-parametrical Man-Whitney U test. Comparisons of PlGF values in mothers and fetal serum and newborns weight and placental weight were made by regression methods and presented graphically. All tests were two tailed, and a probability value of p<.05 was considered to be significant.

Results

In a control group of totally 34 births, 22 births were completed vaginally and 12 by caesarean section. In research group of totally 44 births 15 birth were completed vaginally and 29 by caesarean section. All pregnancies were completed after the 37th week of pregnancy.

Newborn babies and placentas of pregnant women of both the research and the control group were of the similar weight (*Table 1*). There was not a significant difference in the age of pregnant women and the duration of pregnancy. Also, there was not a statistically significant difference in Apgar score after the first and after the fifth minute in two research groups.

Table 1. General data about pregnant women, their newborn babies and placentas of research groups.
Tablica 1. Opći podatci trudnica, njihove novorođenčadi i težine placenti istraživanih skupina.

	Healthy pregnant women group Skupina zdravih trudnica (n=34)	Type-1 DM group Skupina trudnica oboljelih od DM tipa 1 (n=44)	Statistical significance Statistička značajnost (p)
Age of pregnant women (years) Dob trudnica (godine)	29.32±3.7	29.4±4.8	n.s.
Weeks of completion of pregnancy Tjedni dovršenja trudnoće	38.5±1.2	38.3±1.3	n.s.
Placenta weight (g) Težina placente (g)	520.5±105	555.7±154	n.s.
Birth weight Porođajna težina (g)	3330.1±552.3	3565.8±603.6	n.s.
Birth length Porođajna duljina (cm)	50.1±3.5	50.4±2.3	n.s.
Apgar score after 1 min. Apgar indeks nakon 1 min	8.7±2.2	8.3±2.6	n.s.
Apgar score after 5 min. Apgar indeks nakon 5 min	8.9±1.1	8.4±2.2	n.s.

Table 2. PlGF levels (pg/mL) during pregnancy of research groups.
Tablica 2. Razine PlGF pg/mL tijekom trudnoće u istraživanih skupina.

	Healthy pregnant women group Skupina zdravih trudnica	Type-1 DM group Skupina trudnica oboljelih od DM tipa 1	Statistical significance Statistička značajnost (p)
PlGF levels between 8–15 week of pregnancy Razina PlGF između 8.–15. tjedna trudnoće	21.68±4.91 (n=34)	23.16±4.94 (n=44)	n.s.
PlGF levels between 16–20 week of pregnancy Razina PlGF između 16.–20. tjedna trudnoće	62.89±17.70 (n=30)	69.39±21.78 (n=39)	n.s.
PlGF levels between 21–26 week of pregnancy Razina PlGF između 21.–26. tjedna trudnoće	212.88±153.78 (n=32)	268.07±199.58 (n=41)	n.s
PlGF levels between 27–31 week of pregnancy Razina PlGF između 27.–31. tjedna trudnoće	390.41±138.07 (n=33)	440.77±173.03 (n=44)	n.s.
PlGF levels between 32 – 36 week of pregnancy Razina PlGF između 32. – 36. tjedna trudnoće	243.76±112.33 (n=31)	312.43±181.42 (n=42)	n.s.

Table 3. Level of PlGF (pg/mL) in mothers serum and umbilical vein immediately after the birth and pH, pO2 and BE in umbilical artery blood.
Tablica 3. Razine PlGF (pg/mL) u majčinom serumu i serumu vene umbilikalis neposredno nakon porođaja i vrijednosti pH, pO2 i BE u krvi umbilikalne arterije.

	Healthy pregnant women group Skupina zdravih trudnica	Type-1 DM group Skupina trudnica oboljelih od DM tipa 1	Statistical significance Statistička značajnost (p)
PlGF in mother's serum PlGF u majčinom serumu	249.6±193.1	178.0±191.0	n.s.
PlGF umbilical vein serum PIGT u serumu umbilikalne vene	17.6±21,9	12.0±18,2	n.s.
pH umbilical vein blood pH venske umbilikalne krvi	7.261±0.043	7.201±0.073	p=0.01
pO2 umbilical vein blood (kPa) pO2 venske umbilikalne krvi (kPa)	4.12±1.38	2.62±0.90	p=0.001
Base excess in umbilical vein blood (mmol/L) Eksces baze venske umbilikalne krvi (mmol/L)	–7.33±1.56	–8.81±2.34	p=0.01

Placental growth factor was determined in both diabetic and healthy pregnant women from the 8th week of pregnancy till the time of delivery (*Table 2*). P1GF level in diabetic and healthy pregnant women from the 8th till the 15 th week of pregnancy is comparatively low (23.16±4.94 pg/mL : 21.68±4.91 pg/mL), and after the 15th week of pregnancy it increases fast till the 31st week of pregnancy when the value is the highest (440.77± 173.03 pg/mL : 390.41±138.07 pg/mL). After the 31st week of pregnancy there is a decrease of P1GF level. At the time of delivery, P1GF level in the type-1 diabetes group is 178.0±191.0 pg/ml; P1GF level in the control group is 249.6±193.1 pg/mL. Comparing P1GF values between the research groups in defined weeks of pregnancy no statistically significant difference was found (*Table 2*).

A statistically significant correlation (r=0.4; p<0.05) between the newborn's weight and P1GF serum of mother was found. A statistically significant correlation (r=0.5; p<0.05) between the placenta weight and P1GF level of umbilical vein was found as well.

There was no difference in P1GF values in relation to birth completion method (P1GF level in mothers who gave birth by caesarean section was 244.9±188.14 pg/mL : 169.3±159.3 pg/mL of P1GF level in mothers who gave birth vaginally).

P1GF values in an umbilical vein were lower in relation to P1GF serum level of mothers *(Table 3)*. Comparing P1GF serum values of the mother and umbilical vein, a positive and statistically significant correlation coefficient was obtained (r=0.4; p<0.05). A statistically significant correlation (r=0.34; p<0.05) between the placental weight and PlGF level of maternal serum was found too. It was found statistically significant difference between two groups in pH, pO2 and BE in blood of umbilical vein as well *(Table 3)*.

Discussion

Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are key factors in physiological and pathological conditions. VEGF presents its activity through its receptors (tyrosine kinase) VEGFR-1 (Flt-1) and VEGFR-2 (KDR) which can be found in endothelial cells.[13–15] VEGF was localized on a trophoblast and macrophages of fetal and maternal origin. PlGF shows 53% of similarity with VEGF and is isolated from placenta and from media of bigger placental blood vessels.[14] PlGF presents its activity through its receptor VEGFR-1.

Two receptors, VEGFR-1 and VEGFR-2, are considered specific for endothelium. In placenta, endothelial and non-endothelial cells have VEGF receptors. VEGF is proved as a potent stimulator of the endothelial cell proliferation and creates plasminogen activator necessary for proteolytic destruction. PlGF has been proved as a weak chemotaxis stimulator of endothelial cells and proliferation in a physiological concentration of <100ng/mL. VEGF also induces microvascular permeability, while PlGF does not have any action, but intensifies VEGF activity in small amounts.[14,15] This different effect is explained by the fact that PlGF binds to VEGFR-1 but not to VEGF-2. By binding PlGF to VEGFR-1 occurs a non-branching angiogenesis.[16] VEGF and VEGFR-2 are high in early pregnancy and its concentration decreases with the duration of pregnancy, while PlGF and VEGFR-1 at the same time increase. VEGF and VEGFR-2 are involved in the first two trimesters of pregnancy in forming the rich branching capillary network of mesenchymal and immature villi, while PlGF and VEGFR-1 are involved in creating the long weakly branching terminal capillaries in the third trimester. VEGF stimulates extravasation of liquid and proteins; releases nitrate oxide.

In the first half of pregnancy there are only few placental villi, of a diameter of 170 μm. They are built of loose connective stroma which is on the outside covered by a two-layered trophoblast. Blood capillaries are small, situated in the central part of stroma, surrounded by stellate mesenchyme and Hofbauer's cells. Basic functions of a placenta are carried out through placental membrane, which separates mothers from fetal blood. Till the 12th week of pregnancy a placental membrane is built of syncytiotrophoblast, cytotrophoblast, mesenchyme and endothelium of villous blood vessels. By budding of intermediary villi trophoblast, bases of new villi appear in which stroma soon grows in and branches of blood capillaries. In comparison with villi from previous stages of placental development, terminal villi are more numerous and of smaller diameter (about 70μm in the second and about 40μm in the third trimester of

pregnancy). At the beginning of the second trimester, the number of cytotrophoblast and mesenchymal cells of stroma decreases and in the third trimester they mostly disappear.

The vascularisation of villi increases during pregnancy, while the stroma quantity significantly decreases. At the end of pregnancy it is difficult to notice Hofbauer's cells due to sinusoid dilatation of blood capillaries. Intercellular matter contains numerous collagen fibres which make the skeleton of the whole villus tree, from its basal part on a chorionic plate to the top of the smallest terminal villus. However, there are significant differences in the quantity of intercellular matter of stroma and the distribution of its components, depending on the level of branching a villous tree.

In mature intermediary and terminal villi the quantity of intercellular matter of stroma is significantly lower, while fibres are mostly situated circularly, in a thin layer around blood capillaries.

PlGF level in a serum of healthy and diabetic pregnant women increases by four times from the first to the third trimester. This PlGF increase influences the growth of placenta and maintenance of adequate placental circulation. After the 31st week of pregnancy of diabetic and healthy pregnant women, there is a decrease in PlGF level. In pregnant women with pre-eclampsia PlGF values in mother's serum change insignificantly from the 30th week of pregnancy till birth.[17] These values are lower than PlGF serum values of healthy pregnant women.[17–21,25] These differences in PlGF level can be explained by the oxygen level in placenta. It is proved that oxygen is the main regulator of balance between VEGF and PlGF. Hypoxia increases VEGF activity, while hyperoxia decreases it. Successful placentation leads to the low resistance of blood vessels due to spiral artery transformation. Early development of placenta occurs in hypoxia conditions, which leads to stimulation of cytotrophoblast proliferation and it inhibits trophoblast invasion.[22] The real blood flow is established between the 11th – 12th weeks of pregnancy. pO_2 increases and there is a trophoblast invasion as well as spiral artery changes, which leads to decreased resistance in placenta. High oxygen level leads to slowing down of angiogenesis. Hypoxia leads to decrease of PlGF, mRNA and proteins. PlGF stimulates trophoblastic DNA synthesis in the first trimester and increases the number of cells. In pregnant women with pre-eclampsia, mother's oxygenation is normal, but due to damages to uteroplacental circulation, placenta and fetus are hypoxic. In these conditions VEGF increases and PlGF decreases.[16,25] Comparing parameters of acid-base condition of fetuses born by healthy pregnant women and diabetic pregnant women, there was a significant difference in pH values, pO2 and BE. A more frequent chronic fetal hypoxia of diabetic fetuses might be the cause of lower (but not of statistically significant difference) PlGF vessels of mother, as well as of fetal development of blood vessels.

Since mother's diabetes is quite a frequent complication in pregnancy, changes on a placenta result in values in fetal and mothers serum.

The correlation of PlGF serum value of a mother and umbilical vein confirm the hypothesis that PlGF influences the development of uteroplacental blood vessels of mother, as well as of fetal development of blood vessels.

Placentas from pregnancies with badly supervised diabetes are enlarged, fat and plethoric due to fetal hypervolemia and mother's hyperglycemia.[23] In cases of low glucose intolerance in pregnancy, it was established that a placental weight was also increased (as well as fetoplacental weight ratio),[24] while in case of well supervised glycemia level, the placental weight does not differ from the normal one.[23]

Regarding the findings on a placenta from pregnancy complicated by diabetes it can be concluded that its macroscopic and histological features primarily depend on the quality of glucose level regulation in mother's blood and those changes which can be found in it are not specific, but are quite characteristic for diabetes.

Conclusions

PlGF increases from the beginning of pregnancy till the 31st week of pregnancy in both healthy and diabetic pregnant women, after which there is a decrease in PlGF values.

PlGF values in healthy and type-1 diabetes pregnant women do not differ in same weeks of pregnancy.

PlGF values in mother's and fetal serum immediately after birth are a bit lower (but not statistically significant) in diabetic pregnant women in relation to a control group.

A statistically significant correlation coefficient was found between PlGF level and a newborns weight and between PlGF and placenta weight.

A statistically significant correlation coefficient was found between PlGF level of mother's serum and umbilical vein serum.

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