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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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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 PI GF 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. PI GF values in serum of healthy and diabetic pregnant women do not differ in same weeks of pregnancy. PI GF 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 PI GF level and a newborns weight and between PI GF and placenta weight. A statistically significant correlation coefficient was found between PI GF level of mother’s blood and umbilical vein.

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-
progress during pregnancy in healthy pregnant women and pregnant women with type-1 diabetes mellitus.

**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 receive 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/pre eclampsia) 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
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, 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).

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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.

![Figure 2. Linear correlation between PIGF levels of umbilical vein plasma and PIGF of mother’s plasma (r=0.4; p<0.05)](image-url)
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). 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±23.16±4.94 pg/mL : 21.68±4.91 pg/mL), and after the 31st of pregnancy when the value is the highest (440.77±138.07 pg/mL). After the birth vaginally.

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

PlGF values in an umbilical vein were lower in relation to PlGF serum level of mothers (Table 3). Comparing PlGF 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. 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. 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. This different effect is explained by the fact that PlGF binds to VEGFR-1 but not to VEGFR-2. By binding PlGF to VEGFR-1 occurs a non-branching angiogenesis. 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.

The placental functions of a placenta are carried out through placental villi, of a diameter of 170 µm. They are 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
between the 11th–12th weeks of pregnancy. PO2 decreases and PlGF decreases.16,25 Comparing parameters of acid–base condition of fetuses born by healthy pregnant women and diabetic pregnant women, there is a decrease 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 cord serum 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-I 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.

References


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