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PLACENTAL GROWTH FACTOR AND APOPTOSIS AS EARLY PROGNOSTIC FACTOR OF PLACENTAL INSUFFICIENCY

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Summary

Aim is to determine and measurement of placenta growth factor (PLGF), to demonstrate apoptosis in the human placenta in normal and abnormal pregnancies would usefully predict subsequent placental insufficiency. Clinico-functional evaluation of fetoplacental complex and definition of placental growth factor (PLGF) activity in serum of 55 pregnant women has been carried out.

Histological verification of chronic placental insufficiency showed 3 types of patients: Compensatory chronic placental insufficiency, Subcompensatory and Subcompensatory with acute decompensation. Immunohistochemically a cross-sectional study was conducted to determine the expression of antybodies: marker of angiogenesis - CD34 , of apoptosis - P53 and antiapoptotic protein bcl2. Calculation of number of positive cells on 3-d visual areas of slide (200 magnification) took place.

Comparing information of indexes of level of PLGF in the whey of blood, indexes of CD34 positive vessels and apoptosis it is possible to suppose that the investigated data show the dynamics of pathomorphologic changes in a placenta at chronic placenta insufficiency and can be diagnostic criteria.

Key words: human placenta, abnormal pregnancies, angiogenesis, apoptosis.

Objectives. Placental insufficiency is main problem of obstetrics, neonatology and pathomorphology in ante- and perinatal period. Chronic placental insufficiency, also known as placental dysfunction or uteroplacental vascular insufficiency, is an uncommon but serious complication of pregnancy. It contributes to high rate of perinatal morbidity and mortality due to hypoxic condition of the fetus [1,4,10,12].

Essential requirements for successful gestation include the coordinated growth and differentiation of the placenta and the development of a functional placental vasculature [5,11]. However, relatively little have known factors for are responsible for regulating these functions.

Angiogenic growth factor that might be involved in regulating both vascular endothelial cell and trophoblast function is placental growth factor (PLGF)[10,11]. Receptors for PLGF include products of the fms-like tyrosine kinase (flt-1) gene which is expressed in several cell types including endothelial cells and trophoblast during normal pregnancy, and its expression has significantly decreased in obstetric complication presumed to be associated with placental bed hypoxia and ischemia [3,6,7,8]. Accordingly, PLGF can regulate proliferation in first trimester trophoblast, apoptosis in term trophoblast, and it up regulated vascular growth, maturation and permeability [11].

The hypothesis that apoptotic trophoblasts from pregnancies associated with fetal growth restriction caused by preeclampsia use exhibit enhanced expression of the proapoptotic proteins P53 and Bax and diminished expression of the antiapoptotic protein bcl-2 [2,9,15]. More apoptosis in the trophoblast layer of villi than in the trophoblast layer of villi from control pregnancies was found. The enhanced apoptosis correlated with up-regulation of P53, primarily in cytotrophoblast nuclei. The authors speculate that conditions predisposing to placental hypoxia lead to P53-mediated apoptosis in trophoblasts and thereby contribute to placental dysfunction [13,14,15].

However, scientific researches about intercommunication of PLGF, angiogenesis and apoptosis at chronic placenta insufficiency are fragmentary. Researches of these markers are needed for understanding not only of pathogeny of placenta insufficiency, but can be the early diagnostic marker of development of placenta insufficiency.

Aim is to determine and measurement of placenta growth factor, to demonstrate apoptosis in the human placenta in normal and abnormal pregnancies would usefully predict subsequent placental insufficiency.

Materials and methods. Clinico-functional evaluation of fetoplacental complex and definition of PLGF activity in serum of 55 pregnant women has been carried out. We measured PLGF levels in all samples using human PLGF immunoassay (R&D Systems). After the first Doppler examination, 1 venous blood sample (10 mL) was drawn from each woman into tubes containing EDTA. Immediately after sampling, plasma was separated by centrifugation at 4000*g* for 10 minutes and frozen at –80°C. Maternal plasma sFlt1 and PLGF were measured using a commercial ELISA (R&D Systems).

The presence of placenta insufficiency was fixed from data of Dopler examenation.

Histological verification of cross-sectional specimen of placenta with routine staining by hemotoxylin and eosin was carry out.

Immunohistochemically a cross-sectional study was conducted to determine the expression of antybodies: marker of angiogenesis - CD34 (Clone: QBEnd-10, Mouse anti-Human) - strong cytoplasmic reaction with membrane accentuation of virtually all the endothelial cells; marker of apoptosis - P53 (mAb clone DO-7) – the nuclear staining reaction; and antiapoptotic protein bcl2 (mAb clone 124**)** – the strong predominantly cytoplasmic staining. Calculation of number of positive cells on 3-d visual areas of slide (200 magnification) took place.

The pregnant women were subdivided into those:

1. with normal pregnancy (n=10);
2. with placental insufficiency (n=45) and Clinical characteristics of the patients are given in Table 1.

Table 1.

Clinical Data of the Patient Groups

Parameter	Normal outcome (n=10)	Patients With Chronic placental insufficiency (n=45)		
		Compensatory (n=15)	Subcompensatory (n=15)	Subcompensatory with acute decompensation (n=15)
Maternal age	18 to 37	20 to 37	24 to 37	23 to 37
Gestational age, wk	37 to 39	37 to 40	37 to 38	37-38

Results.

Current published reports have surveyed and our own work was review to highlight the expression, function and potential significance of PLGF, angiogenesis, and apoptosis at the human maternal-fetal interface. At histological research of placentas at women compensatory-adaptive processes prevailed with normal pregnancy (Fig.1). At immunohistochemical research the middle amount of the positively staining CD34 vessels in 10 terminals villi was 70,2 ± 3,51 (Fig.2). At immunohistochemical research with the markers of apoptosis P53 amount of positive cells in the epithelium of terminals villi were single, and expression of antiapoptotic bcl2 is total in the epithelium of villi (Fig.3, Fig.4, Tabl.2).

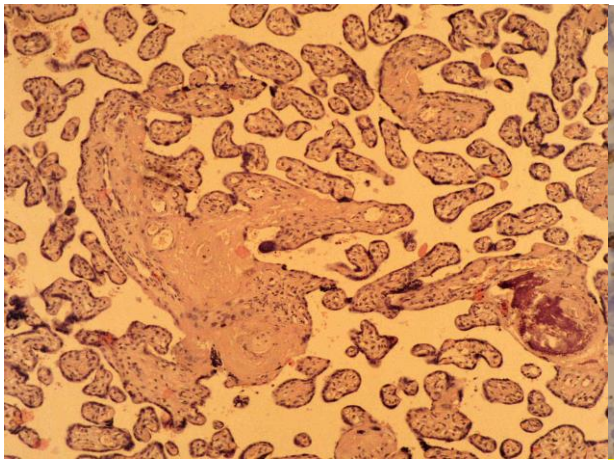

Fig.1. Control group. Compensatory-adaptative processes in normal placenta. H&E. Mg. 10^x.

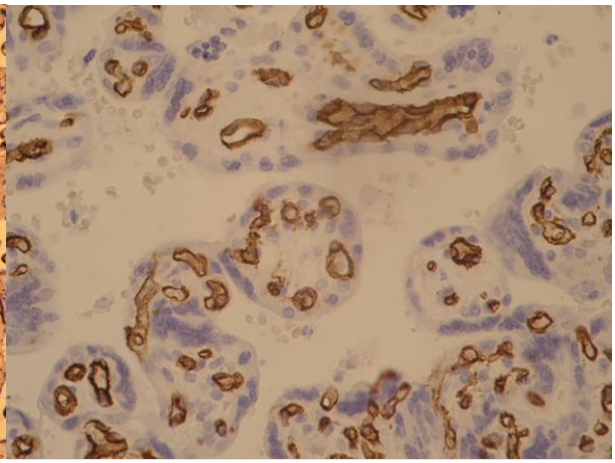

Fig. 2. Control group. IHR. Expression CD34. System of visualization FLEX. Mg. 40^x.

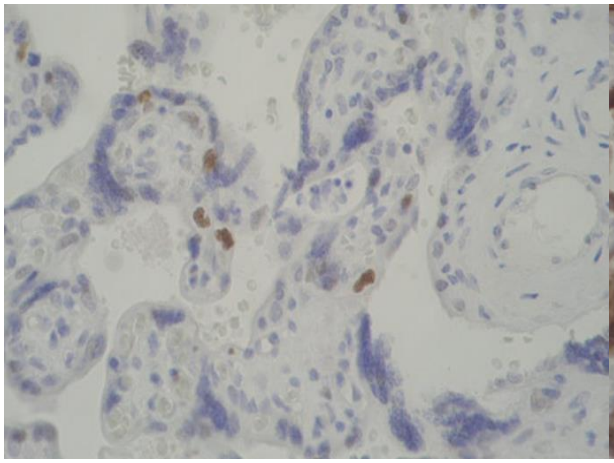

Fig. 3. Control group. IHR. Expression P53. System of visualization FLEX. Mg. 40^x.

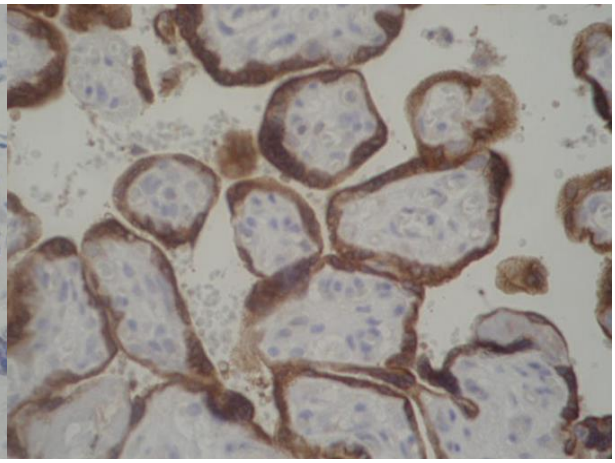

Fig. 4. Control group. IHR. Expression bcl2. System of visualization FLEX. Mg. 40^x.

Table 2.

Prediction of Chronic placental insufficiency

Parame-ter	Normal outcome (n=10)	Patients With Chronic placental insufficiency (n=45)		
		Compensatory (n=15)	Subcompensatory (n=15)	Subcompensatory with acute decompensation (n=15)
PLGF, pg/mL	225,3 ± 1,7	132,3±2,8*	97,4±0,6*	38,8±2,6*
CD34	70,2 ± 3,51	54,7±2,4*	37,9±1,7*	17,3±0,9*
P53	3,1±0,4	8,4±3,2.*	13±0,07*	24,3±1,2*
bcl 2	+++	+++	++	+
* P<0.05				

The high level of PLGF corresponded in the serum of blood (225,3 ± 1,7 pg/mL). In the group of the women with chronic placenta insufficiency marked compensated, subcompensated and decompensated types.

Indexes of PLGF are decreased (132,3±2,8 pg/mL) in the compensated chronic placenta insufficiency. Morphological picture is characterized by the moderato expressed sclerosis of villi, hyperemia of vessels, and deposition of fibrinoid masses in extravillous space. It is the variant of chronic placenta insufficiency most favorable in the prognostic criteria. Expression of P53 at this form increased (8,4±3,2). Indexes of expression of bcl2 did not change. The amount of vessels diminished - 54,7±2,4.

Subcompensated placenta insufficiency is characterized by decrease of PLGF indexes untill to 97,4±0,6 pg/mL. Histologic examination is characterized by the sclerosis of chorion's villi, fybroblastic reaction, absence of sufficient vascularity, proliferation of Langhan's cells, lymphocytic and histiocytic infiltration. The index of expression of P53 is increased (13±0,07), index of bcl2 is decreased, due to absence of expression in most pathologic villi. Expression bcl2 is fragmented. The amount of arterial vessels in the areas of false infarction is decreased (37,9±1,7) and some arteries are narrowed up to obliteration.

The subcompensated chronic placenta insufficiency with acute decompensation is unfavorable of chronic placenta insufficiency, which is characterized by the edema of villi stroma, decreased vascularity, proliferation of syncytiothrophoblasts, focal hemorrhages in intervillous space (Fig. 5). Index of PLGF in this group of pregnancy women is decreased until to 38,8±2,6 pg/mL. Expression of CD34 demonstrates the reduction of arteries– 17,3±0,9 (Fig. 6).

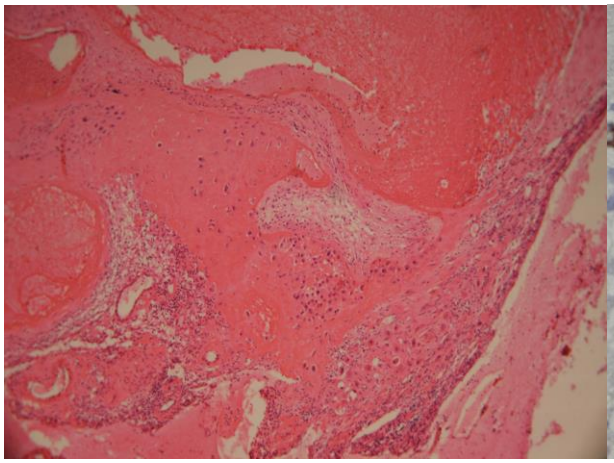

Fig.5. The subcompensated chronic placenta insufficiency with acute decompensation. Fibrinoid necrosis. Hemorrage. H&E. Mg. 10^x.

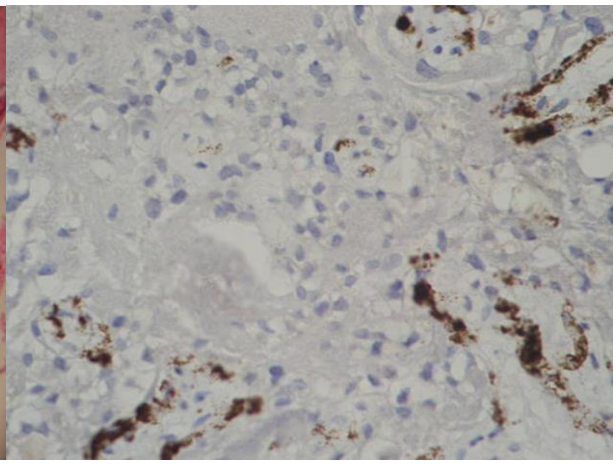

Fig. 6. Subcompensated chronic placenta insufficiency with acute decompensation. Expression CD34 in endothelial sells. IHR. System of visualization FLEX. Mg. 40^x.

Expression of P53 is increased in the areas of pathological changes of villi especially (24,3±1,2), and bcl2 in the same areas has more fragmented character (Fig.7, Fig.8).

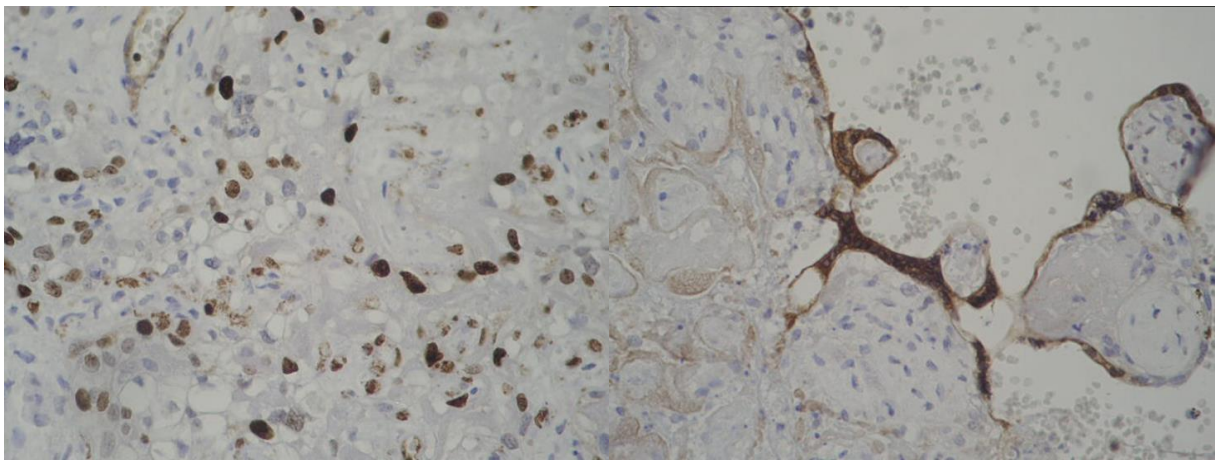

Fig. 7. Subcompensated chronic placenta insufficiency with acute decompensation. Expression P53 in epithelium of the villi. IHR. System of visualization FLEX. Mg. 40^{x}.

Fig. 8. Subcompensated chronic placenta insufficiency with acute decompensation. Expression bcl2 in epithelium of the villi. IHR. System of visualization FLEX. Mg. 40^{x}.

Discussion

Comparing information of indexes of level of PLGF in the whey of blood, indexes of CD34 positive vessels and apoptosis it is possible to suppose that the investigated data show the dynamics of pathomorphologic changes in a placenta at chronic placenta insufficiency and can be diagnostic criteria.

Ahmed A. et all showed, that limited data suggest that excess circulating soluble fms-like tyrosine kinase 1 (sFlt-1), which binds placental growth factor and vascular endothelial growth factor, may have a pathogenic role [11].

No significant difference in the percentage of apoptotic cells was observed comparing the group of normal pregnancies with those of chronic compensatory placenta insufficiency and there was a significant increase in the incidence of apoptosis in chronic subcompensatory placental insufficiency with acute decompensation. This results associated with researches in others authors [9,13]

Serum PLGF levels and number of vessels of terminal villi in patients with placental insufficiency are decreased according the type of chronic placental insufficiency. We can suggest that the growth factor has a role in the endothelial cell activation in the disease. Altered levels of PLGF implicated in the pathogenesis of placental dysfunction and PLGF mediates the endothelial cell activation that is involved in the pathogenesis of the clinical syndrome. Accordingly, PLGF can regulate apoptosis in trophoblast. Ahmed A. et all also concluded, that both early- and late-onset preeclampsia are associated with altered plasma levels of sFlt1 and PlGF. The alterations are more pronounced in early-onset rather than in late-onset disease [11].

The results indicated, that activity of PLGF characterizes initial changes in the fetoplacental complex and is early prognostic factor of possibility of duration placental insufficiency.

Conclusions

Many obstetrics complications are associated with aberrant trophoblast function and inadequate or dysfunctional vasculature within the developing placenta. Decreased PLGF is strongly associated with subsequent early development of placenta insufficiency. The ability of PLGF to influence trophoblast and vascular endothelial cells provides clear impetus for further studies to investigate the biological and clinical significance of PLGF in normal and abnormal pregnancies.

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