THE CHARACTERISTICS OF PLACENTAL ANGIOGENESIS-RELATED MARKERS IN EARLY AND LATE PREECLAMPSIA

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Hypertensive disorders of pregnancy are a leading cause of maternal morbidity and mortality worldwide, accounting for more than 70,000 maternal deaths each year. Of all maternal deaths, 10–15% are directly associated with preeclampsia and its complications.

It has been proven, in clinical and experimental researches that gestational endotheliopathy is a basic mechanism in the development of hypertensive disorders during pregnancy. Gestational endotheliopathy results into ischemia, hypoxia and oxidative stress, and plays the leading role in development of preeclampsia. Preeclampsia has been characterized by some investigators into 2 different disease entities: early-onset preeclampsia and late-onset preeclampsia. Early-onset preeclampsia (EOP) is usually defined as preeclampsia that develops before 34 weeks of gestation, whereas late-onset preeclampsia (LOP) develops at or after 34 weeks of gestation. Although the presenting features overlap, they are associated with different maternal and fetal outcomes, biochemical markers, heritability, and clinical features. To date, no review has analyzed the data focusing on early- versus late-onset preeclampsia.

EOP is the most severe clinical variant of disease occurring 5-20% of all cases of preeclampsia and is associated with neonatal morbidity and mortality. LOP occurring about 75-80% of all cases of preeclampsia; which are associated with maternal morbidity (metabolic syndrome, impaired glucose tolerance, obesity, dyslipidaemia, chronic hypertension), normal birth weight and normal placental volume.

In our previous study, EOP was strongly associated with adverse feto-placental conditions and severe complications. The cause of impaired feto-placental function may be the abnormal invasion of trophoblasts and remodeling of the spiral arteries, which can result in limited blood flow and lead to growth restriction and fetal distress symptoms.

Alterations in circulating angiogenic factors are associated with the diagnosis of preeclampsia and correlate with adverse perinatal outcomes during the third trimester. Angiogenesis-related factors, including sFlt-1 (soluble fms-like tyrosine kinase 1) and PlGF (placental growth factor), soluble endoglin (sENG), are produced by abnormal placentas in higher than normal quantities and released into maternal circulation and play an important role in placental dysfunction; altered levels are detectable several weeks before onset of pregnancy complications

The aim of the study was an analysis of differences between placental angiogenesis in EOP and LOP.

Material and Methods. In the study, women with singleton pregnancy who underwent prenatal assessment at the I-st maternity hospital, Vinnytsya, Ukraine from May 2018 to December 2021 were eligible for inclusion. Our investigation included 40 placentas after delivery among women with preeclampsia (main group) and 40 placentas after physiological delivery in somatically healthy women, who hadn`t complications during pregnancy (control group). Placentas in the main group were divided into two sub-groups (20 in each) – with EOP and LOP. Each group underwent both hystomorphometrical and immunohystochemical investigation with biomarkers CD23, VEGF and PP13. Expession of antigens of CD23, VEGF and PP13 was conducted by immunohystochemical method on generally accepted methodology with the decamouflage of antiangiogens in microwave oven or in citrate buffer (pH 6,0), on water bath during 30 minutes in serial paraffin cuts of placenta, by determination of monoclonal antibodies of class II Clone QBEnd 10 with system of visualization K 801221 EnVision FLEX (universal set EnVision Flex, High pH, Dako).

Microslides were investigated under the microscope Olympus BX 46 with illumination mode according to Keller with ×400 sampling, in order to receive general imagination about the results of immunohystochemical investigation. The quantitative estimation of the results was conducted by microphotos received by the mean of microscopic images fixation system, that consists of microscope Olympus BX 46, digital chamber Olympus UC 30, personal computer on base of Intel Pentium 4 and «Cells entry» software. Photos were made with ×40 sampling (eyepiece 10×, lens 40×), with complete closing of aperture diaphragm, with lifted capacitor in Photo mode and time of display 1/20 sec. Chamber sensitivity-maximal, size of the image-1280×1024 pixels and JPEG graphic picture size (normal). Photography was made from 5 fields of vision for each microslide. Estimation of the expression of investigated biomarker was conducted by systems of computer analysis of microscopic images Morphology 5.2.

After conducting the stages of reactions we estimated brown membrane and cytoplasmic colouring (for biomarker CD23, VEGF, PP13 (clons ab-1, TermoScientific, solution 1:200, Dako Autostainer Instruments). Estimation of expression levels was carried by semiquantitative method on such parameters, as degree of mark spread and colour intensity.

We estimated optical density and area of expression in the entervillious space of placentas. Calculation of relative area of expression was conducted as a relation between area, occupied by immune-positive cells, and general area of cells in vision field; it was expressed in percentage. Optical density of investigated objects was measured in standard units. First index was showing the expression of investigated marker population' cells, second – in separate cells.

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Results PP13 is located in syncitiotrophoblast of the villi of chorion and in multinuclear luminal trophoblast in transformed decidual spiral arterioles. PP13 is secreted by syncitiotrophoblast, and along with decreased levels of expressed PP13 gestational period was complicated by preeclampsia.

Positive immunohystochemical reaction to PP13 was determined in all samples of syncitiotrophoblast of villi of chorion. Investigations showed that expression of PP13 in sub-groups with EOP (1.54±0.13 cells in vision (CiV)) and LOP(3.78±0.22 CiV) was a significant lower ($p < 0.001$) comparing to control group (7.97±0.64 CiV). The smallest area of expression of PP13 biomarker in villi chorion of placenta was determined in subgroup with EOP, 2,5 times bigger it was in LOP ($p < 0.001$) (Figure 1 and Figure 2).

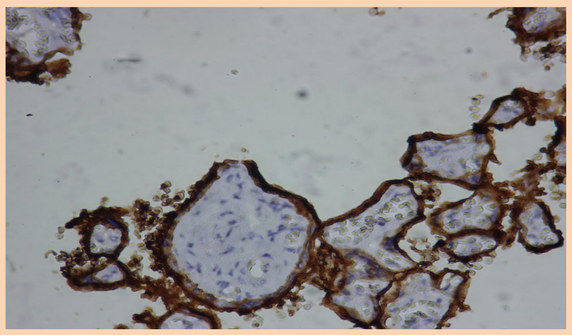

Figure 1. PP13 in placental tissue, in EOP

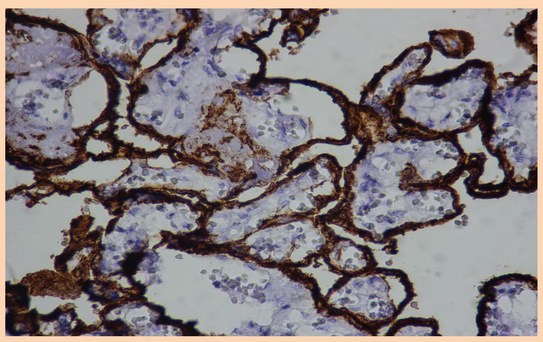

Figure 2. PP13 in placental tissue, in LOP

Estimation of the degree of vascularization of villi of chorion was conducted with the help of VEGF biomarker, which is expressed by endothelial cells. It`s an important parameter of functional activity of placenta. Positive immunohistochemical reaction to VEGF was determined in all samples of endothelia of the capillaries of the villi of chorion. Our investigation showed that expression of VEGF in sub-groups with EOP and LOP was a significant lower also ($p < 0.001$) comparing to a control group (12.45±0.82 CiV). Also results of expressed VEGF biomarker between EOP (2.56±0.32 CiV) and LOP (7.23±0.67 CiV) were significant difference ($p < 0.001$), though, the smallest area of expression of VEGF was determined in sub-group with EOP, it was bigger in group with late PE (Figure 3 and Figure 4).

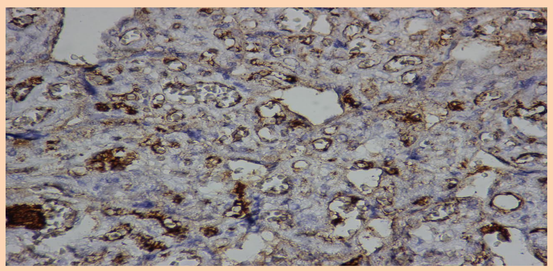

Figure 3. VEGF in placental tissue, in EOP

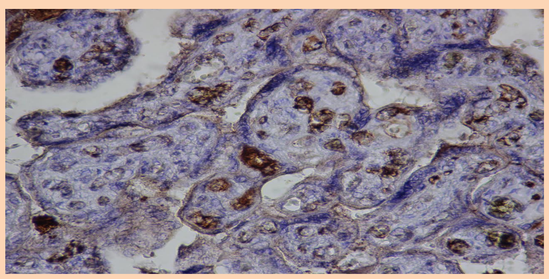

Figure 4. VEGF in placental tissue, in LOP

Expression of CD23 was negative in all samples in endothelia of the capillaries of the villi of chorion and cyncithiotrophoblast among placentas in early (0.18±0.02 CiV) late preeclampsia (0.34±0.04 CiV) and had the same significant authenticity ($p < 0.001$), including in relation to data with placenta after physiological pregnancy (0.52±0.05 CiV) (Figure 5 and Figure 6).

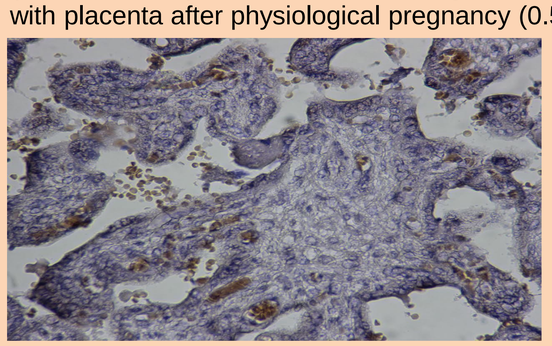

Figure 5. CD23 in placental tissue, in EOP

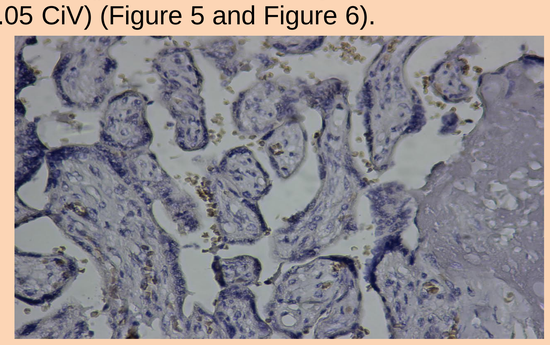

Figure 6. CD23 in placental tissue, in LOP

Conclusion. During immunohystochemical investigation of placenta of two main groups we found statistically significant decrease in expression of biomarker VEGF, PP13, and CD23 in endothelia of vessels of chorion, that can point out on violation of function of vascular system and increased vascular resistance of placental blood stream. In EOP decreased angiogenesis-related markers in placental tissue was more significant ($p<0.001$) than in LOP.

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