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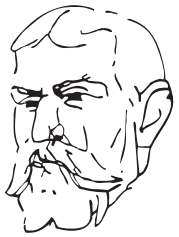

Memory of dr Władysław Biegański

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ORIGINAL ARTICLE

PLACENTAL BIOMARKERS: PP13, VEGF IN DIAGNOSTICS OF EARLY AND LATE PREECLAMPSIA

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ABSTRACT

The aim: To investigate role of CD23, VEGF and PP13 in diagnostics of early and late preeclampsia, and their benefit for prediction of preeclampsia.

Materials and methods: Investigation included 40 placentas from deliveries in women with preeclampsia (main group) and 40 placentas from physiological delivery in somatically healthy women, who had no complications during pregnancy (control group). Placentas in the main group were devided into two sub-groups (20 in each) – with early and late preeclampsia. Each group underwent both hystomorphometrical and immunohystochemical investigation with biomarkers CD23, VEGF and PP13.

Results: 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 early and late preeclampsia was a lot lower comparing to control group (normal pregnancies). Positive immunohystochemical 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 early and late PE was a lot lower comparing to a control group. Immunohystochemical reaction to CD23 was comperatively lower in all samples in endothelia of the capillariesof the villi of chorion and cyncithiotrophoblast.

Conclusions: Determined specialties of the expression of angiogenic factors (PlGF, VEGF, endoglin) and production of PP13, by altered expression of VEGF, PlGF in first trimester of pregnancy, which is associated with lowest production of PP13, accompanied by placental dysfunction and preeclampsia.

KEY WORDS: biomarkers, placenta, early and late preeclampsia, endotheliopathy, PP13, VEGF

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INTRODUCTION

Preeclampsia is one of the leading causes of maternal death – 60 000 annually, and it complicates from 5 to 8% of all pregnancies. It has been proven, in clinical and experimental researches that gestational endotheliopathy is a basic mechanism in the development of hypertensive disorders during pregnancy [1-4]. Gestational endotheliopathy results into ischemia, hypoxia and oxidative stress, and plays the leading role in development of preeclampsia. Preeclampsia that develops in terms less than 34 weeks is called early preeclampsia, after 34 weeks – late preeclampsia. Origin of early preeclampsia is related to inadequate invasion of trophoblast, hypoxia in placenta and release of biologically-active substances, that in future will influence endothelia [5]; in case of late preeclampsia it`s mostly connected with maternal cardiovascular system, that will influence integration of endothelia [1].

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. In vitro diagnostic tests for these biomarkers can improve early diagnosis and facilitate prediction of maternal and fetal outcomes [6,7]. Pooled information on placental perfusion (ultrasonography, mean arterial pressure), clinical characteristics, and biomarker levels (PlGF) can improve first-trimester prediction and preeclampsia diagnosis. Angiogenic factors with or without clinical characteristics can facilitate second-/third-trimester prediction of early-onset and late-onset preeclampsia. Analysis of angiogenic factors with or without uterine Doppler substantially improves sensitivity and specificity for predicting adverse outcomes and iatrogenic preterm delivery [6]. The imbalance of proangiogenic and antiangiogenic factors in circulation is thought to trigger the onset of PE by inducing microangiopathy in target organs such as the kidney, liver, or brain [7].

THE AIM

The aim is to investigate role of CD23, VEGF and PP13 in diagnostics of early and late preeclampsia, and their benefit for prediction of preeclampsia.

MATERIALS AND METHODS

Investigation included 40 placentas from deliveries in women with preeclampsia (main group) and 40 placentas from physiological delivery in somatically healthy wom-

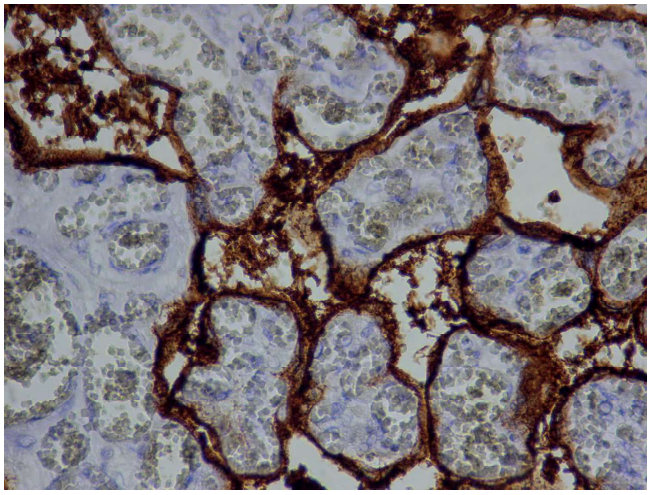

Fig. 1. PP13, control group, positive reaction to biomarker

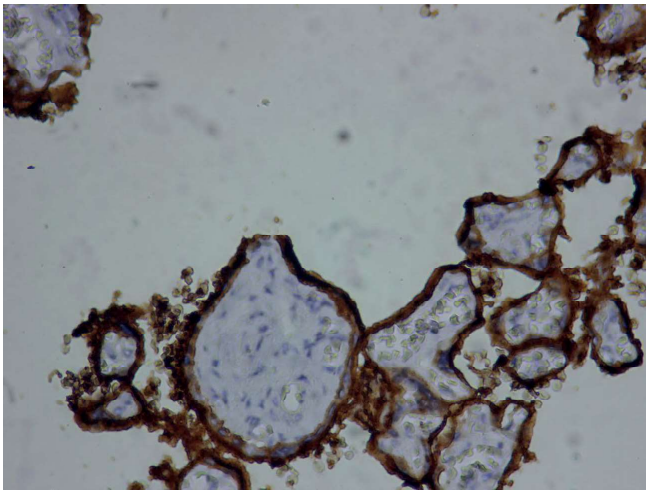

Fig. 2. PP13, early preeclampsia, low positive reaction to biomarker

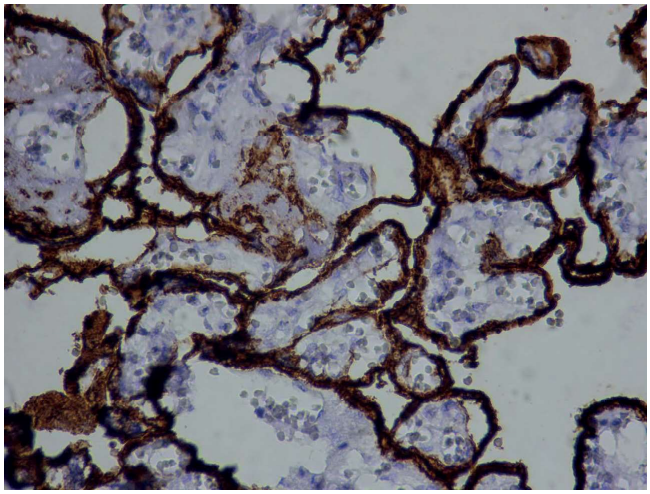

Fig. 3. PP13, late preeclampsia, moderate postive reaction to biomarker

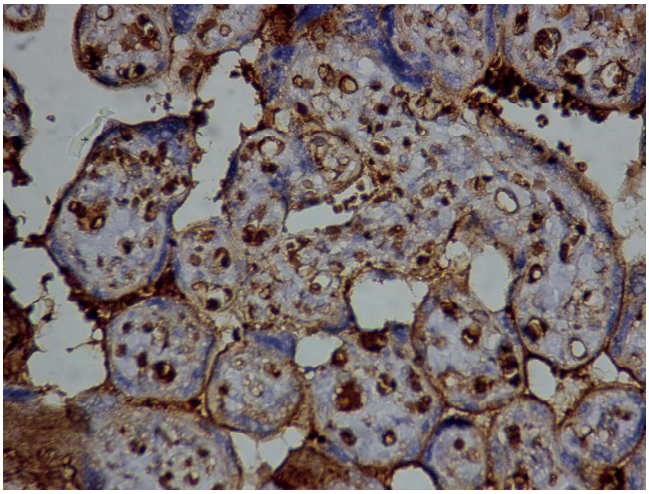

Fig. 4. VEGF, control group, the biggest area of expression

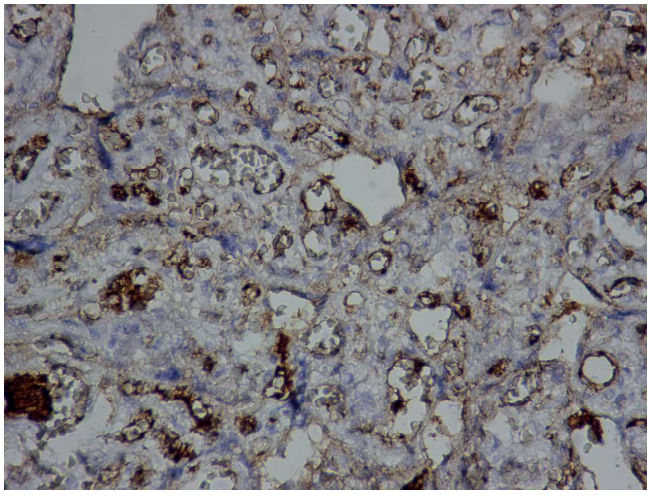

Fig. 5. VEGF, early preeclampsia, the smallest area of expression

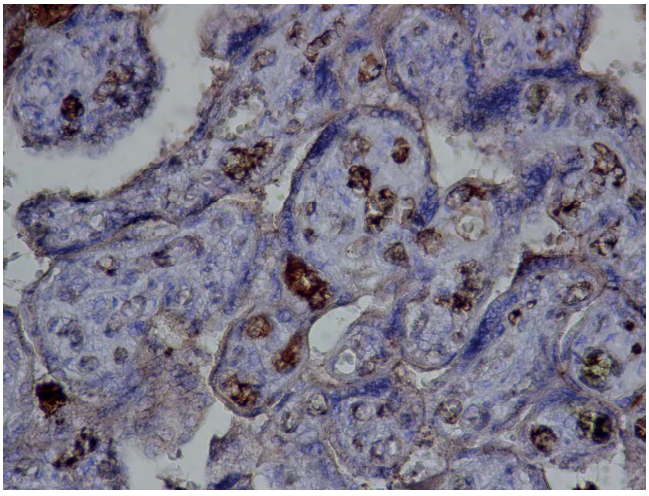

Fig. 6. VEGF, late preeclampsia, moderate index of area of expression

en, who had no complications during pregnancy (control group). Placentas in the main group were devided into two sub-groups (20 in each) – with early and late preeclampsia. 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

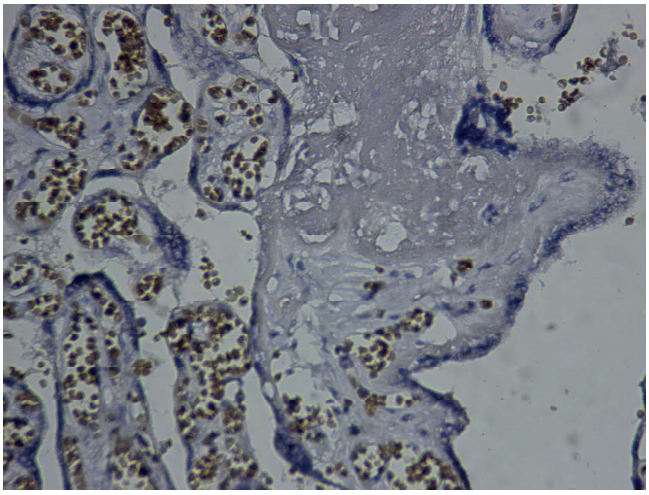

Fig. 7. CD23, control group

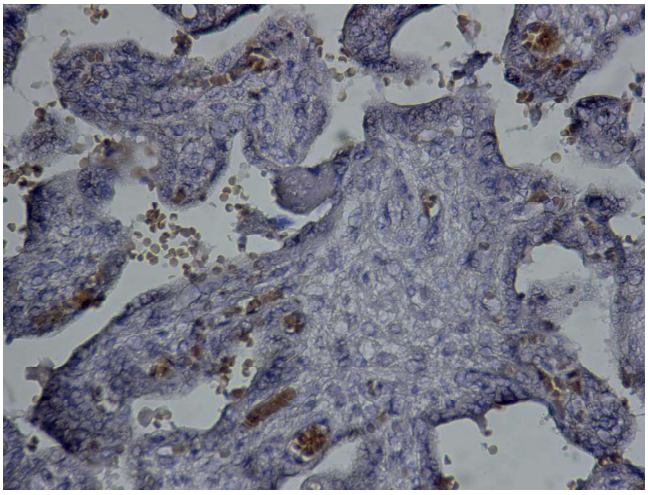

Fig. 8. CD23, early PE

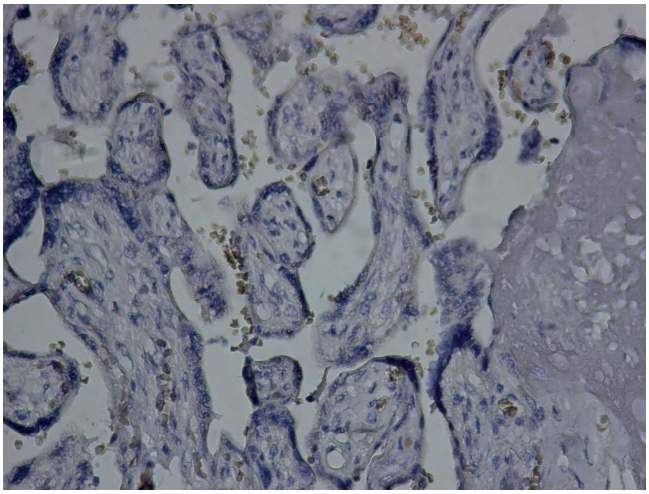

Fig. 9. CD23, late PE

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.

The degree of spreading was determined by counting of number of coloured cell nucleus comparing to total quantity of cells in percentage. Intensity of colouring was estimated by semiquantitative method: 1 point – weak colouring of nucleus, 2 points – moderate colouring of nucleus, 3 points – intense colouring of nucleus.

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 early and late preeclampsia was a lot lower comparing to control group (normal pregnancies). The smallest area of expression of PP13 biomarker in villi chorion of placenta was determined in subgroup with early preeclampsia, a little bigger it was in late preeclampsia and the biggest area was determined in control group (Fig 1,2,3).

Table I. Specialties of expression of PP13, VEGF, CD23 in early, late preeclampsia, and normal pregnancies

	Early PE	Late PE	Normal pregnancy	P
PP13	1.54±0.13	3.78±0.22	7.97±0.64	<0.001
VEGF	2.56±0.32	7.24±0.67	12.45±0.82	<0.001
CD23	0.18±0.02	0.34±0.04	0.52±0.05	<0.001

Table II. Placental biomarkers

Biomarker	Early PE (n=20)	Late PE (n=20)	Normal pregnancy (n=40)
PP13	2	4	16
VEGF	9	6	4
CD23	3	2	1

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 early and late PE was a lot lower comparing to a control group.

Though, the smallest area of expression of VEGF was determined in sub-group with early preeclampsia, it was bigger in group with late PE, and the biggest area of expression was in control group (Fig 4,5,6).

Expression of CD23. Immunohystochemical reaction to this biomarker was much lower in all samples in endothelia of the capillaries of the villi of chorion and cyncithiotrophoblast (Fig 7,8,9).

During immunohystochemical investigation of placenta of two main groups we found decreased expression of biomarker VEGF 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. The results testify to complicated motion of pregnancy, with the development of preeclampsia (Table I).

In early onset preeclampsia evaluated biomarkers PP13 (OR 0.2, 95% CI 0.03, 0.8), VEGF (OR 6.2, 95% CI 1.6, 23.7), CD23 (OR 6.9, 95% CI 0.7, 71.0). In late onset preeclampsia odds were for PP13 (OR 0.4, 95% CI 0.1, 1.3), VEGF (OR 3.9, 95% CI 0.9, 15.8), CD23 (OR 4.3, 95% CI 0.4, 50.9). Obtained data is significantly associated with early onset preeclampsia (Table II).

DISCUSSIONS

In normal pregnancies, serum levels of PP13 slowly rise with gestational age. Several studies have reported that decreased serum levels of PP13 in the first trimester increase the risk of subsequently developing preeclampsia. Measurement of PP13 levels as a first trimester screening marker for preeclampsia may provide an opportunity for identification of women destined to develop early-onset preeclampsia [8].

Moslemi Zadeh et al. conducted a prospective nested case-control study that recruited 1500 pregnant women and 100 women developed preeclampsia and represented the case group. Of 100 women with preeclampsia, 66 cases have mild preeclampsia, while 34 cases have severe pre-eclampsia. Serum PP13 levels along with PAPP-A were measured in the first and second trimesters and were significantly lowered in women who developed preeclampsia ($p< 0.001$). The cumulative value of all the four variables with cut-off points of 238.5 has sensitivity and specificity of 91.0% and area under curve of 0.968. The study concluded that measuring PP13 with PAPP-A in both trimesters of pregnancy is advantageous for the prediction of the incidence of preeclampsia [8,9].

Andraweera P.H. et al. summarized the current knowledge of the roles of the VEGF family in early placentation and of the abnormalities in maternal plasma and placental expression of angiogenic proteins in adverse pregnancy outcomes compared with normal pregnancy. PlGF and sFLT-1 in combination with other clinical and biochemical markers in late first or second trimester appear to predict early-onset preeclampsia with a high sensitivity and specificity. However, VEGF family proteins do not have sufficient power to accurately predict late-onset pre-eclampsia, small-for-gestational age pregnancies or preterm birth. Functional polymorphisms in these angiogenic genes are implicated in pregnancy complications, but their contribution appears to be minor [10].

According to Adi L. Tarca et al. investigation which included 90 patients with a normal pregnancy and 33 patients with early preeclampsia. Two to six maternal plasma samples were collected throughout gestation from each woman. As a result at 22.1-28 weeks of gestation, lower abundance of placental growth factor (PlGF) and vascular endothelial growth factor A, isoform 121 (VEGF-121), as well as elevated sialic acid binding immunoglobulin-like lectin 6 (siglec-6) and activin-A, were the best predictors of the subsequent development of early preeclampsia (81% sensitivity, FPR = 10%); the increase in siglec-6, activin-A, and VEGF-121 at 22.1-28 weeks of gestation differentiated women who subsequently developed early preeclampsia from those who had a normal pregnancy or developed late preeclampsia (sensitivity 77%, FPR = 10%) [11].

Rebecca E. Allen et al. identified 30 studies (65,538 women) for inclusion. Twenty four studies assessed pre-

eclampsia of any onset, 10 studied early onset preeclampsia and seven evaluated late onset preeclampsia (after 34 weeks of gestation). The biomarkers PAPP-A (OR 2.1, 95% CI 1.6, 2.6), PP13 (OR 4.4, 95% CI 2.9, 6.8), sFlt-1 (OR 1.3, 95% CI 2.9, 6.8), pentraxin (OR 5.3, 95% CI 1.9, 15.0) and inhibin-A (OR 3.6, 95% CI 1.7, 7.6) were significantly associated with any preeclampsia. The odds of early onset preeclampsia were significantly increased when the biomarkers PlGF (OR 3.4, 95% CI 1.6, 7.2), PAPP-A (OR 4.8, 95% CI 2.5, 22.5), PP13 (OR 7.5, 95% CI 2.5, 22.5), soluble endoglin (OR 18.5, 95% CI 8.4, 41.0) and inhibin-A (OR 4.1, 95% CI 1.9, 8.8) were abnormal. Two biomarkers, soluble endoglin (OR 2.1, 95% CI 1.9, 2.4) and inhibin-A (OR 1.9, 95% CI 1.4, 2.8) were significantly associated with late onset preeclampsia. Obtained data indicates that abnormal maternal blood biomarkers in early pregnancy are significantly associated with preeclampsia, particularly early onset disease [12].

CONCLUSIONS

Determined specialties of the expression of angiogenic factors (PlGF, VEGF, endoglin) and production of PP13, by altered expression of VEGF, PlGF in first trimester of pregnancy, which is associated with lowest production of PP13, accompanied by placental dysfunction and preeclampsia.

To our opinion biomarkers can not be estimated by themselves, it would be more useful to evaluate them together with Doppler monitoring and pregnancy anamnesis, along with other laboratory tests.

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Conflict of interest:

The Authors declare no conflict of interest.

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