MEDICAL UNIVERSITY - PLEVEN
FACULTY OF MEDICINE, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

DR. NIKOLA KALINOV POPOVSKI

Changes in levels of circulating biomarkers and echographic criteria in hypertensive conditions during pregnancy

ABSTRACT OF DISSERTATION

FOR THE AWARD OF EDUCATIONAL AND SCIENTIFIC DEGREE - DOCTORAL DEGREE

PLEVEN
2021

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FOR THE AWARD OF EDUCATIONAL AND SCIENTIFIC DEGREE - PhD IN SCIENTIFIC SPECIALTY “OBSTETRICS AND GYNECOLOGY”

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PLEVEN
2021

The research for the dissertation was done at the Clinic of Obstetrics and Gynaecology, University Hospital “Dr. Stranski" - Pleven, and at the Department of Clinical Laboratory, Clinical Immunology and Allergology, Sector of Clinical Immunology and Allergology at the Medical University - Pleven.

The dissertation contains 136 typed pages, 9 tables and 23 figures. The references include 334 titles, 14 of which are Cyrillic character set and 320 - in Latin character set.

The dissertation has been discussed and referred for defence by extended professoriate board of the Obstetrics and Gynecology Department, Faculty of Medicine, Medical University of Pleven.

The dissertant is a research assistant at the Department of Obstetrics and Gynecology at Medical University of Pleven, in the Clinic of Obstetrics and Gynecology, University Hospital “Dr. Georgi Stranski" EAD, town of Pleven.

The public dissertation defence will take place on ..... at… hours, hall..... , Medical University of Pleven, ‘St. Kliment Ohridski“ 1
The materials relevant to the dissertation defence are available on the university's website at www.mu-pleven.bg.

CONTENTS

Abbreviations used

1. **ELISA**- enzyme-linked immunosorbent assay
2. **WHO** - World Health Organization
3. **G.w. -** Gestation week
4. **ECM** – extracellular matrix
5. **GH** -gestational hypertension
6. **PE-** preeclampsia
7. **BMI-** body mass index
8. **MMPs**- matrix metalloproteinases
9. **TIMPs-** tissue inhibitors of metal proteinase
10. **ADAMs and ADAMTSs**- dysintegrin-metalloproteinases
11. **MT-MMPs**- Membranno-bound MMPs
12. **EOPE-** early onset of preeclampsia
13. **LOPE**- late occurrence of preeclampsia
14. **PIH**- pregnancy-induced hypertension
15. **NGAL**- neutrophilic gelatinase B-associated lipokaline
16. **IL** -interleukin
17. **TNF** - tumor necrosis factor
18. **sFLT-1**- fms-like tyrosine kinase-1
19. **PlGF**- placental growth factor
20. **sEng**- soluble endoglin
21. **HDP** - hypertensive disorders of pregnancy
22. **pro-MP**- pro-peptide dome-shaped MMP
23. **Norm-Preg**- normal pregnancy
24. **HTN-Preg**- hypertensive pregnancy
25. **RUPP-** reduced uterine perfusion pressure
26. **VEGF**- vascular endothelial growth factor
27. **COL** -collagen
28. **IUGR-** Intrauterine growth restriction
29. **PP-13**- placental protein 13
30. **PAPP-A** -plasma protein A, pregnancy-related
31. **IGF** -insulin-like growth factor

32. **UmA-** umbilical artery
33. **UtA-** uterine artery
34. **PI** -pulsatility index
35. **RI** - resistance index
36. **PCX-** podocalyxin
37. **MAP-** mean arterial pressure
38. **PN-** pulse pressure
39. **ACOG**- American College of Obstetricians and Gynaecologists
40. **ISSHP-** International Society for Study of Hypertension in Pregnancy
41. **IDO-** indleamine 2,3 dioxygenase

I. INTRODUCTION

The hypertensive disorders of pregnancy (HDP) are the most common medical complications affecting 5-10% of the pregnancies on global basis. Their early detection is critical for risk stratification and prevention of further complications. Preeclampsia (PE) complicates around 2-8% of pregnancies around the world [1a]. It is characterized by new hypertension (≥140/90mmHg) and proteinuria (0,3 g in 24-hour urine sample) or end organ dysfunction after 20th gestation week. PE is a major cause of maternal and perinatal morbidity and mortality. This is one of the most common hypertensive pregnancy disorders. Over 2/3 of the pregnancy hypertensive complications are classified as PE [1b]. PE is the most common complication of pregnancy and is among the main causes of maternal mortality in Bulgaria [1c,1d]. Approximately half of the cases of pregnant women with superimposed PE have been shown to have a history of arterial hypertension prior to pregnancy [1e]. Despite this fact, the PE has not yet been fully studied. There are increasing data on the involvement of biomarkers from the matrix metalloproteinases group and their inhibitors in the pathophysiology of preeclampsia [2].

Recent studies in the area of pregnancy hypertension have shown that the extracellular matrix (ECM) plays a very important role in their pathogenesis. The balance between the synthesis and degradation processes of its main components - Type I, III and IV collagen, the basal membrane and the proteoglyans [3] is of paramount importance. The proteins of the matrix metalloproteinases family (MMPs) are involved in both the precisely regulated degradation of the extra-cellulose matrix in physiological processes such as embryonic development, tissue reproduction and remodelling, and in pathological processes. These abnormal conditions are linked to the dysregulated degradation of the ECM. The matrix metalloproteinases play a key role in the degradation processes of many of these components of the EMC, while regulation of MMP activity is achieved through the tissue inhibitors of matrix metalloproteinases (TIMPs). Collagenases (MMP-8 and MMP-13) degrade Type I and III collagen-essential structural components of the uterine wall, while gelatinases (MMP-2 and MMP-9) are important vascular remodelling regulators in normal pregnancy by degradation of type IV collagen [4].

Normal pregnancy has been associated with pronounced haemodynamic and uterine changes that allow adequate blood flow and uterine growth for the growing foetus. The extracellular matrix has been reported to be capable of modulating the trophoblastic invasion and playing a key role in remodelling the decidua of the maternal-foetal interface. There is evidence that abundant placental transfer occurs during normal pregnancy and extensive conversion of spiral arteries to the decidua basis. The extravillous trophoblasts enter the

decidua and stretch the walls of the spiral arteries, replacing the endothelium and muscle wall, and creating expanded, low-resistance vessels that maintain sufficient blood flow and nutrient supply for the developing foetus. These pregnancy-related changes include significant remodelling of uterine placental and vascular structure. It can therefore be concluded that extracellular uterine matrix, placenta and vasculature are being reconstructed during physiological pregnancy.

The matrix metalloproteinases and their inhibitors play an important role in both normal and pathological pregnancy. Normal pregnancy has been associated with pronounced haemodynamic and uterine structure changes that allow adequate uterine-placental blood flow and uterine expansion for the growing foetus. [5]. The matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases are important regulators of the vascular and uterine-placental remodelling in normal pregnancy. In pregnancy hypertension, regulation between MMPs and TIMPs is dropped, MPM expression/activity is abnormal and both normal collagen remodelling of uterine ECM and of spiral arteries are disrupted. The above-listed processes favour generalized vascular damage, uterine-placental hypoperfusion and development of preeclampsia [6,7]. These mechanisms are associated with abnormal vasodilation, placental transfer and impaired MMP/TIMP expression/activity [8,9]. Abnormal expression of uterine-placental integrins, cytokines and MMPs is associated with apoptosis of invasive trophoblast cells, impaired remodelling of spiral arteries and reduced uterine perfusion pressure (RUPP).

MMPs and TIMPs activity can be impaired by endogenous factors, which may alter the MMP/TIMP ratio and may result in pathological process development. Understanding the role of MMPs and TIMPs in uterine-placental and vascular remodelling and function can therefore help to develop new approaches to predict and treat the hypertension status of pregnancy [113].

PE inevitably leads to renal dysfunction due to generalized vascular injury. It is well known that in preeclampsia podocyte and glomerular endothelial damage and dysfunction have been observed. Changes in renal perfusion have been shown to correlate with the severity of PE [5]. The podocalyxin is an indicator for glomerular damage and is also widely expressed on the surface of endothelial cells of many organs such as: heart, lungs and kidney [289, 290] and the kidneys [6a,6b]. The podocalyxin is expressed on the endothelial surface in the whole body and is likely to be released into the circulation during pregnancy as well, in connection with remodelling of the vessels. In PE, changes in renal structure and/or function inevitably occur due to podocyte damage and affected glomerular endothelium. The podocalyxin is a glomerular damage/dysfunction indicator and is also expressed in endothelial cells of many organs other than the kidneys [285, 286]. As PE is systemic

disease with generalized endothelial damage and multi organ involvement, there are increasing assumptions about the contribution of podocalyxin to the pathophysiology of preeclampsia with regard to abnormal organo- and vascular remodelling [7,8]. Therefore, the podocalyxin can be an innovative biomarker and a promising candidate for early indicator for endothelial damage and the risk of development of PE.

Non-invasive echographic methods are useful for examination of the changes in the structure and function of the heart, vessels and assessment of pregnancy. **Transthoracic echocardiography** is a safe, non-invasive technique for evaluation of the cardiac structure and function in pregnancy [266]. Modern ultrasound technologies can demonstrate discrete changes in cardiac geometry and function [267]. The use of echocardiography allows assessment of the myocardial without exposing the pregnant patient to cardiac catheterization or angiography or exposure to X-ray [268]. This is the only technique that can be used with complete safety, can be repeated at frequent intervals and is completely free of patient discomfort [269]. Echocardiography can provide accurate information on structural and functional abnormalities and contributes to understanding the pathophysiology of cardiac changes and haemodynamic consequences of pathological pregnancy.

Doppler ultrasound is an important method both in the assessment of foetal and placental circulation as well as in the prediction of foetal status and outcome of pregnancy [300,301]. It is well known that the doppler ultrasound is an invaluable tool for monitoring high-risk pregnancies. Doppler imaging allows non-invasive assessment of the uterine - placental circulation. The clinical value of doppler velocimetry of the uterine artery **UtA Doppler** is important in predicting adverse outcomes in women at high risk for the development of preeclampsia [302, 303, 304].

Doppler signs of increased placental vascular resistance are frequently associated with limited foetal growth and signs of impending asphyxia (3). The doppler velocimetry of the umbilical artery **UmA Doppler is** currently a routine part of foetal monitoring in high-risk pregnancy. Following initial technical difficulties, the doppler velocimetry of the uterine artery (UTA) has proved its value in predicting the outcome of a high-risk pregnancy, and in recent years it has been reported to be comparable to dopplerography of UmA in this respect [305]. In late pregnancy, abnormal UtA doppler and decreased venous volume of umbilical blood flow are considered to be indicators of reduced placental perfusion [306]. UtA Doppler screening during the first trimester and mid-pregnancy have also proved their value in predicting an adverse outcome in high-risk pregnancies [307a,307b]. Increased mean pulsatility index from the two uterine arteries above the 95th percentile in Doppler examination of pregnancies with foetus retardation were associated with the birth of a child

with a weight below the 10th percentile [308]. The placental vascular impedance is usually expressed by pulsatility index (PI), resistance index and systolic/diastolic relationship to UmA and UtA.

In order to reduce PE cases, it is important to identify high-risk women for early detection of PE and the implementation of prompt therapy. Despite the intensive search for indicators for early detection and prediction of PE, the clinical efficacy of these indicators showed low predictive value in practice. There are numerous potential biomarkers for preeclampsia-eclampsia but their efficacy is unsatisfactory and comparisons are difficult due to heterogeneity between different examinations. There is an urgent need for high-quality, large-scale studies of PE markers to improve the diagnosis, prognosis and treatment of high-risk women.

The aetiology and pathogenesis of preeclampsia are not fully understood. The attention of researchers and clinicians should therefore be focused on research of new biomarkers to diagnose and predict PE and echographic research, and on clarifying the pathogenesis of PE. Understanding the role of MMPs, TIMPs and podocalyxin and uterine-placental and vascular remodelling and function of PE can contribute to the understanding of the pathogenesis of PE. Given the high perinatal and infant morbidity and mortality and the risks to the foetus and pregnant women, more detailed studies on these markers are necessary to improve the diagnosis and prognosis of women at high risk of developing preeclampsia.

The objects of this dissertation are the role of serum matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, podocalyxin and mother echographic parameters in the pathogenesis of the preeclampsia, as well as the options for disease diagnosis and prognosis by examining these parameters.

Conclusions, justifying the objective and the tasks:

1. The preeclampsia is the primary cause of maternal and perinatal morbidity and mortality and complicates in around 2-8% of pregnancies around the world. Despite this fact, the PE has not yet been fully studied.

2. Early detection of preeclampsia is critical for risk stratification and behavior. The use of appropriate indicators would allow early detection of PE and prompt therapy.

3. In order to reduce PE cases, it is important to identify high-risk women. For this reason, clinicians need more accurate examinations to predict PE.

4. The extracellular matrix plays a very important role in the pathophysiology of preeclampsia through the synthesis and degradation processes of its essential components - Type I, III and IV collagen, the basal membrane and the proteoglycans regulated mainly by matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs).

5. There is an increasing number of literature data on the participation of MMPs and TIMPs in the pathogenic mechanisms of preeclampsia.

6. The podocalyxin is an indicator for glomerular damage. This marker has not only a renal expression, but is also expressed on the surface of endothelial cells of many organs other than the kidneys. As preeclampsia is a systemic disease characterized by generalized endothelial damage/dysfunction and multiple organ involvement, more detailed studies on the role of PCX serum in the pathogenesis of preeclampsia regarding abnormal organ and vascular remodelling are required and the possibility to use it as an indicator of diagnosis and prognosis of PE.

7. More detailed studies of the ECM biomarkers and the ultrasound indicators of preeclampsia are required.

II. OBJECTIVE AND TASKS

OBJECTIVE OF THE STUDY: Research of the role of serum matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, podocalyxin and mother echographic parameters in the pathogenesis of the preeclampsia, as well as the options for disease diagnosis and prognosis by examining these indicators.

TASKS OF THE STUDY:

1. Determination of the mean pulsatility index of the uterine and umbilical artery in preeclampsia and in normal pregnancy.

2. Determination of the mean pulsatility index of the uterine and umbilical artery over the duration of the manifestations of preeclampsia.

3. Determination of the mean pulsatility index of the uterine and umbilical artery according to the severity of the preeclampsia.

4. Examination of maternal echocardiography parameters in preeclampsia.

5. Determination of serum levels of collagenase MPM-8, -13 in preeclampsia.

6. Determination of serum levels of gelatase MPM-2, -9 in preeclampsia.

7. Determination of serum levels of TIMP-1, -2 in preeclampsia.

8. A study on the role of serum podocalyxin in the pathogenesis of preeclampsia and its potential as a marker for early diagnosis and prediction of preeclampsia.

III. MATERIAL

- **Clinical contingent**

Patients

All patients from the study were from the Clinic of Obstetrics and Gynecology at the Dr. Georgi Stranski University Hospital for Active Treatment - Pleven, Department of Obstetrics and Gynecology at the Medical University - Pleven. All procedures were followed in accordance with the ethical standards of the Human Research Committee (institutional and regional), as well as the Helsinki Declaration of 1975 revised in 2000. The study was approved by the Ethics Committee of Research at the Medical University of Pleven. The study covered the period from July 2020 to August 2021. The voluntary participation of all subjects studied was verified by written informed consent and serum was taken from each subject. The study group included 55 pre-eclampsia patients with mean age of 24.9±6 years; and a control group of 35 healthy women with normal pregnancies at 24.7±5.4 years of age (Table 1). The selected persons of the core group complied with the following eligibility and illegibility criteria:

The eligibility criteria for participation in the study were as follows: Pregnant women between 20 and 42 with clinical symptoms and laboratory criteria for preeclampsia; age over 18 years and under 40 years, maintenance of current diet and exercise during the study; signing informed consent to participate in the study; absence of metabolic syndrome and/or diabetes mellitus; hypertension parameters (grade, stage, complications); presence/absence of risk factors; presence/absence of hypertensive organ damage (brain, kidney, cardiac, ocular, peripheral), signed informed consent to participate in the study.

The illegibility criteria were as follows: diabetes, kidney and heart disease, signs of chorioamnionitis, presence of a foetus with chromosome abnormality, refusal to sign informed consent to participate in the study. Criteria for the selection of the control group: healthy women with normal pregnancies, between 20 and 42 years of age over 18 and under 40 years old, signed informed consent to participate in the study.

- **Classifications used**

The classification of the American College of Obstetricians and Gynecologists Convention 2019 [309], commonly adopted for clinical practice and research purposes has been used and the following diagnostic criteria have been applied:

Blood pressure

- Systolic blood pressure ≥140 or diastolic blood pressure ≥90mmHg in two separate measurements at a distance of at least 4h. after 20th gestation week in women with a previous normal blood pressure.
- Systolic blood pressure ≥160 or diastolic blood pressure ≥110mmHg.

and

Proteinuria

- ≥300mg in 24 h urine or this amount extrapolated from collected urine over a set interval of time or
- protein/creatinine ratio of 0.3 mg/dL or greater or
- test strip 2+ (used only if other quantitative methods are not available)

OR, IN THE ABSENCE OF PROTEINURIA, NEW HYPERTENSION WITH EMERGING ONE OF THE FOLLOWING CONDITIONS:

•Thrombocytopenia (platelet count less than 100 000x10^9/L).

•Abnormal liver function indicated by elevated blood concentrations of liver enzymes (twice the upper limit of normal concentration).

•Renal failure (serum creatinine concentrations greater than 1,1 mg/dL or a doubling of serum creatinine concentration in the absence of other renal diseases).

•Pulmonary edema

•New onset headache, which is not affected by medication, cannot be explained by any other diagnosis or visual disturbances.

According to American College of Obstetricians and Gynecologists 2013 [310] and the International society for the Study of Hypertension in Pregnancy 2014 [311] depending on the time range, the preeclampsia is classified as early-onset preeclampsia (EOPE) requiring

delivery before 34th gestation week, and late-onset preeclampsia (LOPE) - during or after the 34th gestation week.

According to the definition of American College of Obstetricians and Gynecologists 2019 [309], the retransmission is classified as severe PE where systolic blood pressure≥160 mmHg or diastolic blood pressure≥110 mmHg, or one of the following severe characteristics increasing the risk of morbidity and mortality:

•Thrombocytopenia (platelet count less than 100 000x10^9/L).

•Abnormal liver function as manifested by increased blood concentrations of liver enzymes (twice the upper limit of normal concentration) and severe persistent epigastric pain or right upper abdominal quadrant pain not responding to medical treatment and unexplained by other diagnosis.

•Renal failure (serum creatinine concentrations greater than 1,1 mg/dL or a doubling of serum creatinine concentration in the absence of other renal diseases).

•Pulmonary edema

•New onset headache, which is not affected by medication, cannot be explained by any other diagnosis or visual disturbances.

IV METHODS

IV 1 Clinical method- anamnesis and physical status

- **Anamnesis -** Personal and ID data, Anamnesis morbi (current illness), Anamnesis vitae (anamnesis of life), social history, harmful habits, Anamnesis familae (family history), examination of complaints by systems (systematic questioning). Evaluation of subjective complaints, cardiovascular risk factors, co-morbidities and therapy.
- **Physical status-** view, palpation, percusion, auscultation. Measurement of height with hand-arm and weighing by medical scales.
- **Midwifery medical history and obstetrics status**
- **Clinical method/Anthropomometric measurements** – height, weight, weight prior to pregnancy, BMI calculation (BMI = kg/m2)

IV 2 Documentary and sociological method:

- Analysis of documents – disease history, discharge summaries issued after hospitalization, lab reports with results from examinations of the patients.

IV 3 Laboratory tests:

Complete blood count, electrolytes, biochemistry-glucose, urea, creatinine, uric acid, total cholesterol, triglyceride, HDL, LDL, ASAT, ALAT, LDH, CPK, CK-MB, total protein, albumin. The laboratory tests were performed at the central clinical laboratory of Dr. Georgi Stranski University Hospital for Active Treatment - Pleven. Venous blood was collected in the morning hours between 8 and 10 hours in sterile, cooled vacutainers, centrifuged at about 1500 revolutions and the resulting serum and stored at minus 20˚C until analysis was carried out to ensure comparability of results.

IV 4 Immunological tests

The analysis of the markers of the dissertation work was performed using the immunoenzyme quantitative ELISA method. All the requirements and procedures laid down by the manufacturer concerned have been complied with. Serum levels of MMP-2,-9,-8,-13 and TIMP-1 and -2 were tested using the following ELISA kits: Human total MP-8 Quantikine ELISA Kit, total MMP-2 Quantikine ELISA Kit, Human MMP-9 Quantikine ELISA Kit, Human TIMP-1 Quantikine ELISA kit, Human TIMP-2 Quantikine ELISA kit- R&D systems and Human MMP-13 ELISA kit Reagent Genie; whereas for the podocalyxin - RJ-HUF0095 Human Podocalyxin ELISA kit (Reagent Genie). The immunological tests were performed at the Department of Clinical Laboratory, Clinical Immunology and Allergology, sector of Clinical Immunology and Allergology.

IV.5 Non-invasive methods of heart, vascular and pregnancy testing

IV 5.1 Measurement of blood pressure- Measurement of blood pressure according to WHO recommendations: after a 10-minute sitting position with a upper arm cuff. A standard manual sphygmomanometer with a difference of up to 2 mmHg was used. 3 measurements with a 2 minute interval between them were carried out. The average value was taken as reference value. The measurements were made on both arms, the higher value being taken as the reference.

IV 5.2 ECG- standard 12-channel ECG. The standard ECG included 12 leads – 6 peripheral from the extremities and 6 of the pericordial region. Peripheral limb leads are:

- Three bipolar leads - I, II and III, known as standard or Einthoven leads. Three single pole (unipolar) leads – to register electrical potential (potential difference) between one point on the body,from one limb and the sum of the other electrodes respectively. They are indicated by three letters: aVR - right hand lead (R- right-hand side), aVL - left hand lead (L- from left-hand), aVF - left foot lead (F - foot)
- The pericordial (thoracic) leads are single-pole leads and are marked with the letter V and the Arab digits from 1 to 6 (V1; V2; V3; V4; V5 and V6).

IV 5.3. Echocardiography

Transthoracic echocardiography with a Philips 4 MHz transducer performed by one and the same echographer. All measurements were made according to the European Association of Cardiovascular Imaging (EACVI) and The American Society of Echocardiography (ASE) criteria for Cardiac Chamber Quantification by Echocardiography [312]. A single-dimensional EhoCG-M-type (1-dimensional echoCG), two-dimensional echocardiography (B-type), M-Mode, Doppler pulsed wave were used. Values for all echocardiographic parameters were

measured as a mean of 3 consecutive cardiac cycles. The total LV function assessment was performed using the ejection fraction indicator calculated by the modified Simpson formula. The left ventricular end systolic and diastolic dimensions and volumes were measured via direct 2D access. The rates of mitral blood flow were measured by apical 4-cavity section. The diastolic function was assessed by measuring the transmitral diastolic (E-wave) and atrial (A-wave) velocities and calculation of the E/A ratio. Using tissue Doppler, early (e′) and late (a′) diastolic mitral annular velocities were measured from the septum of the mitral annulus in apical 4-cavity section with an equalised septal annular movement. *Doppler echocardiography*- monitoring of blood flow through cardiac cavities and large vessels. Determination of cardiac output as well as the structure, thickness and functioning of cardiac valves.

IV 5.4 Transabdominal echography to determine Doppler PI on umbilical and uterine artery

o Doppler PI on the uterine arteries - the Doppler test is a non-invasive method for examination of the uteroplacental circulation. After three positive consecutive wave curves the PI of both uterine arteries is measured and the mean of the two is calculated. This study was conducted during abdominal ultrasound using a high-grade Philips Affiniti 70G equipment with 4-6 Mhz volumetric transducer. The mean value of the pulsatility index of the two uterine arteries was measured by Doppler Velosimetry and a color and pulse map was performed. The wave curves of blood flow velocity have been recorded with 3.5 MHz and 5 MHz through a 3.2 MHz through transabdominal convexate transducer. Transabdominal access was used and the probe was inserted in both inguinal areas. All methodological recommendations were followed: "The Doppler test shall be performed in a supine position of the pregnant woman. The transducer shall be placed parallel to the anterior uterine wall in its isthmus part so that the a. iliaca comunis is traced back to the site of the bifurcation. Then the transducer is directed medially in the same layout as the image of the uterine artery appears. The examination window is placed approximately 1 cm medially from the place where the uterine artery crosses a a. iliaca externa. After each uterine artery has been identified, a pulse wave Doppler was inserted, with a 2 mm wide acoustic window to cover the entire vessel. Special attention was paid to the angle of insonation which must be less than 30 degrees. The maximum systolic pressure in the vessel should be not less than 60 cm/s in order to accurately evaluate the correct vessel and not its branches. The depth is to be adjusted so that the acoustic window is in the lumen of the vessel. Its width varies according to the diameter of the lumen of the vessel being examined. At this time, the

recording of wavy curves and sound signals with a typical uterine arteries is starting. High pulsatility was observed in high-resistance vessels. Apart from index measurement, the wave blood flow curves can also be categorized by qualitative criteria: absence of end-diastolic blood flow, post-systolic incision, etc.. Pathological findings in the uterine artery were observed in the complicated pregnancies with hypertension, preeclampsia, intra-fetal growth retardation, congenital thrombophilia, etc." [280]

o Doppler PI of umbilical artery- evaluation is performed during foetal resting. If possible, keep Tlb<0.5 or at least <1 by reducing the acoustic output. A free loop is determined from the umbilical cord of Color Doppler. The high color PRF is used to avoid aliasing and conservative gain. The transducer is placed in the umbilical cord section which is passed parallel to the Doppler beam. The Doppler beam shall not be focused on the foetus eyes. The spectral Doppler baseline, the PRF and the velocity of the spread is optimized for a large waveform. If the EDV is close to the base line, the filter shall be low enough to indicate EDV. The test is interpreted as an abnormal test at >95 percentiles [313].

IV 6 Statistical methods

The study data were processed with software STATGRAPHICS statistical packages; SPSS and EXCEL for Windows. The results are described in tables, graphs and numerical indicators for structure, frequency, mean values, correlation factors, etc. The significant results, assumptions and conclusions are determined at p<0,05. The following statistical methods of analysis have been applied:

1. Description of qualitative variables
2. Description of quantitative variables

Two main types of means, namely algebraic means (arithmetic mean) and position means (median, mode, quartiles, percentiles), were used to measure the central trend. The tests used to check the normality of distribution and the equality of the deviations are Stnd. Skyness & Stnd. Kurtosis. For significant differences between groups, the t-test of Student and ANOVA of mean ± SD was used in normal distribution cases (LSD, Tukey HSD, Scheffe, Bonferroni, Newman-Keuls, Duncan) and K-W, median H-test (M) in cases other than normal distribution, together with the first and third quartile Q1 and Q3; (Twenty-fifth and seventy-fifth percentile P25 and 75P).

3. Variance analysis

The following descriptive numerical characteristics are used to measure variability: Range of variation order - the difference between the extreme values /maximum and minimum/; standard deviation - the mean variation of the arithmetic mean and interquartile ranges.

4. Parametric methods for checking hypotheses – only applicable to quantities at normal or near normal distribution.

- t-criterion of Student - used to compare means, coefficients and proportions.
- Dispersion analysis - one-factor and multifactor dispersion analysis has been used to examine significant differences between results in the groups by Fisher's F-criterion.

5. Non-parametric methods for checking hypotheses – applicable to quantitative and qualitative variables, regardless of the form of the distribution. The criterion of Pearson (Chi-squared) and the criteria of Kruskall-Wallis were used.

6. A correlation analysis has been used to examine the relationship between changes in the dependent variable and the relevant changes in the factors studied. Where an actual link exists, it is represented by the coefficient of correlation r, which has a numerical value and a /+ or -/ sign. The numerical value characterizes the force of the correlation, and the symbol in front of the number indicates the direction of the link, with a positive correlation sign /+/, and with a negative sign. For qualitative alternative, the coefficient of correlation of Pearson has been used and a

special factor influence criterion as a measure of the force of the relationship between a factor and the forecast of the PE, called financial (OR - odds ratio). The odds ratio shall be used as an approximate measure of risk ratio for disease outcomes depending on a particular risk factor or group of risk factors. For the quantitative variables the coefficient of Pearson has been used, and for the categorical variables, displayed in an ordinal scale, the quantitative variables and in case of one quantitative variable and one qualitative variable the ranking coefficient of Spirman has been used.

7. Regression analysis

The individual action of each factor has been determined by applying a logistic regression analysis to describe the relationship between one dependent variable and one or more independent variables. An economic biomedical acceptable prognostic model, as established for patients with PE, has been developed through logistic regression analysis. This model describes the relationship between disease outcome and a number of independent variables. The logistic regression analysis was used to determine a minimum of a number of factors (model) by which the probability of a patient falling into the event group or the event-free group can be predicted. The Kaplan and Meier method has been used and a non-parametric survival model has been created. Patients who have not reached any of the end-points for the follow-up period have been removed (sensored).

8. Test $\chi 2$ and Fisher's test to check hypothesis of correlation between categorical variables.

9. A binary logistic regression analysis – for quantitative assessment of the dichotomy exit factors and calculation of the probabilities of these outcomes.

10. One-factor variance analysis (ANOVA with post-hoc tests Tukey, Scheffe, Bonferroni, Newman-Keuls, Duncan) – to check hypothesis of difference between several independent samples.

Table 1. Clinical data on patients and controls

	Normal pregnancy	**Preeclampsia**	**P**
Age	24.7±5.4	24.9±6	p>0.05
BMI	26.7±4.2	34±7.3*	p<0.001*
Pregnancy	2(2) **	2(2)**	
Parity	1(2) **	1(2) **	
SBP (mmHg)	116.1±9.55	157.8±22*	p<0.001*
DBP (mmHg)	75.3±7.76	100.5±10*	p<0.001*
Anamnesis of PE	0/ 35	23/ 55	
Anamnesis of AH	1/ 35	26/ 55	
AH before pregnancy	0/ 35	15/ 55	
PP	40.8±7.32	57.3±16.1*	p<0.001*
Average BP	88.8±7.69	119.7±13.1*	p<0.001*
Urea	2.96±0.78	3.75±1.63*	p=0.01*
Creatinine	75.78±14.45	73.33±15.33	p>0.05
Uric acid	205.6±40.2	326.8±105.93*	p<0.001*
Total protein	68.89±3.16	58.71±8.78*	p<0.01*
Albumin	37.31±2.78	31.67±4.98*	p<0.01*
ASAT	8.43±2.33	20.67±7.82*	p<0.01*
ALAT	9.83±2.50	27.76±8.25*	p<0.01*
LDH	369±70.78	435.25±80.74*	P=0.04*
PLT	237.26±61.12	228.74±88.53	p>0.05
CPK	83.1±23.77	130.5±46.8*	P<0.05*
CK-MB	15.3±3.3	24.3±7.9*	P<0.05*
Number	(n=35)	(n=55)	

BMI- body mass Index; SBP-systolic blood pressure; DBP-diastolic blood pressure; PE-preeclampsia; AH-arterial hypertension; PP-pulse presure; ASAT-aspartate aminotransferase; ALAT-alanine aminotransferase; LDH-lactate dehydrogenase; PLT-platelets; CPK-creatine phosphokinase; CK-MB-isoenzymatic creatine phosphokinase. Data were adjusted as mean±SD; *p<0.05; **Data were provided by means of median (first and third quartile Q1 and Q3; twenty-fifth and seventy-fifth percentile P25 and 75P

V. RESULTS

V.1. RESULTS FROM DOPPLER MEASURING OF THE MEAN PULSATILITY INDEX OF UMBILICAL AND UTERINE ARTERY IN PREECLAMPSIA

In analysing the distribution of patients in the preeclampsia study group (n=55) depending on the time of manifest and severity of the disease, the following results were obtained: 41 the case of early vs. 14 cases of late preeclampsia and 29 cases of mild vs. 26 cases of severe preeclampsia. The mean pulsatility index of uterine artery and the mean pulsatility index of umbilical artery were significantly higher in preeclampsia versus normal pregnancy, respectively, of 1.19±0.44 vs. 0.79±0.12 (F=28.15; p=0.0001) and 1.24±0.35 vs. 1.09±0.14 (F=5.87; p=0.02) (Table 2).

Table 2. Mean pulsatility index of uterine artery and mean pulsatility index of umbilical artery in preeclampsia versus normal pregnancy

	Normal pregnancy	**Preeclampsia**	**P**
Umbilical artery mean PI (UmA)	1.09±0.14	1.24±0.35	P=0.02*
Uterine artery mean PI (UtA)	0.79±0.12	1.19±0.44	P=0.0001*

UtA- mean pulsatility index of uterine artery, UmA- mean pulsatility index of umbilical artery **The values are mean ±SD**

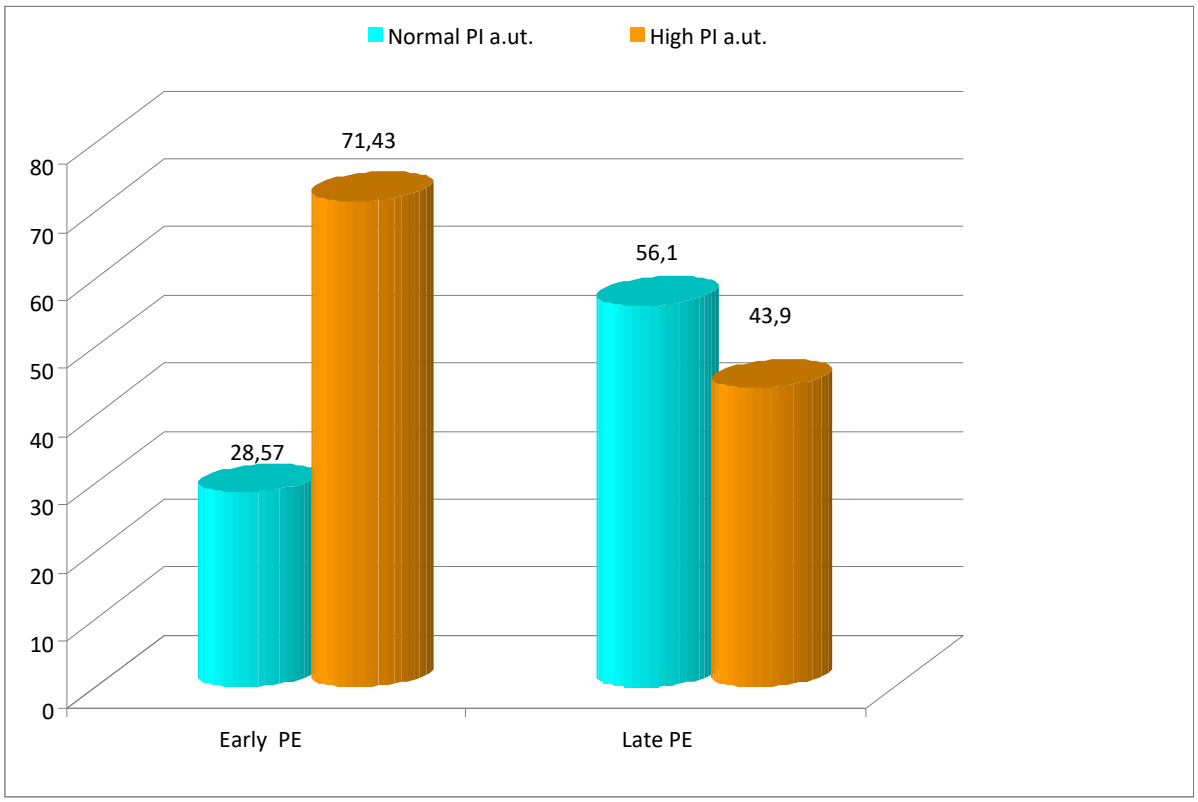
V.2. RESULTS OF THE DETERMINATION OF MEAN PULSATILITY INDEX OF A.UTERINA AND A. UMBILICALIS BY THE TIME OF PREECLAMPSIA MANIFESTATION

2.1 DETERMINATION OF MEAN PULSATILITY INDEX OF A. UTERINA IN EARLY AND LATE PREECLAMPSIA

In early preeclampsia high UtA PI was detected in 71.43% of cases, while 28.57% had a normal index ($\chi 2=3.16$; $p=0.04$).

In late preeclampsia high UtA PI was detected in 43.9% of cases, while 56.1% had a normal index **(Figure 1)**.

Figure 1 Percentage ratio of mean pulsatility rate of a. uterina (normal vs. high) in women with early and late preeclampsia

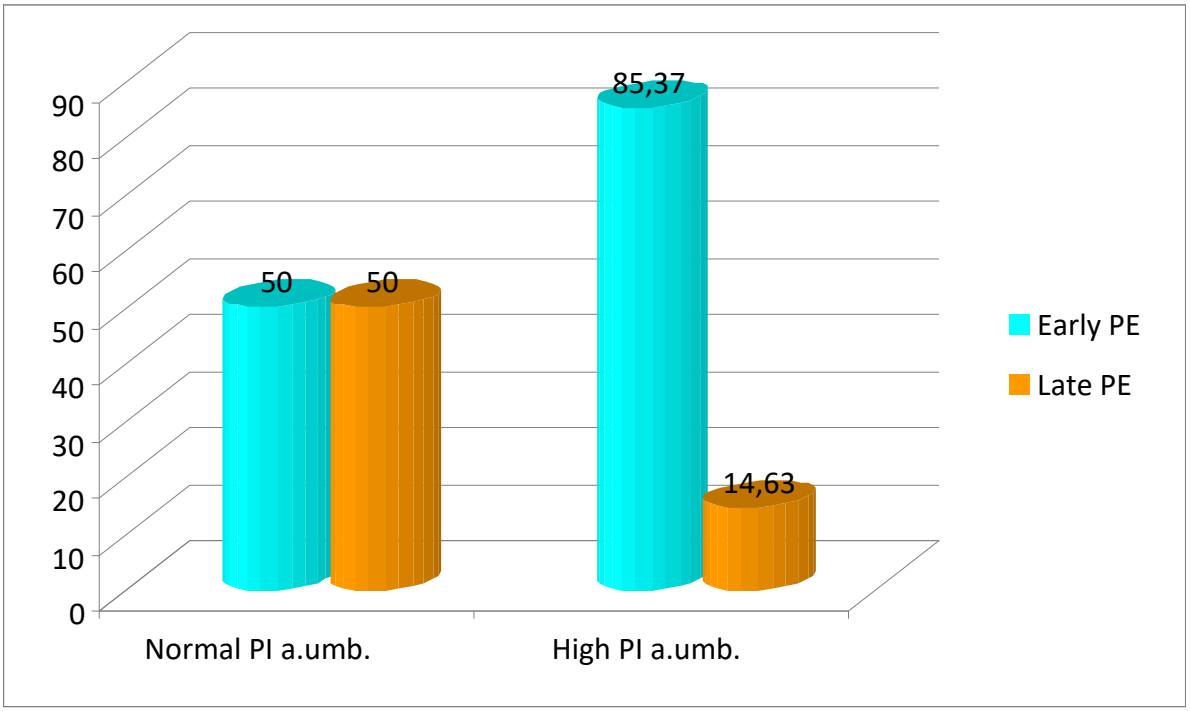


2.1 DETERMINATION OF MEAN PULSATILITY INDEX OF A. UMBILICALIS IN EARLY AND LATE PREECLAMPSIA

In early preeclampsia high UmA PI was detected in 50% of cases, while 50% had a normal index.

In late preeclampsia high UmA PI was detected in 85.37% of cases, while 14.63% had a normal index ($\chi 2=7.23$; $p=0.003$) (Figure 2)

Figure 2 Percentage ratio of mean pulsatility rate of a. umbilicalis (normal vs. high) in women with early and late preeclampsia



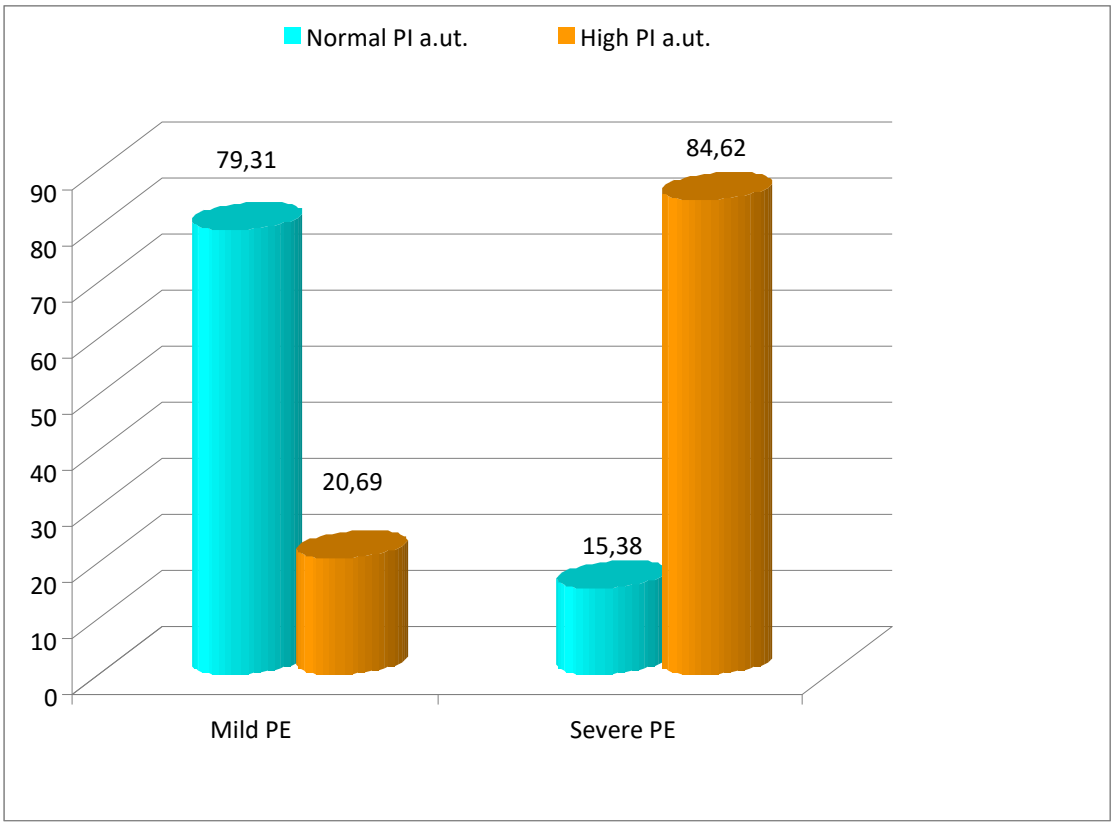
V.3. RESULTS OF THE DETERMINATION OF MEAN PULSATILITY INDEX OF A.UTERINA AND A. UMBILICALIS ACCORDING TO THE SEVERITY OF THE PREECLAMPSIA

3.1 DETERMINATION OF MEAN PULSATILITY INDEX OF A. UTERINA IN EARLY AND LATE PREECLAMPSIA

In mild preeclampsia high UtA PI was detected in 20.69% of the cases, while 79.31% had a normal index.

In severe preeclampsia high UtA PI was detected in 84.62% of cases, while 15.38% had a normal index ($\chi 2=22.42$; $p=0.0001$) (Figure 3)

Figure 3 Percentage ratio of mean pulsatility rate of a. uterina (normal vs. high) in women with mild and severe preeclampsia

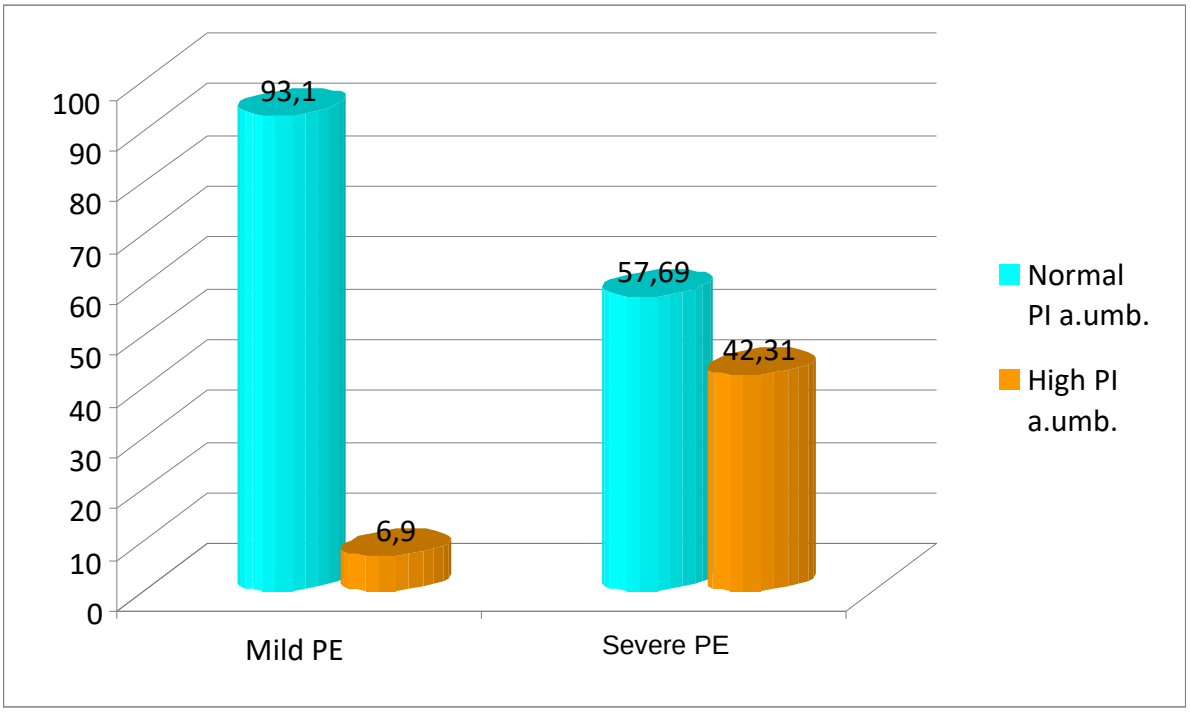


3.2 DETERMINATION OF MEAN PULSATILITY INDEX OF A. UMBILICALIS IN MILD AND SEVERE PREECLAMPSIA

In mild preeclampsia high UmA PI was detected in 6.9% of cases, while 93.1% had a normal index ($\chi 2=7.66$; p=0.006).

In severe preeclampsia high UmA PI was detected in 42.31% of cases, while 57.69% had a normal index (Figure 4).

Figure 4 Percentage ratio of mean pulsatility rate of a. umbilicalis (normal vs. high) in women with mild and severe preeclampsia



V.4. RESULTS OF THE ECHOCARDIOGRAPHIC EXAMINATION IN PREECLAMPSIA

LVEDD, LVEF, IVS, LVPWD, EF%, E/e' parameters were significantly higher in preeclampsia versus normal pregnancy ($p<0.05$) (Table 3).

Table 3. Echocardiographic evaluation of women with preeclampsia and normal pregnancy

	Normal pregnancy	**Preeclampsia**	**P**
LVEDD	46.06±1.51	47.67±2.83*	p=0.003*
LVEF	28.23±1.48	29.84±2.43*	p=0.0007*
IVS	9.47±0.86	10.74**±**0.93*	p<0.01*
LVPWD	9.03±1.04	10.4±1.31*	p<0.001*
EF%	9.03±1.04	64.69±5.14*	p=0.0002*
E/e'	9.64±1.02	11.76±0.77*	p<0.001*
Number	**35**	**55**	

LVEDD-left ventricular end-diastolic diameter, LVEF-left ventricular end-systolic diameter, IVS-ventricular septal thickness, LVPWD- posterior left ventricular wall thickness, EF%-left ventricular ejection fraction, *p<0.05, **the values are mean ±SD**

V.5. RESULTS OF DETERMINATION OF MMP-8,-13 COLLAGENASES IN PREECLAMPSIA

The conducted examinations showed that serum levels of MMP-8 in women with normal pregnancy were lower than those with preeclampsia of 1,75 (1,36÷3,2) vs. 1,8 (1,26÷3,0), and these values are not significant (p>0,05) (Table 4; Fig. 5). MMP-13 levels in patients with preeclampsia were higher than in women with normal pregnancy 0,18 (0,16÷0,2) vs. 0,17 (0,15÷0,2) and these values are not significant (p>0,05) (Table 4; Fig. 6).

Table 4. Serum MMP-8, -13 values in women with normal pregnancy and in patients with preeclampsia

	Women with normal pregnancy	**Preeclampsia**	**P**
MMP-8 (ng/ml)	1.75 (1.36÷3.2)	1.8 (1.26÷3.0)	p>0.05
MMP-13 (ng/ml)	0.17 (0.15÷0.2)	0.18 (0.16÷0.2)	p>0.05

Values are presented as median (M), along with first and third quartile Q1 and Q3 (twenty-fifth and seventy-fifth percentile P25 and 75P).

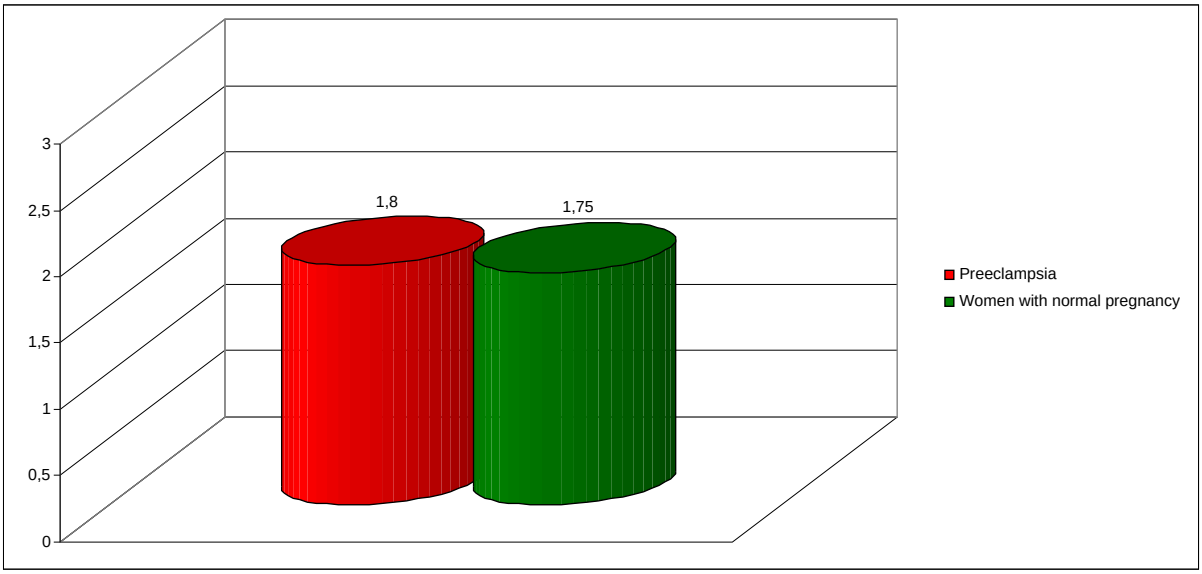


Figure 5 Serum levels of MPM-8 in women with preeclampsia and normal pregnancy

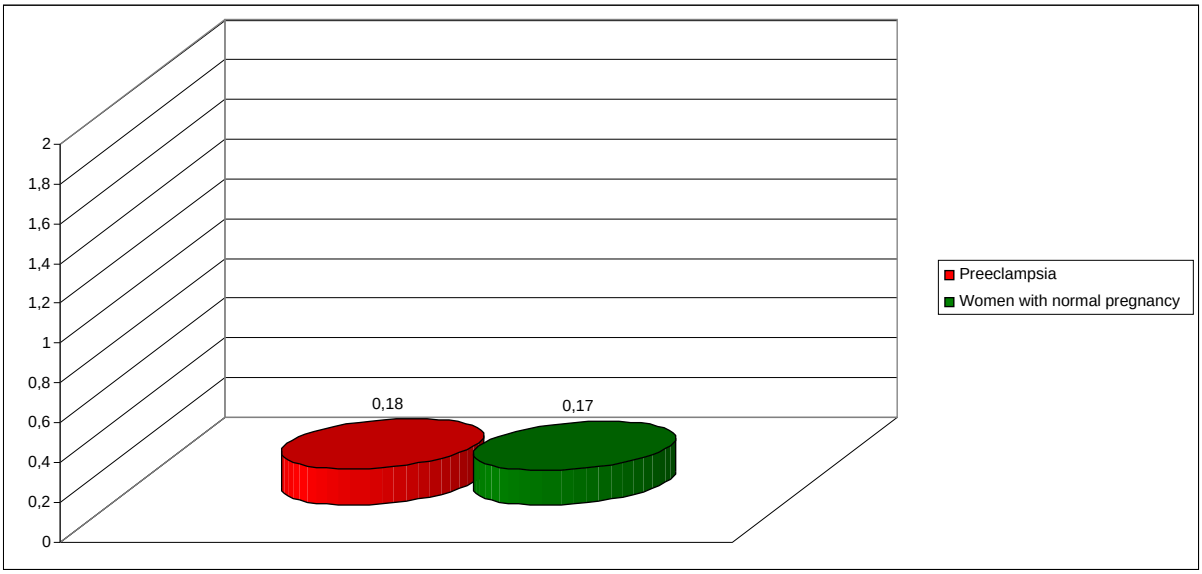


Figure 6 Serum levels of MPM-13 in women with preeclampsia and normal pregnancy

V.6. RESULTS OF DETERMINATION OF GELATINASE MMP-2,-9 IN PREECLAMPSIA

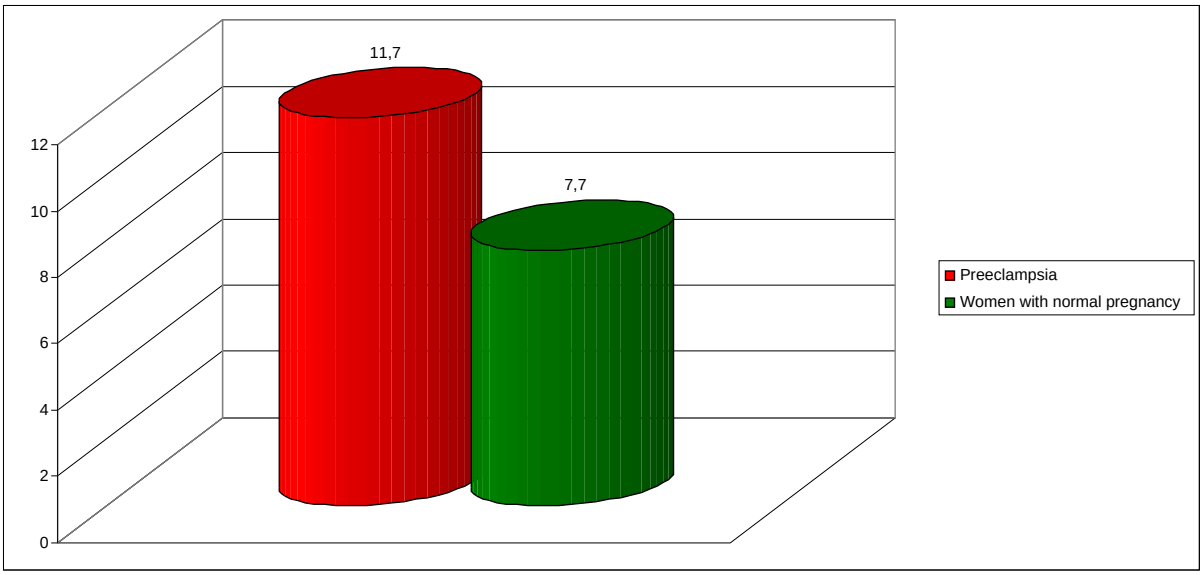
The conducted examainations showed that serum levels of MMP-2 in preeclampsia were statistically significantly higher compared to the levels in women with normal pregnancies of 11.7 (9.1÷15.5) vs. 7.7 (6.5÷13.4), (KW=5.78; P=0.02) (Table 5; Fig. 7). MMP-9 levels in women with normal pregnancy were higher than the levels in patients with preeclampsia 4.54 (3.47÷6.57) vs. 3. 95 (3÷ 3. 06) and these values are not significant (p>0,05) (Table 5; Fig. 9).

Table 5. Serum MMP-2, -9 values in women with normal pregnancy and in patients with preeclampsia

	Women with normal pregnancy	**Preeclampsia**	**P**
MMP-2 (ng/ml)	7.7 (6.5÷13.4)	11.7 (9.1÷15.5)*	p=0.02*
MMP-9 (ng/ml)	4.54 (3.47÷6.57)	3.95 (3÷7.06)	p>0.05

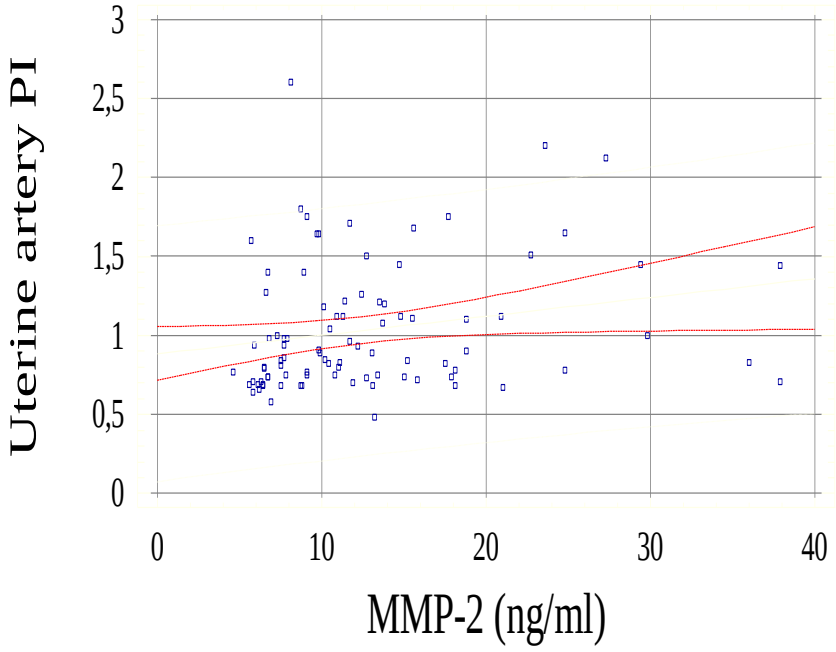
Values are presented as median (M), along with first and third quartile Q1 and Q3 (twenty-fifth and seventy-fifth percentile P25 and 75P).

Figure 7 Serum levels of MPM-13 in women with preeclampsia and normal pregnancy



MPM-2 showed correlation with the mean pulsatility index of uterine artery (UtA) (r=0.21; p=0.04) (Fig. 8).

Figure 8 Linear regression analysis describing the relationship between MPM-2 and UtA PI



A statistically significant relationship exists between MPM-2 and UtA PI on a 95% confidence level (r=0.21; p=0.04)

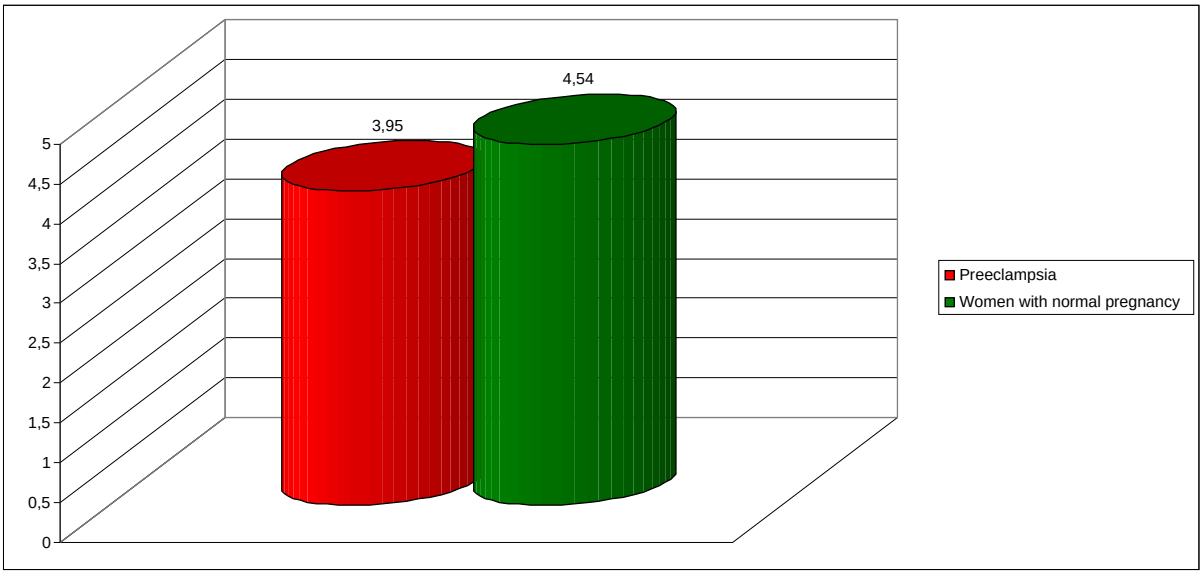


Figure 9 Serum levels of MPM-13 in women with preeclampsia and normal pregnancy

V.7. RESULTS OF DETERMINATION OF TIMP-1, -2 IN PREECLAMPSIA

The conducted examinations showed that serum levels of TIMP-1 in women with normal pregnancy were lower than those in patients with preeclampsia of 3.02 (1.28÷ 1.58) vs. 2.41 (1.01÷ 4) and these values are not significant (p>0,05) (Table 6; Fig. 10). TIMP-2 levels in women with normal pregnancy were higher than the levels in patients with preeclampsia 0.69 (0.41÷1.08) vs. 0.86 (0.45÷1.51) and these values are not significant (p>0,05) (Table 6; Fig. 11).

Table 6. Serum TIMP-1, -2 values in women with normal pregnancy and in patients with preeclampsia

	Women with normal pregnancy	**Preeclampsia**	**P**
TIMP-1 (ng/ml)	3.02 (1.28÷3.58)	2.41 (1.01÷4)	p>0.05
TIMP-2 (ng/ml)	0.69 (0.41÷1.08)	0.86 (0.45÷1.51)	p>0.05

Values are presented as median (M), along with first and third quartile Q1 and Q3 (twenty-fifth and seventy-fifth percentile P25 and 75P).

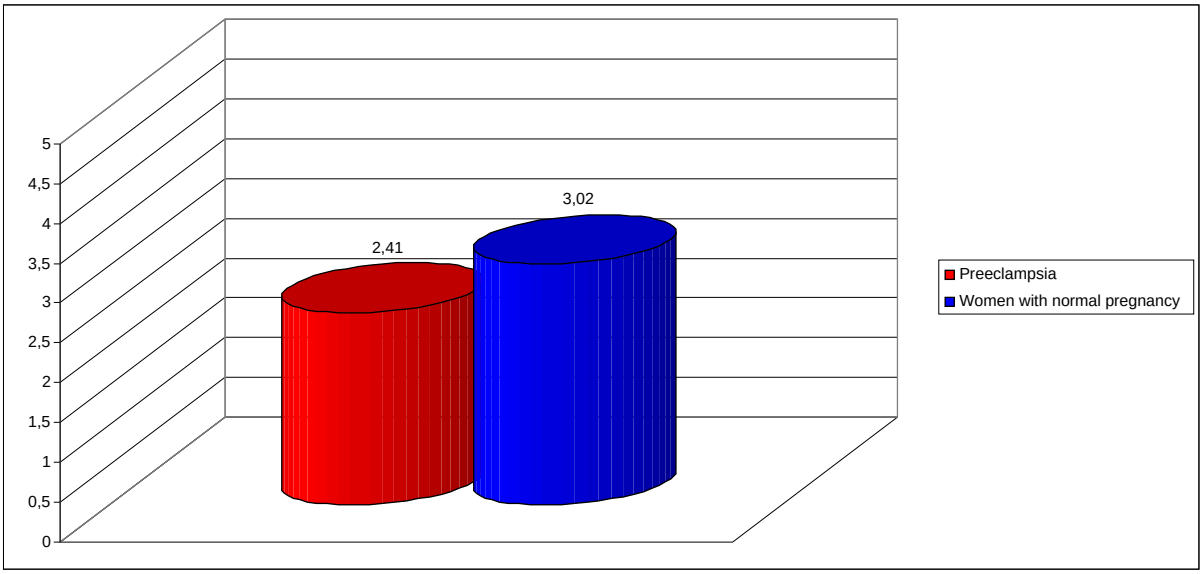


Figure 10 Serum levels of MPM-13 in women with preeclampsia and normal pregnancy

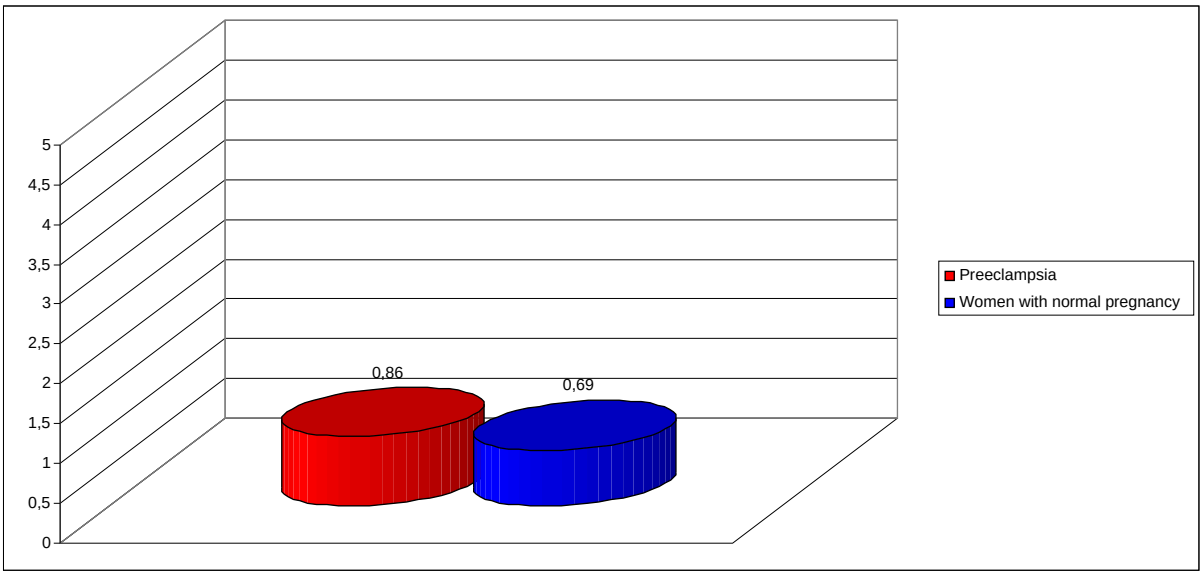


Figure 11 Serum levels of MPM-13 in women with preeclampsia and normal pregnancy

V.8. RESULTS OF SERUM PODOCALYXIN DETERMINATION IN PREECLAMPSIA CASES

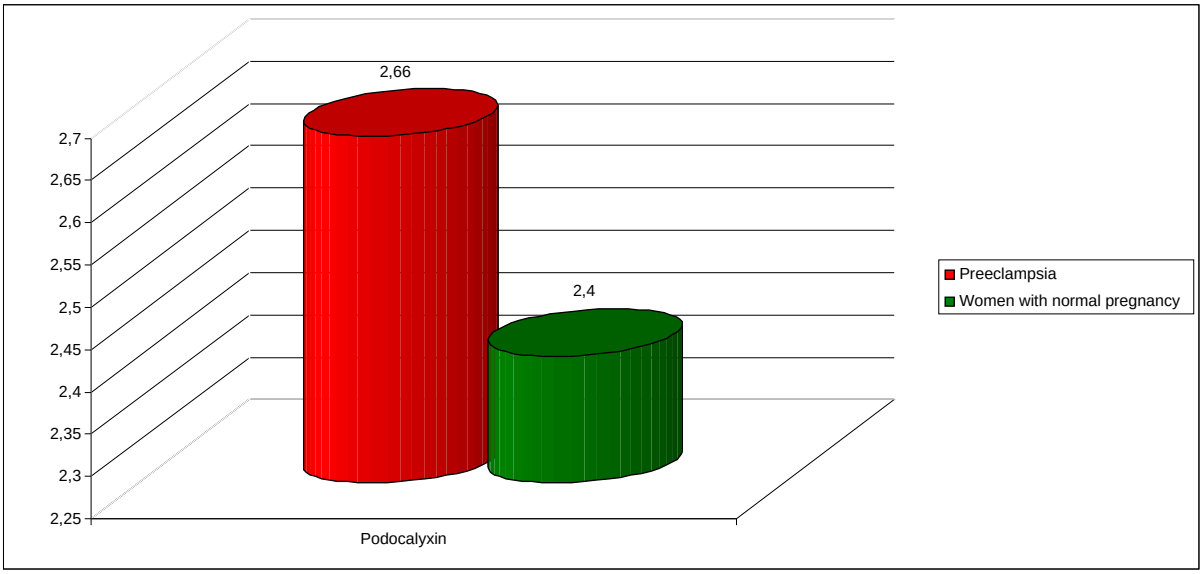
The conducted examinations showed that the serum levels of podocalyxin in patients with preeclampsia were statistically significantly higher compared to the levels in women with normal pregnancies of 2.66±0.67 vs 2.40±0.33, (F=4.59; P=0.03) (Table 7; 12).

Table 7. Serum levels of podocalyxin in women with normal pregnancy and in patients with preeclampsia

	Women with normal pregnancy	**Preeclampsia**	**P**
Podocalyxin	2.40±0.33	2.66±0.67*	P=0.03*

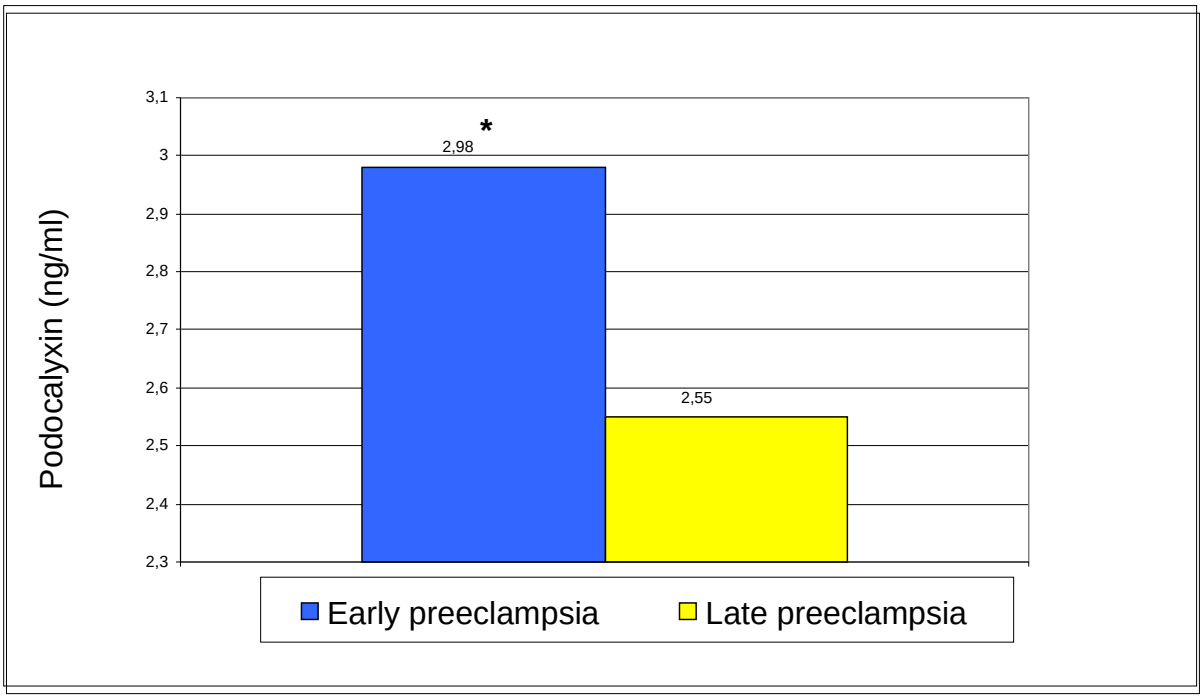
Values are represented as mean ±SD

Figure 12 Serum levels of podocalyxin in women with normal pregnancy and in patients with preeclampsia



The serum levels of podocalyxin in women with early preeclampsia were statistically significant higher than in women with late preeclampsia: 2.98±1.06 vs. 2.55±0.43 ng/ml (p=0.03) **(**Figure 13).

Figure 13 Serum levels of podocalyxin in women with early and late preeclampsia



A threshold value of 3ng/ml for serum podocalyxin was defined, which distinguishes preeclampsia from normal pregnancy.

The logistic regression model showed statistical significance: OR=3.226; (95% CI=1.084÷9.599); (p=0.019). The developed prognostic model shows that elevated serum podocalyxin levels indicate a 3-fold higher likelihood of preeclampsia compared to healthy pregnant women without elevated levels (Table 8).

Table 8. Logistic regression model for assessing the relationship between podocalyxin and the development of preeclampsia

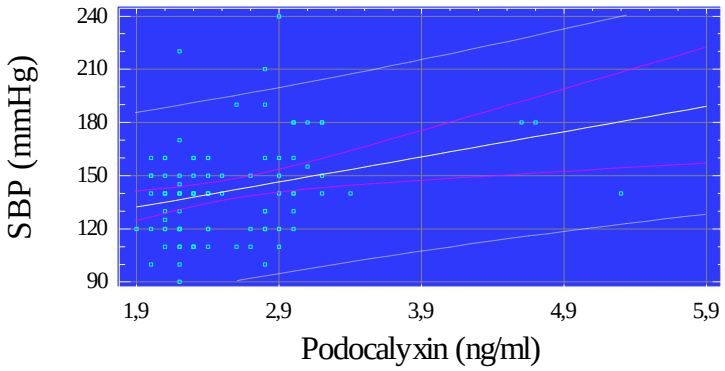
Variable	**β**	**SE**	**df**	**Sig.**	**Exp (β)**	**95% CI for Exp (β)**	**P**
Podocalyxin	1. 171	0. 556	1	0. 035	3. 226	1.084÷9.599	0.019*

* $p < 0.05$

The podocalyxin showed correlation with systolic blood pressure (r=0.30; p=0.004), diastolic blood pressure (r=0.35; p=0.0007), uric acid (r=0.32; p=0.002), CPK (r=0.22; 0.03) and CK-MB (r=0.21; p=0.04), the mean pulsatility index of uterine artery (r=0.30; p=0.004), the mean pulsatility index of umbilical artery (r=0.21; p=0.047), the inter-chamber septal thickness (r=0.32; p=0.002) and the rear wall thickness of the left ventricle (r=0.28; p=0.007).

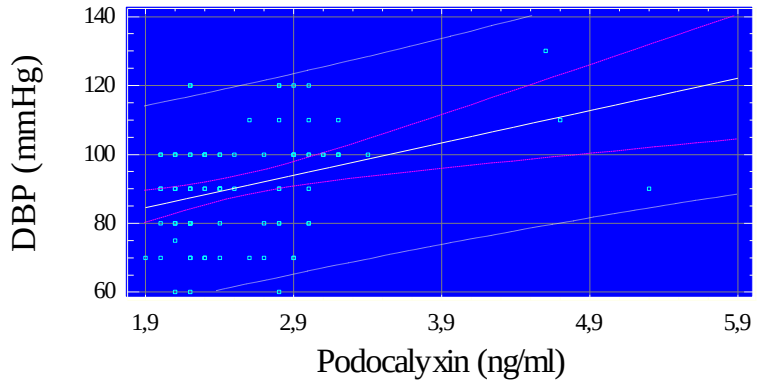
A statistically significant relationship exists between podocalyxin and MAP (mean arterial pressure) on a 99% confidence level (r=0.21; p=0.04). (Figure 14)

Figure 14 Linear regression analysis describing the relationship between podocalyxin and MAP.



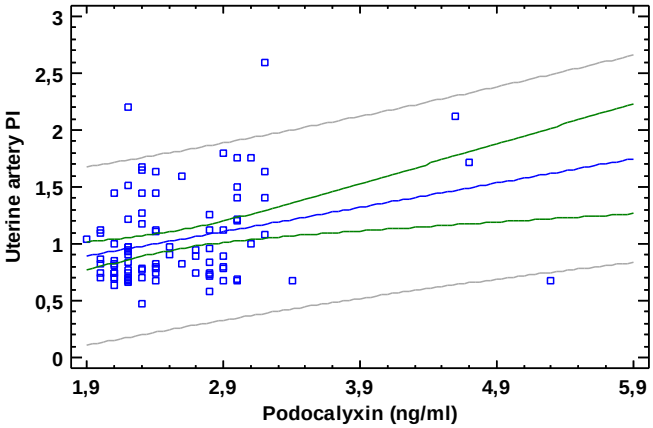
A statistically significant relationship exists between podocalyxin and DAP (diastolic arterial pressure) on a 99% confidence level (r=0.35; p=0.0007). (Figure 15)

Figure 15 Linear regression analysis describing the relationship between podocalyxin and DAP.



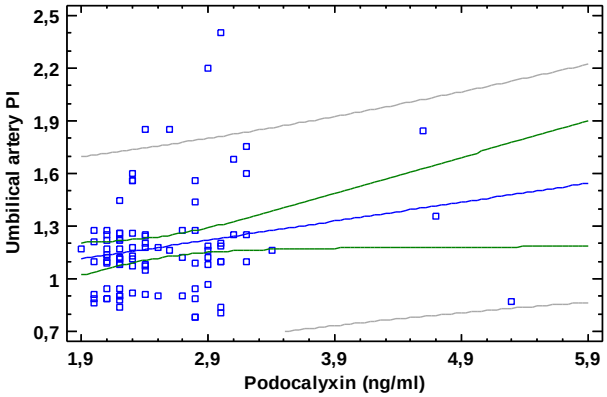
A statistically significant relationship exists between podocalyxin and mean pulsatility index of the uterine artery on a 99% confidence level (r=0.30; p=0.004). (Figure 16)

Figure 16 Linear regression analysis describing the relationship between podocalyxin and mean pulsatility index of uterine artery



A statistically significant relationship exists between podocalyxin and mean pulsatility index of the umbilical artery on a 99% confidence level (r=0.21; p=0.047). (Figure 17)

Figure 17 Linear regression analysis describing the relationship between podocalyxin and the mean pulsatility index of the umbilical artery



VI. DISCUSSION:

MMPs and their TIMPs inhibitors are important vascular reactivity mediators. More and more studies have shown that changes in their activity play an important role in the pathophysiology of preeclampsia.

Abnormal change in MMP/TIMP ratio in preeclampsia indicates that the ECM metabolism is impaired. In normal pregnancy, the increase of the MMP/TIMP ratio induces the metalloproteinases activity and favors the ECM degradation. This is important for the cytotrophoblast invasion of the uterine wall. The significant decline in the above ratio implies an environment that acts against the myometrial invasion. Subsequent placental perfusion disorder may result in relative or absolute foetal hypoxia and abnormal placenta function and hypertension during pregnancy, which may result in preeclampsia.

In this study we have monitored the role in the pathogenesis and the diagnosis and prognosis of the preeclampsia of serum matrix metalloproteinases, their inhibitors, podocalyxin and mother's echographic parameters.

The results obtained suggest that certain MMP play an important role in the process of vascular remodelling, development and progression of the preeclampsia. These data can be used to explain abnormal structure and function of the vascular wall, responsible for vascular remodelling.

Our study gives the prospect that the non-invasive serological marker podocalyxin is related to the structural and functional irregularities of the uterine and umbilical arteries, and it is possible to play a role in pathological vascular remodelling in preeclampsia. This is also confirmed by the correlation between the serum values of podocalyxin with Doppler ultrasound indicators mean pulsatility arterial and umbilical artery.

A threshold of serum podocalyxin was found to distinguish preeclampsia from normal pregnancy. Further more, elevated serum podocalyxin levels indicate a 3-fold higher likelihood of preeclampsia compared to healthy pregnant women without elevated levels. The determination of serum podocalyxin may provide important diagnostic and prognostic information for preeclampsia patients.

1. MEASURING OF THE MEAN PULSATILITY INDEX OF UMBILICAL AND UTERINE ARTERY IN PREECLAMPSIA

Doppler UtA PI eprovides evaluation of the blood flow rate in the uterine arteries and thus can identify women at increased risk of developing complicated pregnancy associated with uterine placental insufficiency (mainly in the second trimester) prior to clinical manifestation [313]. Doppler UtA PI is associated with the development of PE and adverse events during pregnancy [314]. The use of UtA PI to predict PE has been extensively studied in the IInd and Ist trimester of pregnancy. Abnormal placental function, which characterizes PE, is associated with increased blood flow resistance of the uterine placental circulation. This is evidenced by ultrasonography examination, showing increased UtTA PI. It is known that this procedure has a higher diagnostic and predictive value when used in II rather than in the I trimester of pregnancy and plays a major role in the predictive of severe or early PE in a low-risk population [315]. Studies have shown that increased UtA PI has a sensitivity of 78% and a specificity of 95% for prediction of severe PE when applied during the IInd trimester [316].

Doppler UmA PI is a marker of the foetal status and can be useful for foetal monitoring in high-risk pregnancies. It evaluates the impedance in the foetal-placental circulation and indirectly measures blood flow into the placental vasculature [317,318,319]. Increased UmA PI is a measure of abnormal blood flow in the umbilical artery, placental insufficiency, and subsequent intrauterine delayed foetal development or for suspected PE. The determination of UmA PI reduces perinatal morbidity and mortality in high-risk obstetric situations [320,321,322,323].

In analysing the distribution of patients in the preeclampsia study group (n=55) depending on the time of manifest and severity of the disease, the following results were obtained: 41 the case of early vs. 14 cases of late preeclampsia and 29 cases of mild vs. 26 cases of severe preeclampsia. The mean pulsatility index of uterine artery and the mean pulsatility index of umbilical artery were significantly higher in preeclampsia versus normal pregnancy.

2. ANALYSIS OF THE DETERMINATION OF MEAN PULSATILITY INDEX OF A.UTERINA AND A. UMBILICALIS ACCORDING TO THE TIME OF PREECLAMPSIA MANIFESTATION

The placenta plays a key role in the aetiology of preeclampsia. Although pathogenesis is still unclear, preeclampsia is clinically determined by primary placental damage [324,40]. Early preeclampsia "placental PE" is a result of impaired trophoblast invasion of spiral arteries, causing placental ischaemia and oxidative stress. Placental histology of early PE or delayed foetal development often demonstrated abnormal changes in fork branches supporting this theory [325]. The early PE was observed less frequently (0.4-1%) than late PE, but it was responsible for more severe disease events due to premature and delayed foetal development [326,327,328]. In addition, increased maternal cardiovascular morbidity was observed in the long term. Late preeclampsia - 'maternal PE' - was secondary to cardiovascular and metabolic predisposition of the mother to generalized endothelial dysfunction and had similar risk factors for cardiovascular diseases as in adults - AH (Arterial hypertension), obesity, glucose intolerance and dyslipidemia [329]. The placenta in such cases may appear to be normal or have minimal histological changes. Therefore, UtA PI may remain in normal values. One of the major pathogenetic differences between early and late PE was that early PE was defined to be associated with placental damage, while at the late PE the placenta was usually normal in clinical-pathological testing. Consequently, both the early and late PE result from the same low-perfusion factor, which has many different causes.

Our data showed that in early preeclampsia high UtA PI was detected in 71.43% of cases. These results confirm impaired trophoblast invasion of spiral arteries, causing placental ischaemia and oxidative stress. Despite that early PE was observed less frequently than late PE, it was responsible for more severe disease events due to prematurity and delayed foetal development. In addition, increased maternal cardiovascular morbidity was observed in the long term.

In late preeclampsia high UtA PI was detected in 43.9% of cases, indicating that the cardiovascular and metabolic predisposition of the mother to endothelial dysfunction plays a greater role in the pathogenesis of late PE.

In late preeclampsia in 85.37% of cases high UmA PI was detected in the examined population, indicating significant foetus - placental circulation disturbance. In early preeclampsia high UmA PI was detected in 50% of cases, which confirmed the evidence that determination of UmA PI reduces perinatal morbidity and mortality in highly risky obstetric situations.

3. ANALYSIS OF THE DETERMINATION OF MEAN PULSATILITY INDEX OF A.UTERINA AND A. UMBILICALIS ACCORDING TO THE SEVERITY OF THE PREECLAMPSIA

In severe preeclampsia high UtA PI was detected in 84.62% of the examined patients. These results confirm that determination of Doppler UtA PI can identify women at increased risk of developing complicated pregnancy associated with uterine placental deficiency [330,273,274]. The established regularities suggest that the higher the severity of PE, the more of impaired uterine placental circulation is present and it leads to placental ischemia, reduced uterine perfusion pressure, and abnormal ECM vascular and uterine remodelling. The high rate of cases (84.62%) with severe PE, where high UtA PI is found present an argument in favour of this hypothesis.

In mild preeclampsia, 93.1% of cases were with normal UmA PI. The above does not indicate significant disturbance of foetus - placental circulation and intrauterine delayed foetal growth. This also confirms data from a number of studies that the determination of UmA PI reduces perinatal morbidity and mortality in high-risk obstetric situations.

4. ECHOCARDIOGRAPHIC EXAMINATION IN PREECLAMPSIA

Results from transthoracic echocardiography in preeclampsia showed diastolic blood dysfunction, increased left ventricular end diastolic diameter, left ventricular end systolic diameter, ventricular septum thickness and left ventricular rear wall thickness compared to the controls. Although these parameters fall within the reference limits, the listed echocardiographic parameters were significantly higher in preeclampsia compared to these in women with normal pregnancy. Furthermore, the inter-ventricular septum thickness and the posterior wall thickness of the left ventricle showed correlation with podocalyxin levels.

In a large number of studies, echocardiographic assessment of patients with preeclampsia has been reported to show findings such as: increased ventricular mass, left ventricular hypertrophy, enlargement of left atrium and diastolic dysfunction [275,276,331, 277,278,322,333].

In this study, a link was found between PCX and specific echocardiographic parameters such as the inter-ventricular septum thickness and left ventricle posterior wall thickness. These results suggest possible interaction between PCX and certain cardiac structures. However, in order to find concrete structural changes, more specific methods such as immunohistochemistry or immunocytochemistry, with tissue sampling analysis and evaluation of podocalyxin expression in tissue samples are required. This would help to assess exactly which tissues release the podocalyxin serum in preeclampsia.

The above factors are involved in the central routes of development and the progression of preeclampsia. **The evidence found in our study suggest that interaction between PCX and specific cardiac structures may contribute to pathological cardiovascular changes, development of hypertension during pregnancy and subsequent preeclampsia.**

5. DETERMINATION OF MMP-8, -13 COLLAGENASES IN PREECLAMPSIA

In our study, the serum MPM-8 levels in preeclampsia were examined for the first time. Patients showed higher levels of MPM-8 and the differences against the women with normal pregnancy were not significant.

Collagenases MMP-8 is responsible mainly for the degradation of interstitial collagen types I, II and III. Current results suggest that the absence of significant changes in the concentration of this metalloproteinases is associated with its decreased activity (indicating and delayed exchange of this type of collagen). **Taken together, these data raise the prospect that MMP-8 is not actively involved in the pathogenesis of preeclampsia.**

Regarding the MMP-13, it was found that its levels in preeclampsia patients were higher than in women with normal pregnancy, these values were not significant. The results of M. Laskovska [146], who in 2017 established statistically non-significantly increased MMP-13 levels in both early and late PE versus uncomplicated pregnancy control group.

6. DETERMINATION OF MMP-2, -9 GELATINASES IN PREECLAMPSIA

While examining the MMP-2 in PE, we found that the serum levels of MMP-2 in women with preeclampsia were statistically significantly higher than the levels in women with normal pregnancy. In addition, MMP-2 showed correlation with the mean pulsatility index of the uterine artery. These results indicate abnormal type IV collagen exchange in patients with preeclampsia and abnormal remodelling. The following authors established increased MMP-2 values through ELISA: Laskovska 2017 (in sera) [146], Palay (in plasma) [153], Montegrana (in sera) [156], Narumia 2001 (in plasma) [232].

Elevations in MMP-2 in PE may contribute to endothelial cell activation, increased vascular MMP-2 expression, cleavage of endothelin-1 and vasoconstriction with increased transmural pressure. As a result, vascular reactivity and vascular remodelling were impaired. The increased levels of TIMP-2 were also in support of this hypothesis. The elevated MMP-2 values in PE indicate enhanced type IV collagen degradation of the vascular basal membrane - a process which should not be observed at this stage of pregnancy. As a result, reduced vasodilation, increased vasoconstriction and development of hypertension was observed during pregnancy complicated by preeclampsia. Moreover, elevated MMP-2 levels are also associated with increase in UtA PI, which indicates for favoured vasoconstriction, increased vascular resistance, placental ischemia, hypertension development and preeclampsia. Elevated MMP-2 levels may contribute to vascular dysfunction secondary to hypertension-related mechanical stress on the vascular wall. Thus, the disrupted regulation of the MMP-2/TIMP-2 complex plays a major role not only in remodelling of the spiral arteries by controlling cyto-trophoblast invasion, but also influencing the structure/function of uterine arteries also after 20th gestation week.

The MMP-2 gelatinase is responsible for the degradation of Type I collagen and mostly of Type IV, while MMP-9 cannot directly lye Type I collagen and degradesType IV collagen. MMP-2 is an important regulator of vascular remodelling during pregnancy. AMR-2 dysregulation is associated with abnormal vasodilation, placental perfusion and uterine enlargement in preeclampsia. Therefore, the

increased levels of MMP-2 in our study are mainly related to the pathological degradation of Type IV collagen, a major structural component building the basal membrane. As activated MMP-2 may increase vasoconstriction levels and decrease vasodilation levels, we link current results with compromised remodelling of uterine arteries, increased perfusion pressure, placental ischemia, and the development of pregnancy hypertension that is complicated by preeclampsia. This hypothesis is also supported by the correlation found between MMP-2 and the increased values of the mean pulsatility index of the uterine artery, demonstrating increased vascular resistance.

Remodelling of uterine spiral arteries is important for the normal course of pregnancy. In this respect, the balance between MMPs and TIMPs plays an important role in maintaining vascular vasodilation. In preeclampsia there was abnormal exchange of Type IV collagen and MMP-2 dysfunction and affected strucutures of the uterine and the uterine arteries. Although the pathogenesis of preeclampsia is not fully understood, abnormal placental perfusion leading to reduced uterine perfusion pressure is a pathogenetic pathway that is important for the development of maternal endothelial dysfunction. How exactly the impaired MMP-2/TIMP-2 balance results in abnormal uterine placental remodelling and vasoconstriction associated with placental ischemia and preeclampsia and what the mechanisms of the dysregulated MMP-2 are questions of great importance. It requires larger and long term studies with more specific methods and serial measurements of these parameters at different time points, which would allow a more precise determination of the link of the above-mentioned indicators with the appearance of preeclampsia. This would clarify not only the participation of MMP-2 in the development of preeclampsia but also the possibility of MMP-2 as a potential future therapeutic target.

The following studies [146,151,153,162,233] also suggest that MMP-2 can be activated by various factors in preeclampsia. Increased plasma levels of MMPs may result in modification of a vasoactive peptides. Activated MMP-2 is able to raise vasoconstriction levels and reduce vasodilation levels.

Our study gives the prospect that MMP-2 is relevant to pathologically increased vascular resistance of the uterine arteries in preeclampsia.

The results with respect to MMP-9 indicate that the levels in women with preeclampsia were lower than those in women with normal pregnancy, and these values were not significant. There are no unequivocal results in the literature regarding the MMP-9. The researchers Louison and co-authors 2014 [233] examined plasma of women with PE and GH (gestational hypertension), using the ELISA method and found lowered MMP-9 levels. The results of Thaibgy and collaborators are similar. 2005 [234] examination of patients with GH (in plasma) as well as for SCOPE 2014 study (in plasma) [236] for PE as well as for Montegrana (in sera) [156] for PE. The opposite results were received by Paley and collaborators, [153] in examination of plasma of women with GH and PE, where increased MMP-9 activity in GH cases is registered. Ab Hamid and co-authors 2012 [235] using the ELISA method, conducted examination of sera from women with GH were conducted and elevated MMP-9 levels were established. The results of Meng and collaborators were similar. 2016 [238] but these included patients with PE, and found elevated serum MMP-9 levels using the ELISA method.

The MPM-9 genotypes and haplotypes were associated with changes in plasma MMP-9 and TIMP-1 plasma concentrations in preeclampsia. It has been proven, that MMP-9 genetic polymorphisms (g.-1562C>T and g.-90(CA)13-25 can modify plasma MMP-9 and TIMP-1 levels and the response to antihypertensive therapy in patients with preeclampsia and gestational hypertension [153]. The plasma MMP-9 concentrations are not affected by g.-1562C>T genotypes or haplotypes in cases of GH and PE, except in cases of polymorphism g.-90(CA)13-25: Patients with GH and LH-genotype for this polymorphism had higher MMP-9 levels than those with other genotypes. In addition, the T allele for g.1562C>T polymorphism and H4 haplotype (combining T and H allele) are related to GH and lack of response to antihypertensive therapy in GH. H2 haplotype (combining C and H allele) is associated with a lack of response to antihypertensive therapy in PE, but not in cases of GH. The results of the current study on the lower serum MMP-9 in patients versus controls were consistent with those of the Louison and co-authors. 2014(examination of patients

with PE and GH) as well as for SCOPE 2014 study (in plasma) [233 236] in PE as well as for Montegrana (in sera in PE) [156]. A possible explanation for the absence of statistical significance in our study is the assumption that in the examined population of preeclampsia patients the MMP-9 genotype g.-1562C>T prevails, where MMP-9 levels do not increase significantly. This also sets new horizons for future research into genetic polymorphisms of MMP-9 in women with preeclampsia in Bulgaria that include both quantitative measurement of the indicator and genetic analysis.

7. DETERMINATION OF TIMP-1, -2 IN PREECLAMPSIA

The tissue inhibitor of matrix metalloproteinases (TIMP-1) is a major endogenous inhibitor of MMP-9. The results of the TIMP-1 determination in this study showed lower serum TIMP-1 levels in preeclampsia cases compared to the levels in women with normal pregnancy, with no significant difference. The TIMP-1 values established were in contrast to the reports of the researchers Louison and collaborators 2014 [233] for tested plasma of women with PE and gestational hypertension (GH) by ELISA, where elevated TIMP-1 levels in patients with preeclampsia with TG genotype were established, whereas Gupta and collaborators 2016 [147] tested sera of women with PE by ELISA and did not establish differences in second and third trimesters with respect to TIMP-1. Montegrana and collaborators 2009 [15] as well as Paley and co-authors 2008, 2012 [152, 153] examined serum and plasma of patients with PE and found elevated TIMP-1 levels. Our results are in line with those of Gupta and collaborators 2016. The lower serum levels of TIMP-1 in preeclampsia, although insignificant, may be due to the fact that TIMP-1 polymorphisms (g.–9830T>G, rs2070584) modify the plasma MMP-9 and TIMP-1 levels and the response to antihypertensive therapy in both PE and GH. The patients with PE and TG-genotype had higher TIMP-1 levels than those with TT-genotype. G-allele and GG-genotype are associated with both PE and response to antihypertensive therapy in PE, but not in GH. A possible explanation for the absence of significant difference of TIMP-1 in patients versus controls in the examined population with preeclampsia is the probable predomination of TT-genotype in which TIMP-1 levels do not increase

significantly [153]. In addition, there is an option for a certain difference in the gestational age of patients at the point of sampling time in the different studies.

It is known that TIMP-2 primarily inhibits MMP-2. Our data present increased TIMP-2 values in preeclampsia compared to normal pregnancy, with no significant difference. These results confirm the established TIMP-2 values for preeclampsia in previous studies. Elevated TIMP-2 levels were detected, using the ELISA method, by Paley authors too (in plasma of GH and PE), Montegrans (in serum in PE cases). It is curious that even without a significant increase in TIMP-2, the MMP-2/TIMP-2 ratio is changed, favoring the expression of MMP-2. The MMP-2 activity in this case may be modulated by the interaction between nitric oxide and reactive oxygen radicals or other tissue inhibitor which raises the question whether MMP-2 in preeclampsia is only regulated by TIMP-2 or that balance is secured through routes, for example, in combination with TIMP-3 or TIMP-4 or with inhibitors unknown so far.

8. EXAMINATION OF SERUM PODOCALYXIN IN PREECLAMPSIA CASES

Our study is one of the few studies that assessed the podocalyxin in preeclampsia. The results of this study are in line with previous reports demonstrating that the determination of serum podocalyxin (PCX) levels can contribute to the PE diagnostic process, and this is largely true for the determination of early preeclampsia [264,265,296,334].

However, our data indicate **for the first time that a threshold value for serum podocalyxin may distinguish preeclampsia from normal pregnancy.**

In this study, women with normal pregnancy, where podocalyxin levels are greater than 3 ng/ml, were found **to be at higher risk for the development of preeclampsia. Further more, elevated serum podocalyxin levels indicate a 3-fold higher likelihood of preeclampsia compared to healthy pregnant women without elevated levels.**

In addition, a correlation between podocalyxin and systolic and diastolic blood pressure was found **to reveal a possible contribution of PCX to the pathophysiology of hypertension during pregnancy and the development of preeclampsia.** Elevated podocalyxin levels can play **an important role in the**

process of increasing blood pressure during pregnancy, thus contributing to the development of preeclampsia.

The correlation between maternal echocardiographic parameters of the inter-ventricular septum thickness, left ventricular posterior wall thickness, and podocalyxin showed **interaction between podocalyxin and these cardiac structures. Podocalyxin may favour changes in specific cardiac structures leading to hypertension during pregnancy, resulting in preeclampsia.**

This study has shown important results. This was the first study that showed a significant relationship between PCX levels and blood pressure values. Our data show that podocalyxin can play an important role in raising blood pressure during pregnancy. The above factors are involved in the central routes of development and the progression of preeclampsia. Given this, we assume that **the podocalyxin may be involved in the pathogenic mechanisms of hypertension during pregnancy, and is a promising marker of diagnosis and prediction of preeclampsia.**

VII. CONCLUSIONS

1. The mean pulsatility index of the uterine and umbilical artery were determined, using Doppler, in preeclampsia and in normal pregnancy. The mean pulsatility index of uterine artery and the mean pulsatility index of umbilical artery are significantly higher in preeclampsia versus normal pregnancy.

2. In early preeclampsia high mean pulsatility index of the uterine artery was detected in 71.43% of cases, while 28.57% of the cases had a normal index. These results confirm disrupted trophoblast invasion of spiral arteries in early preeclampsia. In late preeclampsia a high mean pulsatility index of the umbilical artery was detected in 85.37% of cases, while 14.63% had a normal index indicating significant foetus - placental circulation disturbance in late preeclampsia.

3. In severe preeclampsia high mean pulsatility index of the uterine artery was detected in 84.62% of cases, while 15.38% of the cases had a normal index. The established regularities suggest that the higher the severity of PE, the more of impaired uterine placental circulation is present and it leads to placental ischemia, reduced uterine perfusion pressure, and abnormal ECM vascular and uterine remodelling. In mild preeclampsia high mean pulsatility index of the umbilical artery was detected in 6. 9% of cases, while 93.1% had a normal index, which confirmed the evidence that determination of UmA PI reduces perinatal morbidity and mortality in highly risky obstetric situations.

4. Hemodynamics changes in preeclampsia have been assessed by transthoracic echocardiography. The following were found: diastolic blood dysfunction, increased left ventricular end diastolic diameter, left ventricular end systolic diameter, ventricular septum thickness and left ventricular rear wall thickness. Although these parameters fall within the reference limits, the listed echocardiographic parameters were significantly higher in preeclampsia compared to these in women with normal pregnancy.

5. Determination of serum levels of collagenase MMP-8, -13 in preeclampsia. The conducted examinations have shown that serum levels of MMP-8, -13 do not show a statistically significant difference in preeclampsia compared to normal pregnancy.

6. Determination of serum levels of gelatinases MMP-2, -9 in preeclampsia. MMP-9 levels in women with normal pregnancy were higher than the levels in patients with preeclampsia, and these values were not significant. Conducted studies have shown that serum levels of MMP-2 in patients with preeclampsia were statistically significantly higher compared to the levels in women with normal pregnancy. A correlation between changes in serum levels MMP-2 and mean pulsatility index of the uterine artery was also found. MMP-2 has a relevance to pathogenetic mechanisms in the development of preeclampsia.

7. TIMP-1, -2 were examined. The conducted examinations have shown that serum levels of TIMP-1, -2 do not show a statistically significant difference in preeclampsia compared to normal pregnancy.

8. Conducted studies have shown that serum levels of podocalyxin in patients with preeclampsia were statistically significantly higher compared to the levels in women with normal pregnancies. A threshold value of 3ng/ml for serum podocalyxin was defined, which distinguishes preeclampsia from normal pregnancy. The developed logistic regression model showed that elevated serum podocalyxin levels indicate a 3-fold higher likelihood of preeclampsia compared to healthy pregnant women without elevated levels. PCX is involved in the pathophysiology of hypertension during pregnancy and the development of preeclampsia. The relationships between Doppler ultrasound indicators-mean pulsatility index of the uterine and umbilical artery and podocalyxin revealed vascular changes associated with pathological remodelling of these arteries in preeclampsia.

VIII. CONTRIBUTION

Original contributions

1. The frequency distribution of the mean pulsatility index of the uterine and umbilical artery over the duration of the manifestations of preeclampsia and according to its severity has been analysed.

2. A study of the podocalyxin in patients with preeclampsia has been conducted. For a first time a threshold value of 3ng/ml for serum podocalyxin was defined, which distinguishes preeclampsia from normal pregnancy. The developed logistic regression model showed that elevated serum podocalyxin levels indicate a 3-fold higher likelihood of preeclampsia compared to healthy pregnant women without elevated levels. Based on these results, this study provides grounds for considering that serum podocalyxin may be used as an indication of diagnosis and prediction of preeclampsia.

3. For the first time, changes in podocalyxin levels have been found to play a role in the blood pressure increase during pregnancy, thus related to the pathogenesis of preeclampsia. The correlation between podocalyxin and the systolic and diastolic blood pressure is the evidence for that.

4. For the first time, an interaction between PCX and specific cardiac structures is indicated, based on an open correlation between maternal echocardiographic parameters, the inter-ventricular septum thickness and the posterior wall thickness of the left ventricle and the podocalyxin. The relation between Doppler ultrasound indicators mean pulsatility index of the uterine and umbilical artery and podocalyxin revealed vascular changes associated with pathological remodelling of these arteries in preeclampsia.

5. For the first time in Bulgaria, serum levels of gelatinases MMP-2, -9, collagenases MMP-8, -13 and tissue inhibitors were determined: TIMP-1, -2 in preeclampsia.

6. A correlation between changes in serum levels MMP-2 and mean pulsatility index of the uterine artery was found for a first time. It has been established that MMP-2 is related to pathologically elevated vascular resistance in preeclampsia, vascular remodelling, development and progression of preeclampsia, with the above mentioned metalloproteinases playing an important role in the pathogenetic mechanisms for the development of this hypertensive state of pregnancy.

7. MMP-8 in preeclampsia was examined for a first time . The analysis performed have shown that serum levels of MMP-2 do not show a statistically significant difference in preeclampsia compared to normal pregnancy. MMP-8 has been found not to be directly involved in the pathogenesis of preeclampsia.

Confirming contributions

1. It has been confirmed, that the mean pulsatility index of uterine artery and the mean pulsatility index of umbilical artery were significantly higher in preeclampsia, compared to normal pregnancy.

2. Examination of maternal echocardiography parameters in preeclampsia have been conducted. Although these parameters fall within the reference limits, the examined echocardiographic parameters were significantly higher in preeclampsia compared to these in women with normal pregnancy.

3. It is confirmed that serum levels of podocalyxin in patients with preeclampsia were significantly increased compared to these of women with normal pregnancy, with podocalyxin levels in women with early preeclampsia being statistically significantly higher than in women with late preeclampsia.

IX. PUBLICATIONS RELATED TO DISSERTATION

1. **Popovski N**, Nikolov A. Biomarkers for Early Detection of Hypertensive Disorders in Pregnancy: Current Applications and Future Directions- The Role of Extracellular Matrix. Biomedical Journal of Scientific & Technical Research 2019;16 (2): 1-3 ISSN:2574-1241

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3. **Popovski NK**, NikolovAG, Popov YD, Blazheva S. Relationship between elevated maternal serum podocalyxin concentrations with blood pressure values and routine laboratory parameters in preeclampsia. Journal of Biomedical and Clinical Research. Issue 2, volume 14, 2021, **(under print)** (ISSN: 1313-9053)

4. **Popski N**, Nikolov A, Popov Y, Blazheva C. Comparison of serum levels of MMP-2,-9,-8 and their inhibitors: TIMP-1 and 2 in women with preeclampsia and normal pregnancy. Obstetrics and Gynecology, volume 60, issue 2, 2021; 17-23 ISSN: 0324 0959

5. Popovski NK, Popov. Y. Nikolov AG Atypical case of surgical corrected combined atrial septal defect with persistent left-right shunt in preeclampsia patient. Obstetrics and Gynecology 2020; 59(1):
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X. SCIENTIFIC COMMUNICATIONS RELATED TO DISSERTATION

1. **Popovski N**, Nikolov A. Fulminant severe HELLP syndrome in early onset preeclampsia: A case report. Pregnany Hypersensision, volume 17, Supplement 1, pages S23-S24 **(IF-1.992)** ISSN: 2210-7789. EuroISSHP Conference 2019, Lund, Sweden, October 2, 2019 - October 4, 2019

2. **Popovski N**, Nikolov A. Gestational Hypertension: Current Immunological Markers and Future Trends. Journal of Biomedical and Clinical Research. Vol. 12 number 1, Supplement 2, 2019, 25 (ISSN: 1313-9053) Jubilee Scientific Conference
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3. **Popski N**, Nikolov A. Prognostic value of biomarkers for hypertensive pregnancy disorders -current application and new trends. Abstracts from XXXVII National Midwifery Conference 20-23 June 2019 Riviera, Obstetrics and Gynecology 2019 Suppl. 9, ISSN ISSN0324-0959

4. **Nikola K. Popovski**, Asparuh G. Nikolov. Diminished serum TIMP-2 levels are associated with severe preeclampsia: a cross sectional study. Proceedings of BAFA's Annual International Scientific Conference, 08 Oct 2021, 365-371 ISSN 2738-716X

Participation in university research projects

1. Project N1/2019- Changes in serum markers of cardiac extracellular matrix (cardiac collagen exchange) and myocardial remodelling in patients with essential hypertension and heart failure with suppressed marginal ejection fraction. Head: Assoc. Prof. Dr. Asparuh Nikolov

2. Project 1/2020- Changes in the levels of circulating Type I collagen, III degraded biomarkers, copeptin, podocalyxin and echographic criteria in hypertensive conditions during pregnancy. HEAD: Assoc. Prof. Dr. Asparuh Nikolov

XI QUOTATIONS

1. Alka C. Bapat, Rupesh Kashikar, Shashi R. Goyal. Role of dual biomarkers and uterine artery doppler study in predicting PIH and IUGR in antenatal patients registered in a tertiary care centre. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2021 Mar;10(3):982-987

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