folia medica

Folia Medica 63(6):948-57
DOI: 10.3897/folmed.63.e60055

Original Article

Urinary Nephrin and Podocalyxin Levels as Predictors of Pre-eclampsia in High-Risk Pregnant Women

Irena Kostovska, Katerina T. Trajkovska, Ognen Kostovski, Danica Labudovic

Department of Medical and Experimental Biochemistry, Faculty of Medicine, Ss Cyril and Methodius University, Skopje, Republic of North Macedonia

Corresponding author: Irena Kostovska, Department of Medical and Experimental Biochemistry, Faculty of Medicine, Ss Cyril and Methodius University, 50 Divizija 6, 1000 Skopje, Republic of North Macedonia; E-mail: irenakostovska22@yahoo.com

Received: 26 Oct 2020 ♦ **Accepted:** 8 Feb 2021 ♦ **Published:** 31 Dec 2021

Citation: Kostovska I, Trajkovska KT, Kostovski O, Labudovic D. Urinary nephrin and podocalyxin levels as predictors of pre-eclampsia in high-risk pregnant women. Folia Med (Plovdiv) 2021;63(6):948-57. doi: 10.3897/folmed.63.e60055.

Abstract

Introduction: Pre-eclampsia (PE) is characterized by new-onset hypertension and proteinuria. Damage of podocyte cells has been reported in pre-eclamptic women, thus podocyte specific proteins such as nephrin and podocalyxin could be useful biomarkers in PE.

Aim: To investigate the role of urinary nephrin (u-nephrin) and urinary podocalyxin (u-PDX) levels in predicting PE in women with a high-risk pregnancy.

Materials and methods: We included 101 pregnant women in this study and allocated them into three groups: group 1 included pregnant women at high risk of developing PE (n=41), group 2 - pregnant women with PE (n=30), and group 3 was the controls including healthy pregnant women (n=30). The inclusion criteria for women with PE were de novo hypertension >140/90 mm Hg, proteinuria >300 mg/24 hours, and presence of edema after 20 weeks of gestation, while the exclusion criteria were a history of renal diseases and pregnant women younger than 18. Inclusion criteria for the group of women with a high-risk pregnancy was gestational week >15, a history of PE in a previous pregnancy, pre-existing diabetes type 1 or 2, pre-existing hypertension, multiple gestations, prior placental abruption, obesity women, nulliparity, maternal age >35 years, and a family history of PE. The study was conducted from March 2016 to May 2017 in the Medical Faculty at the Institute of Medical and Experimental Biochemistry in Skopje. Urine samples were used to measure the nephrin and podocalyxin levels using immunoenzyme assay, creatinine and microalbumin. Blood samples were collected for biochemical analyses.

Results: U-nephrin levels were elevated in 96.7% of women with PE, and 73% of women with a high-risk pregnancy. U-PDX levels were elevated in 63% of the women with PE and 100% of the women with a high-risk pregnancy. U-nephrin and u-PDX levels were significantly increased in women with a high-risk pregnancy and women with PE compared with a control group (p<0.001). A significant difference was found between the subgroups of pregnant women classified according to gestational age in their u-nephrin and u-PDX levels. There was a significant positive correlation between the levels of both markers and glomerular filtration rate, and significant negative correlation between the levels of both markers and gestational age. ROC analysis revealed that the cut-off value of 304.6 ng/ml of u-nephrin had a sensitivity (Se) of 96.7%, specificity (Sp) of 96.7% (for both Se and Sp 95% confidence interval (CI) 82.8-99.9), while the cut-off value of 59.5 ng/ml of u-PDX had a sensitivity of 100% and Sp of 93.3% (Se - 95% CI 88.4-100, Sp - 95% CI 77.9-99.2), in distinguishing women with PE and healthy pregnancies. Both markers showed excellent clinical utility (CUI≥0.81), for u-nephrin (CUI+ and CUI− is 0.934), for u-PDX (CUI+ is 0.938; CUI− is 0.933).

Conclusions: U-nephrin and U-PDX levels could be useful as predictors of PE in women with a high-risk pregnancy.

Keywords

high-risk pregnancy, nephrin, podocalyxin, pre-eclampsia

INTRODUCTION

Pre-eclampsia (PE) is a leading cause of maternal and perinatal morbidity and mortality worldwide, particularly in low- and middle-income countries due to poor antenatal care of pregnant women. Pre-eclampsia affects 2–8% of pregnancies, overall, 10-15% of all direct maternal deaths are associated with PE in low and middle-income countries.[1-3] In the past decade, the incidence of PE has increased as a result of the increased prevalence of predisposing factors, namely maternal age, chronic hypertension, PE in a previous pregnancy or a family history of PE, diabetes, pre-pregnancy obesity, and multiple gestations.[4] PE is defined as the presence of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg on two occasions at least 4 hours apart in previously normotensive women, and is accompanied by one or more of the following new-onset conditions at or after 20 weeks of gestation:

1. Proteinuria (≥300 mg/24 hours; protein:creatinine ratio of ≥30 mg/mol; or a urine dipstick protein of ≥2+);

2. Evidence of other maternal organ dysfunction, including acute kidney injury (creatinine ≥90 μmol/L; 1 mg/dL); liver involvement (elevated transaminases, alanine aminotransferase or aspartate aminotransferase >40 IU/L) with or without right upper quadrant or epigastric abdominal pain; neurological complications (e.g. eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, and persistent visual scotomata); or hematological complications (thrombocytopenia - platelet count <150 000/μL, disseminated intravascular coagulation, hemolysis);

3. Uteroplacental dysfunction (such as intrauterine growth restriction, abnormal umbilical artery Doppler waveform analysis, or stillbirth).[5]

PE clinically has been recognized since the time of Hippocrates, but the etiology and pathophysiology of this disease remains enigmatic.[6] PE is still a significant public health threat, and the only effective treatment remains delivery of the baby, by induction of labour or by prelabour cesarean section (CS), but novel therapeutic strategies such as aspirin and statins have been recommended as a preventive therapy for preterm pre-eclampsia. Accordingly, early prediction of PE in women with a high-risk pregnancy might prevent progression of disease and reduce maternal and fetal morbidity and mortality. A high-risk pregnancy is defined by presence of one or more of the following risk factors: a family history of pre-eclampsia, nulliparity, multiple pregnancy, advanced maternal age, in vitro fertilization, maternal comorbidities such as diabetes mellitus, chronic hypertension, obesity, chronic kidney disease, history of acute kidney injury or systemic lupus erythematosus, previous placental abruption or intrauterine fetal growth restriction, trisomy 13, and molar pregnancies.[7] PE screening should be performed in the first trimester of pregnancy, including assessment of risk for development of PE, measurement of blood pressure, ultrasound Doppler of uterine arteries, and blood and urine biochemical analysis.[8] Over the years, numerous studies have attempted to define biomarkers for PE screening, diagnosis, and treatment. A screening test need to be highly sensitive and specific and must provide an adequate positive predictive value. Today, several potential biomarkers have been described, alone or in combination, that might meet these criteria such as increased levels of soluble fms-like tyrosine kinase 1 (sFlt-1) and reduced levels of placental growth factor (PlGF)[9,10], serum alpha-fetoprotein (AFP)[11], reduced plasma levels of placental protein 13 (PP13) and urinary glycosaminoglycans/proteoglycans (GAGs and PGs)[12,13], low maternal serum levels of pregnancy-associated plasma protein-A (PAPP-A)[14], endoglin, cystatin C and free fetal hemoglobin (HbF)[15], vascular endothelial growth factor (VEGF), P-selectin, A disintegrin and metalloprotease 12 (ADAM12), Pentraxin 3 (PTX3)[16], and kidney markers[17]. Although different biomarkers have been described as promising screening tools of PE, data came often with heterogeneous results, therefore, reliable biomarkers for prediction of PE in women with high risk-pregnancy are required.

Endothelial dysfunction plays a major role in the pathogenesis of PE, and multiorgan involvement including kidneys.[18] Recent data suggest that podocyte damage has an important role to play in the renal involvement in PE including structural damage of podocyte cells due to increased oxidative stress[19] dysregulation, and detachment from the glomerular basement membrane (GBM) and their shedding through urine – podocyturia.[20] Podocyturia had a significantly greater sensitivity and specificity (100% Se and 100% Sp) for the subsequent diagnosis of PE than any single angiogenic marker, or a combination thereof, thus podocyturia may allow prediction of PE in women with high-risk pregnancy.[18,21] Podocytes are terminally differentiated visceral epithelial cells, which form the glomerular filtration barrier together with the opposing fenestrated endothelium in the vascular space and GBM in between. Podocyte cells form the final barrier to plasma protein leakage. Nephrin as the main component of filtration slit-diaphragm forms a physical barrier, while podocalyxin as a sialoglycoprotein forms an electrostatic barrier to plasma proteins.[22] Nephrin and podocalyxin appear in urine in the early course of renal dysfunction in PE and may precede the microalbuminuria rendering them as possible useful predictive markers of PE. The most recent studies have described the role of urinary nephrin and podocalyxin as predictive biomarkers of PE.[23-26] To our knowledge, simultaneous measurement of urinary nephrin (u-nephrin) and urinary podocalyxin (u-PDX) in a pregnancy complicated by PE, various high-risk pregnancies, and a healthy pregnancy has not been attempted.

AIM

This study aimed to evaluate the potential role of u-nephrin and u-PDX in predicting PE in women with a high-risk pregnancy.

Folia Medica

MATERIALS AND METHODS

In this cross-sectional study, a total of 101 pregnant women were included and classified into three groups: pregnant women at high risk of developing pre-eclampsia (n=41), pregnant women with pre-eclampsia (n=30), and healthy pregnant women (n=30) as a control group. The study was conducted from March 2016 to May 2017 in the Medical Faculty at the Institute of Medical and Experimental Biochemistry in Skopje, following the ethical principles of the current Declaration of Helsinki, and approved by the Ethical Committee of the Faculty of Medicine in Skopje, North Macedonia (No 03-5515/8 from 09.12.2015). Written informed consent was obtained for each participant before enrolment into the study. Women with PE were recruited from the University Clinic of Gynecology and Obstetrics at the Medical Faculty in Skopje. Inclusion criteria for this group of women were de novo hypertension >140/90 mm Hg, proteinuria >300 mg/24 hours, and presence of edema after 20 weeks of gestation.[5] This group consisted of 10 women in the second trimester (15-26 weeks of gestation) and 20 women in the third trimester (27-38 weeks of gestation) of pregnancy. Exclusion criteria were a history for renal diseases and pregnant women younger than 18. The women with PE were used to determine whether u-nephrin and u-PDX levels are elevated in all women with PE and to test the diagnostic performance of u-nephrin and u-PDX in women with PE. The women with a high-risk pregnancy were selected from the University Clinic of Gynecology and Obstetrics at the Medical Faculty in Skopje, and from the Primary Health Care Offices in Skopje, North Macedonia. Inclusion criteria for this group of women were gestational week >15, a history of PE in a previous pregnancy, pre-existing diabetes type 1 or 2 (diagnosed at least 20 weeks before pregnancy), pre-existing hypertension (blood pressure >140/90 mm Hg detected at least 20 weeks before pregnancy), multiple gestations, prior placental abruption, obesity women (BMI >30 kg/m^2), nulliparity, maternal age >35 years, and a family history of PE.[27] This group consisted of 18 women in the second trimester and 23 women in the third trimester of pregnancy. The healthy controls were selected from the Primary Health Care Offices and this group consisted of 15 pregnant women in the second and 15 pregnant women in the third trimester of pregnancy. The control group consisted of healthy pregnant women or women with low-risk pregnancy which means there were no active complications and that there were no maternal or fetal factors that increased the risk for complications.

As materials, we used the first midstream morning urine and venous blood. Urine samples (10 ml) were collected in plastic clean tubes, without preservatives. Blood samples were collected in 1.5 ml Eppendorf tubes, and centrifuged for 10 min at 3.000 rpm. First, a chemical urinalysis was performed using dipsticks. Then, urinary microalbumin was measured by the immunoturbidimetric method while the urinary creatinine concentration was measured by Jaffe's reaction on biochemical analyzer ChemWell (2910 Awareness Technology, Inc.). The residual urine samples after centrifugation were stored at −80°C for future measurement of the u-nephrin and u-PDX. Urinary microalbumin-to-creatinine ratio (UM/CR) was determined as the urinary microalbumin concentration divided by the urinary creatinine concentration (mg/g). The glomerular filtration rate (eGFR) was estimated by the Cocroft and Gault formula.[28] U-nephrin and u-PDX were estimated using commercially available ELISA kits (Exocell Inc., Philadelphia, PA). Urine samples were diluted with dilution buffer provided by the ELISA kits, and samples were measured in duplicate. The method for estimation of u-nephrin and u-PDX was an indirect competitive ELISA, wherein polyclonal antibodies against u-nephrin/u-PDX were used. U-nephrin/u-PDX (antigens) and immobilized nephrin/PDX antigens (at the bottom of polystyrene plates) competed for anti-u-nephrin/anti-u-PDX rabbit antibodies. Anti-rabbit horseradish peroxidase (HRP) conjugate was used for detection of bounded antibodies. Unbounded antibodies were removed by washing, and the remaining bounded antibodies to immobilized u-nephrin/u-PDX antigens were measured photometrically at 450 nm wavelength. The intensity of the colour was inversely proportional to the concentration of u-nephrin/u-PDX. The concentration of u-nephrin/u-PDX was read from a standard curve constructed using commercially available standards. The values were expressed as ng/ml.

The blood samples were used for measurement of urea, creatinine, glucose, total protein, and albumin. Biochemical parameters were measured photometrically on biochemical analyzer ChemWell. Medical history and information on age, height, weight, blood pressure, and glycaemic control were obtained by completing questionnaires administered to the patients.

Statistical analysis

Statistical analyses were performed using the statistical package SPSS, version 17.0 and MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium). Kolmogorov-Smirnov test was first performed for assessing whether data were normally distributed or not. All data were not normally distributed, so we used non-parametric statistical tests, Kruskal-Wallis (one-way analysis of variance, ANOVA) to compare the differences between the groups in terms of clinical data between more than two groups, and Mann-Whitney U test - to compare the differences in the groups in terms of clinical data between two groups. Data were expressed as mean ± SD or median. The association between u-nephrin and u-PDX, and eGFR and gestational age were calculated by the Spearman rank-order correlation. The diagnostic performance of u-nephrin and u-PDX in predicting PE was tested and compared by receiver operative characteristic (ROC) analysis. Differences between groups were considered to be statistically significant at $p<0.05$.

RESULTS

Comparison of clinical and biochemical data among groups of pregnant women

We compared the clinical and biochemical data of the groups of pregnant women and found statistically significant differences between groups for the following data: body mass index (BMI), blood glucose, UM/CR, systolic blood pressure (SBP), diastolic blood pressure (DBP), albumin, blood urea, blood creatinine, u-nephrin, and u-PDX. Regarding age, total proteins in serum, and eGFR we did not found statistically significant differences between the evaluated groups of pregnant women. Post-hoc analysis showed statistically significant differences between women with PE and women with a high-risk pregnancy in their blood glucose (p=0.042), UM/CR (p=0.004), u-nephrin (p<0.001), and u-PDX (p<0.001). The comparison of the clinical and biochemical data of the groups of pregnant women is shown in **Table 1**.

Comparison of the u-nephrin / u-PDX levels of the groups of pregnant women

The level of u-nephrin was statistically significantly elevated in groups of women with PE and high-risk pregnancy compared to healthy subjects (p<0.001). The results are shown in **Fig. 1**. The level of u-PDX was statistically significantly elevated in groups of women with PE and women with a high-risk pregnancy compared to healthy subjects (p<0.001). The results are shown in **Fig. 2**.

Comparison of the u-nephrin/u-PDX levels of the subgroups of pregnant women according to gestational age

We found statistically significant differences in the u-nephrin and u-PDX levels between women with PE and women with a high-risk pregnancy in the second and third trimester (u-nephrin, p=0.016, u-PDX p=0.050).

Correlation between u-nephrin/u-PDX levels and eGFR

The correlation between u-nephrin/u-PDX levels and eGFR in groups of pregnant women was weak negative and not statistically significant for u-nephrin (Spearman ρ=−0.152, p=0.129), and also for u-PDX (Spearman ρ=−0.194, p=0.051).

Correlation between u-nephrin/u-PDX levels and gestational age

We found a weak positive and statistically significant correlation between u-nephrin levels and gestational age (Spearman ρ=0.321, p=0.001), also between u-PDX levels and gestational age (Spearman ρ=0.259, p=0.009).

Measures of diagnostic accuracy and clinical utility of u-nephrin and u-PDX in women with PE

Non-parametric ROC analysis was used to assess the diagnostic accuracy of u-nephrin and u-PDX and to calculate

Table 1. Comparison of clinical and biochemical data among groups of pregnant women

	Pregnant women with PE	Women with a high-risk pregnancy	Healthy pregnant women	Kruskal-Wallis
	n=30	n=41	n=30	*p* value
Age (years)	27.7±4.7	29.1±5.6	29.4±6.0	0.376
BMI (kg/m^2)	29.3±4.6	29.9±3.6	25.7±3.3	<0.001
Blood glucose (mmol/L)	5.2±0.5	5.9±1.2	4.6±0.5	<0.001
UM/CR (mg/g)	214.3±160.3	169.0±271.7	15.8±11.0	<0.001
SBP (mm/Hg)	151.8±13.8	145.6±26.6	119±5.4	<0.001
DBP (mm/Hg)	95.1±7.4	90.1±13	77.3±6.0	<0.001
Total proteins (g/L)	67.6±7.2	69±6.2	68.3±5.5	0.562
Albumin (g/L)	34.2±5.1	35.4±4.7	39.1±4.1	0.001
Blood urea (mmol/L)	5.5±1.2	6.3±2.3	5.0±1.9	0.045
Blood creatinine (μmol/L)	70.7±9.2	69.4±11.9	56.8±4.6	<0.001
eGFR (mL/min/1.73 m^2)	91.3±15.1	97.5±26.9	95.5±9.6	0.372
u-nephrin (ng/ml)	1846.7±1248.2	455.9±235.4	151.6±90.1	<0.001
u-PDX (ng/ml)	164.3±93.1	98.7±75.4	27.2±28.8	<0.001

Results are shown as mean ± SD. BMI: body mass index; UM/CR: urinary microalbumin to creatinine ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: (estimated glomerular filtration rate); u-nephrin: urinary nephrin; u-PDX: urinary podocalyxin

Folia Medica

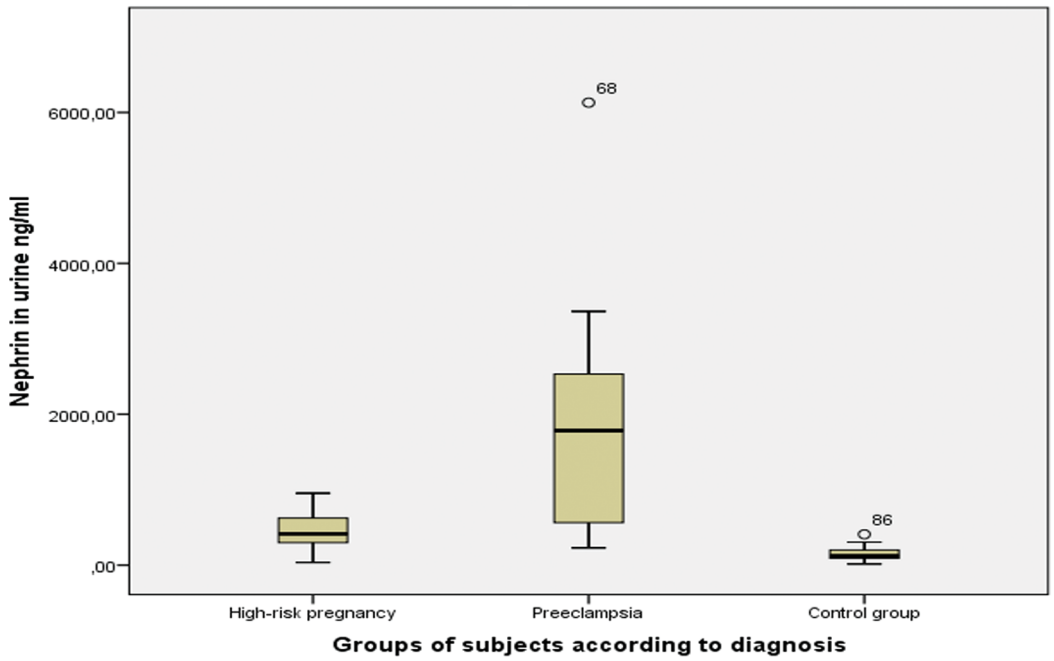

Figure 1. Levels of u-nephrin in groups of pregnant women.

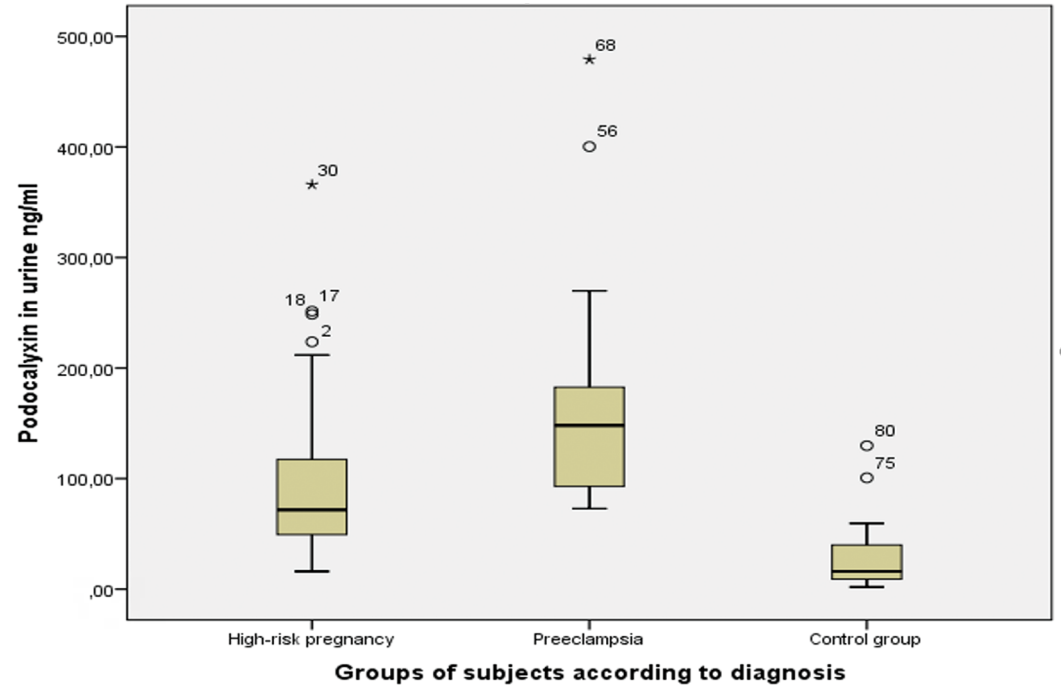

Figure 2. Levels of u-PDX in groups of pregnant women.

the optimal cut-off value of u-nephrin and u-PDX with maximum sensitivity and specificity for prediction of PE. The optimal cut-off value was obtained from the maximum Youden Index (sensitivity + specificity – 1).[29] The clinical utility indexes (CUI+ and CUI–) for u-nephrin and u-PDX were obtained using the Clinical Utility Index Calculator (CUI+ CUI–) created by Dr Alex J. Mitchell.[30] The results are shown in **Table 2** and **Fig. 3**.

Elevated u-nephrin and u-PDX levels in groups of pregnant women

Using ROC analysis, we determined the optimal cut-off for u-nephrin (>304.6 ng/ml), and u-PDX (>59.5 ng/ml). The u-nephrin level was higher than the cut-off value in 95% of women with PE, and in 73% of women with a high-risk pregnancy, while u-PDX level was higher than the cut-off value

in 100% of women with PE and in 63% of the women with a high-risk pregnancy. The results are shown in **Figs 4, 5.**

DISCUSSION

Key findings of our study are high percentage of women with a high-risk pregnancy and women with PE with elevated levels of u-nephrin and u-PDX, statistically significant difference in the u-nephrin levels, respectively u-PDX levels among studied groups of pregnant women and subgroups of pregnant women divided according to gestational age, weak positive but significant correlation between u-nephrin/u-PDX levels and gestational age, high diagnostic sensitivity and specificity, and excellent clinical utility of u-nephrin and u-PDX in prediction of PE. PE as secondary nephropathy includes podocyte damage which leads to proteinuria, and the clinical diagnosis is established principally on the basis of de novo hypertension and proteinuria after 20 weeks' gestation. Recent studies suggested that podocyte shedding in the urine occur earlier than microalbuminuria and proteinuria, indicating that podocyturia is an early sign for PE[31], and more sensitive and specific marker than angiogenic markers[18], with a sensitivity and specificity of 100% in early diagnosis of PE.[20] Elevated u-nephrin and u-PDX levels are associated with podocyte damage in women with PE[23,32], hence, we aimed this study to investigate the role of u-nephrin and u-PDX as predictors of PE in women with a high-risk pregnancy.

Table 2. Diagnostic accuracy and clinical utility of u-nephrin and u-PDX in women with PE

	u-nephrin	u-PDX
AUC	0.990	0.973
95% CI	0.922-1	0.895-0.998
Significance level p (Area=0.5)	<0.0001	<0.0001
Youden index J	0.9333	0.9333
Cut-off value	>304.6	>59.5
Sensitivity (%)	96.7	100
Specificity (%)	96.7	93.3
NPV (%)	96.7	100
PPV (%)	96.7	93.8
Diagnostic effectiveness (accuracy) (%)	96.7	96.7
CUI (+)	0.934	0.938
CUI (−)	0.934	0.933

AUC: the area under the ROC curve; CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value; CUI: clinical utility index; u-nephrin: urinary nephrin; u-PDX: urinary podocalyxin

The results of our study showed significant difference in the u-nephrin and u-PDX levels between the groups of pregnant women. It is particularly important that we found a statistically significant difference in the u-nephrin and u-PDX levels between women with high-risk pregnancy and pregnant women with PE. Similar findings are present in the study of Wang et al. wherein both u-nephrin and u-PDX levels were significantly higher in the women with PE compared to normal pregnancy group.[23] A significantly higher u-PDX levels in women with PE were obtained in another study.[26] It is important to highlight that we found elevated u-nephrin levels in 96% of women with PE, and 73% of women with a high-risk pregnancy, while u-PDX was elevated in 100% of women with PE and 63.4% of

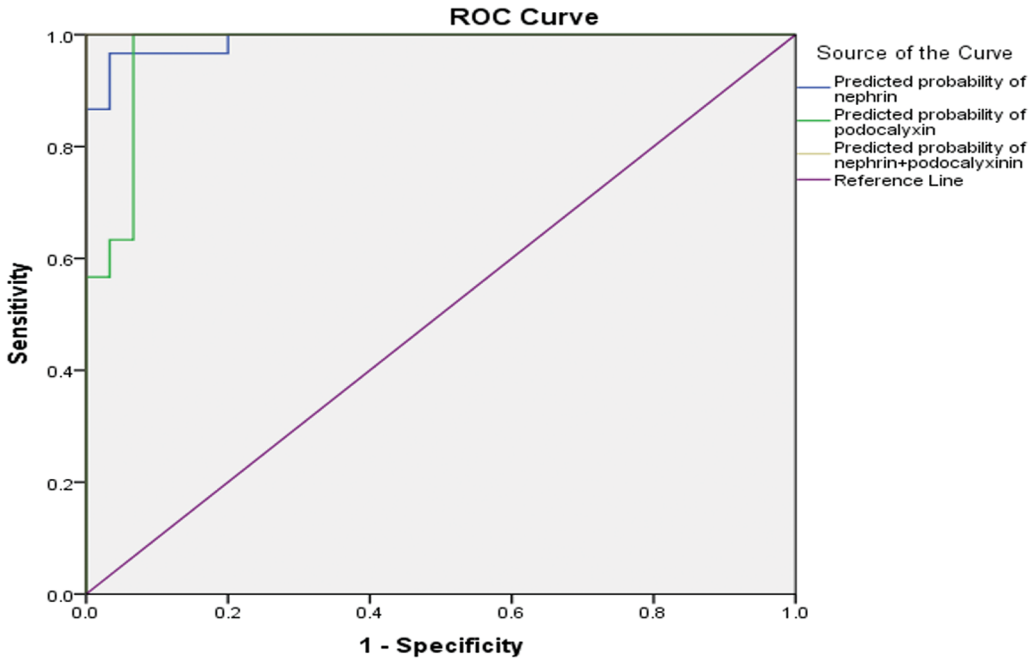

Figure 3. ROC curve of u-nephrin and u-PDX in women with PE.

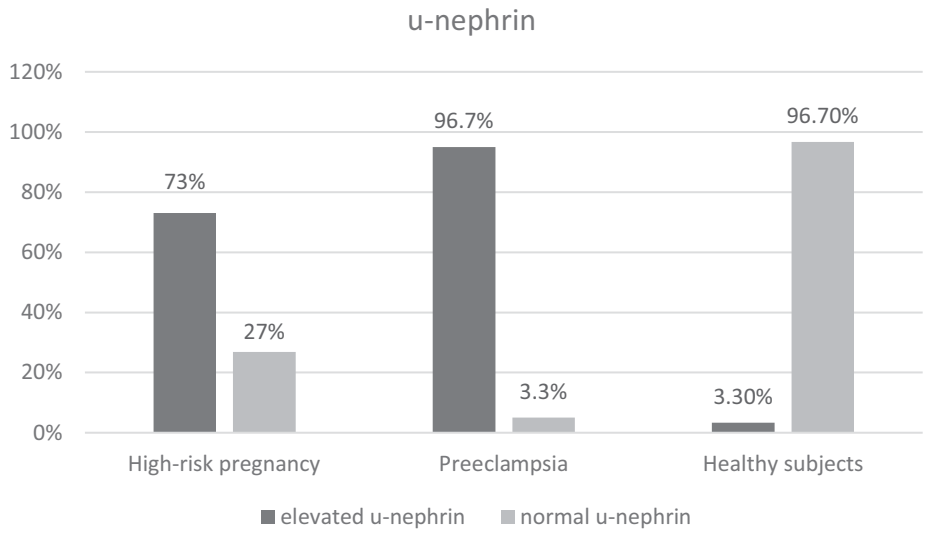

Figure 4. Percentage of pregnant women with elevated u-nephrin level in studied groups.

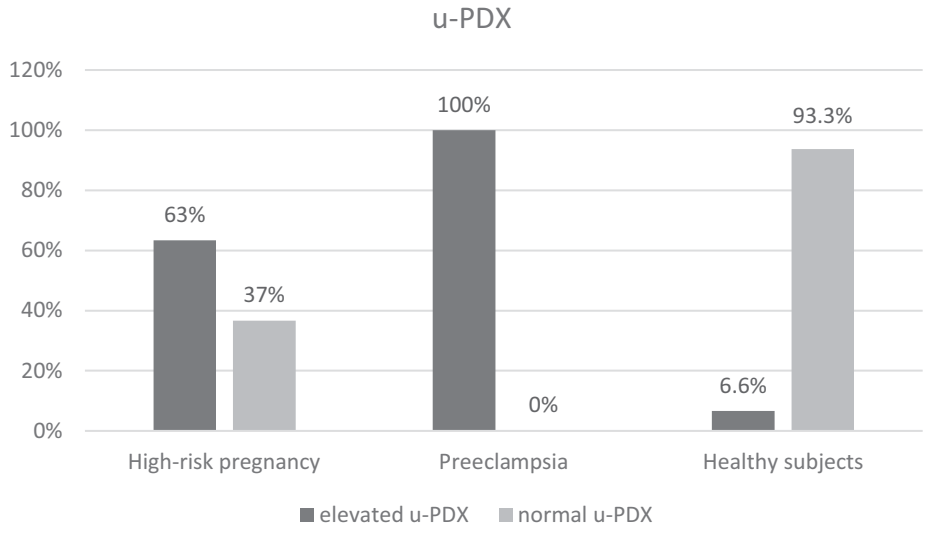

Figure 5. Percentage of pregnant women with elevated u-PDX level in studied groups.

women with a high risk of developing PE. Similar results were demonstrated by another study[18], although measurement of podocyte cell in urine was performed. Comparison of u-nephrin levels, respectively u-PDX levels, showed significant differences among subgroups of pregnant women classified according to gestational age, these findings are in line with another study wherein a significant rise of u-nephrin levels compared to the controls was observed in the third trimester of pregnancy in women who subsequently developed PE.[25] Yung YJ et al. detected that the more advanced the gestational age was, the higher were the levels of u-nephrin.[33] We observed weak positive but significant correlation between u-nephrin levels, respectively u-PDX levels, and gestational age. All these obtained results indicate the significance of both markers in predicting PE in women with a high-risk pregnancy.

Studies performed on normal pregnant women in all trimesters have shown a progressive increase in GFR, while in women with PE, a significant decline in GFR was observed.[34] In our study, no significant correlation between u-nephrin levels, respectively u-PDX levels and GFR was observed. In the study of Amin et al. no significant correlation between eGFR and u-PDX was found.[35] The literature data on correlation between u-nephrin levels and GFR are limited.

ROC analysis showed diagnostic accuracy of 96% of u-nephrin, and 95% of u-PDX in women with PE. Both markers have 100% accuracy in discriminating between women with PE and healthy pregnant women. U-nephrin had the highest accuracy in distinguishing PE from healthy pregnancies with a cut-off value of 304.6 ng/ml, sensitivity of 96.7%, specificity of 96.7%, while u-PDX had the highest accuracy in distinguishing PE from healthy pregnancies with a cut-off value of 59.5 ng/ml, sensitivity of 100% and specificity of 93.3%. In a recent study it was found that

u-nephrin had a sensitivity of 57% and specificity of 58% in diagnosing PE.[36] Similar results are reported in the study of Yang et al. for u-nephrin in predicting PE, wherein sensitivity, specificity, PPV, and NPV were 67%, 83%, 58%, and 76%, respectively.[33] Another study reported sensitivity of 64% and specificity of 77% of u-PDX in making the diagnosis of PE.[26] Both markers showed excellent clinical utility (CUI≥ 0.81) as diagnostic tests in distinguishing healthy pregnant women from women with PE.

There are two major limitations in this study: these are the small sample size and the cross-sectional nature of study. Therefore, there is a need for large, prospective studies in order to further assess the value of these markers in predicting PE. If further studies confirm that u-nephrin and u-PDX or both are markers for prediction of PE, they could be implemented in clinical and laboratory practice as routine biomarkers used to predict PE in women with a high-risk pregnancy.

CONCLUSIONS

U-nephrin and u-PDX levels could be useful as predictors of PE in women with a high-risk pregnancy.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Poon LC, Shenan A, Hyett JA, et al. The international federation of gynecology and obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. Int J Gynaecol Obstet 2019; 145:1–33.
2. Andarge RB, Anshebo AA, Halil HM, et al. Prevalence and associated factors of pre-eclampsia among pregnant women at antenatal booking in the Halaba Kullito General Hospital, Southern Ethiopia. J Women's Health Care 2020; 9:496.
3. Salam RA, Das JK, Ali A, et al. Diagnosis and management of pre-eclampsia in community settings in low and middle-income countries. J Family Med Prim Care 2015; 4(4):501–6.
4. Bartsch E, Medcalf KE, Park AL, et al. High risk of pre-eclampsia identification group. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. BMJ 2016; 353:i1753.
5. Webster K, Fishburn S, Maresh M, et al. Diagnosis and management of hypertension in pregnancy: summary of updated NICE guidance. BMJ 2019; 366:l5119.
6. Armaly Z, Jadaon JE, Jabbour A, et al. Preeclampsia: novel mechanisms and potential therapeutic approaches. Front Physiol 2018; 9:973.
7. Phipps EA, Thadhani R, Benzing T, et al. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. Nat Rev Nephrol 2019; 15(5):275–89.
8. Song WL, Zhao YH, Shi SJ, et al. First trimester Doppler velocimetry of the uterine artery ipsilateral to the placenta improves ability to predict early-onset preeclampsia. Medicine (Baltimore) 2019; 98(16):e15193.
9. Agrawal S, Cerdeira AS, Redman C, et al. Meta-analysis and systematic review to assess the role of soluble FMS-Like Tyrosine Kinase-1 and Placenta Growth Factor ratio in prediction of preeclampsia: The SaPPPhirE Study. Hypertension 2018; 71(2):306–16.
10. Tsiakkas A, Cazacu R, Wright A, et al. Maternal serum placental growth factor at 12, 22, 32 and 36 weeks' gestation in screening for pre-eclampsia. Ultrasound Obstet Gynecol 2016; 47(4):472–7.
11. Bredaki FE, Mataliotakis M, Wright A, et al. Maternal serum alpha-fetoprotein at 12, 22 and 32 weeks' gestation in screening for pre-eclampsia. Ultrasound Obstet Gynecol 2016; 47:466–71.
12. De Muro P, Capobianco G, Lepedda AJ, et al. Plasma PP13 and urinary GAGs/PGs as early markers of pre-eclampsia. Arch Gynecol Obstet 2016; 294:959–965.
13. Galvis-Ramírez MF, Quintana-Castillo JC, Bueno-Sanchez JC. Novel insights into the role of glycans in the pathophysiology of glomerular endotheliosis in preeclampsia. Front Physiol 2018; 9:1470.
14. Litwińska E, Litwińska M, Oszukowski P, et al. Biochemical markers in screening for preeclampsia and intrauterine growth restriction. Ginekol Pol 2015; 86(8):611–5.
15. Daskalakis G, Papapanagiotou A. Serum markers for the prediction of preeclampsia. J Neurol Neurophysiol 2015; 6:264.
16. Park HJ, Shim SS, Cha DH. Combined screening for early detection of pre-eclampsia. Int J Mol Sci 2015; 16(8):17952–74.
17. Craici IM, Wagner SJ, Weissgerber TL, et al. Advances in the pathophysiology of pre-eclampsia and related podocyte injury. Kidney Int 2014; 86(2):275–85.
18. Craici IM, Wagner SJ, Bailey KR, et al. Podocyturia predates proteinuria and clinical features of preeclampsia: A longitudinal prospective study. Hypertension 2013; 61(6):1289–96.
19. Wang Y, Zhao S, Gu Y, et al. Loss of slit protein nephrin is associated with reduced antioxidant superoxide dismutase expression in podocytes shed from women with preeclampsia. Physiol Rep 2018; 6(13):e13785.
20. Kwiatkowska E, Stefanska K, Zielinski M, et al. Podocytes - the most vulnerable renal cells in preeclampsia. Int J Mol Sci 2020; 21:5051.
21. Panek-Laszczyńska K, Konieczny A, Milewska E, et al. Podocyturia as an early diagnostic marker of preeclampsia: a literature review. Biomarkers 2018; 23(3):207–12.
22. Kostovska I, Tosheska Trajkovska K, Cekovska S, et al. Nephrin and podocalyxin - new podocyte proteins for early detection of secondary nephropathies. BANTAO J 2016; 14(1):11–6.
23. Wang Y, Zhao S, Loyd S, et al. Increased urinary excretion of nephrin, podocalyxin, and βig-h3 in women with preeclampsia. Am J Physiol Renal Physiol 2012; 302(9): F1084–9.
24. Son GH, Kwon JY, Lee S, et al. Comparison of serum and urinary nephrin levels between normal pregnancies and severe preeclampsia. Eur J Obstet Gynecol Reprod Biol 2013; 166(2):139–44.
25. Jung YJ, Cho HY, Cho S, et al. The level of serum and urinary nephrin in normal pregnancy and pregnancy with subsequent preeclampsia. Yonsei Medical Journal 2017; 58(2):401.
26. Palacios de Franco Y, Velazquez K, Segovia N, et al. Urinary podocalyxin as a marker of preeclampsia in a Hispanic population. Int J Physiol Pathophysiol Pharmacol 2014; 6(2):115–24.
27. Rana S, Lemoine E, Granger JP, et al. Preeclampsia: pathophysiology,

challenges, and perspectives. Circ Res 2019; 124(7):1094–112.
28. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16(1):31–41.
29. Habibzadeh F, Habibzadeh P, Yadollahie M. On determining the most appropriate test cut-off value: the case of tests with continuous results. Biochem Med 2016; 26(3):297–307.
30. Mitchell AJ. Sensitivity × PPV is a recognized test called the clinical utility index (CUI+). Eur J Epidemiol 2011; 26(3):251–2.
31. Garovic VD. The role of the podocyte in preeclampsia. Clin J Am Soc Nephrol 2014; 9:1337–40.
32. Furuta I, Zhai T, Ishikawa S, et al. Association between nephrinuria, podocyturia, and proteinuria in women with pre-eclampsia. J Obstet Gynaecol Res 2017; 43:34–41.
33. Yang GY, Lee KA, Park MH, et al. Urinary nephrin: A new predictive marker for pregnancies with preeclampsia and small-for-gestational age infants. Obstet Gynecol Sci 2013; 56(1):22–8.
34. Gonzalez Suarez ML, Kattah A, Grande JP, et al. Renal disorders in pregnancy: Core Curriculum 2019. Am J Kidney Dis 2019; 73(1):119–30.
35. Amin AF, Abou-Taleb H, Gamal M, et al. Evaluation of podocalyxin level in pre-eclampsia with severe features' patients: a cross-sectional study. Int J Reprod Contracept Obstet Gynecol 2019; 8:3255–8.
36. Jim B, Mehta S, Qipo A, et al. A comparison of podocyturia, albuminuria and nephrinuria in predicting the development of preeclampsia: A Prospective Study. PLoS One 2014; 9(7):e101445.

Уровни нефрина и подокаликсина в моче как предикторы преэклампсии у беременных из группы высокого риска

Ирена Костовска, Катерина Т. Трайковска, Огнен Костовски, Даница Лабудович

Кафедра медицинской и экспериментальной биохимии, Медицинский факультет, Университет имени Св. Св. Кирилла и Мефодия, Скопье, Республика Северная Македония

Адрес для корреспонденции: Ирена Костовска, Кафедра медицинской и экспериментальной биохимии, Медицинский факультет, Университет имени Св. Св. Кирилла и Мефодия, ул. „50-та Дивизия" № 6, 1000, Скопье, Республика Северная Македония; E-mail: irenakostovska22@yahoo.com

Дата получения: 26 октября 2020 ♦ **Дата приемки:** 8 февраля 2021 ♦ **Дата публикации:** 31 декабря 2021

Образец цитирования: Kostovska I, Trajkovska KT, Kostovski O, Labudovic D. Urinary nephrin and podocalyxin levels as predictors of pre-eclampsia in high-risk pregnant women. Folia Med (Plovdiv) 2021;63(6):948-57. doi: 10.3897/folmed.63.e60055.

Резюме

Введение: Преэклампсия (ПЭ) характеризуется появлением артериальной гипертензии и протеинурии. Сообщалось о повреждении подоцитов у женщин с преэклампсией, и специфические для подоцитов белки, такие как нефрин и подокаликсин, могут быть полезными биомаркерами ПЭ.

Цель: Изучить роль нефрина в моче (u-nephrin) и подокаликсина в моче (u-PDX) в прогнозировании ПЭ у женщин с высоким риском беременности.

Материалы и методы: В данное исследование была включена 101 беременная женщина, которые были разделены на три группы: 1-я группа - беременные с высоким риском развития ПЭ (n = 41), 2-я группа - беременные с ПЭ (n = 30), и группа 3 – контрольная, состоявшая из здоровых беременных женщин (n = 30). Критериями включения женщин с ПЭ были гипертензия de novo > 140/90 mmHg., протеинурия > 300 мг / 24 часа и наличие отеков после 20 недель беременности, в то время как критериями исключения были наличие в анамнезе заболевания почек и беременных женщин в возрасте моложе 18 лет. Критерии включения для женщин с беременностями высокого риска: гестационная неделя > 15, ПЭ в анамнезе во время предыдущей беременности, существующий ранее диабет 1 или 2 типа, существующая ранее гипертензия, многоплодная беременность, существующая ранее отслойка плаценты, тучные женщины, нерожавшие, возраст матери> 35 лет и семейный анамнез ПЭ. Исследование проводилось с марта 2016 года по май 2017 года на медицинском факультете Института медицинской и экспериментальной биохимии в Скопье. Образцы мочи использовались для исследования уровней нефрина и подокаликсина с помощью иммуноферментного анализа, креатинина и микроальбумина. Были взяты образцы крови для биохимического анализа.

Результаты: Уровень u-nephrin увеличился на 96.7% у женщин с ПЭ и на 73% у женщин с беременностями высокого риска. Уровни u-PDX были увеличены на 63% у женщин с ПЭ и на 100% у женщин с беременностями высокого риска. Уровни u-nephrin и u-PDX были значительно повышены у женщин с беременностями с высоким риском и у женщин с ПЭ по сравнению с контрольной группой (p<0.001). Была обнаружена значительная разница между подгруппами беременных женщин, классифицированных по сроку беременности в соответствии с их уровнями u-nephrin и y-PDX. Обнаружена значимая положительная взаимосвязь между уровнями обоих маркеров и скоростью клубочковой фильтрации и значительная отрицательная корреляция между уровнями обоих маркеров и гестационным возрастом. Анализ ROC показал, что предельное значение 304.6 ng/ml u-nephrin имело чувствительность (Se) 96.7%, специфичность (Sp) 96.7% (как для Se, так и для Sp 95% доверительный интервал (CI) 82.8-99.9), в то время как предельное значение 59.5 ng/ml для u-PDX имело чувствительность 100%, а Sp - 93.3% (Se - 95% CI 88.4-100, Sp - 95% CI 77.9-99.2), при различении женщин с ПЭ и здоровая беременность. Оба маркера показали отличную клиническую пользу (CUI ≥ 0.81) для u-nephrin (CUI + и CUI- 0.934), для u-PDX (CUI + 0.938; CUI- 0.933).

Заключение: Уровни U-нефрина и u-PDX могут быть полезными предикторами ПЭ у женщин с беременностями высокого риска.

Ключевые слова

беременность высокого риска, нефрин, подокаликсин, преэклампсия

Folia Medica