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Original Scientific Paper

URINARY NEPHRIN AS AN EARLY BIOMARKER OF HYPERTENSIVE NEPHROPATHY

Irena Kostovska[1], Katerina Tosheska Trajkovska[1], Danica Labudović[1], Ognen Kostovski[2] and Goce Spasovski[3]

[1]Department of Medical and Experimental Biochemistry, Faculty of Medicine, Ss Cyril and Methodius University, Skopje, North Macedonia;
[2]Department of Digestive Surgery, Faculty of Medicine, Ss Cyril and Methodius University, Skopje, North Macedonia;
[3]Department of Nephrology, Faculty of Medicine, Ss Cyril and Methodius University, Skopje, North Macedonia

SUMMARY – Hypertensive nephropathy (HN) is characterized by kidney damage due to chronic high blood pressure. Podocytes play a crucial role in the pathogenesis of HN, thus, nephrin could be important in the early diagnosis of HN. The aim of the study was to investigate the association of urinary nephrin (u-nephrin) levels with clinical and laboratory characteristics in patients with HN and to test diagnostic relevance of u-nephrin as an early biomarker of HN. In this cross-sectional study, 114 subjects were recruited, 84 patients with chronic hypertension (CH) and 30 healthy controls. All patients with CH were classified according to the urinary microalbumin/creatinine ratio (UM/CR) and according to the chronic kidney disease (CKD) stage. Urine samples were collected to estimate the u-nephrin level by ELISA and to determine UM/CR. Blood samples were used for biochemical analyses. We found elevated u-nephrin in 78.3% of normoalbuminuric subjects with CH. The levels of u-nephrin increased gradually with the stage of CKD. ROC curve plotted for u-nephrin showed 89.7% sensitivity and 88.8% specificity, while UM/CR showed a sensitivity of 44.8% and specificity of 86.1% to detect HN in the early stage. It is concluded that u-nephrin can be useful as an early biomarker of HN.

Key words: *Nephrin; Hypertensive nephropathy; Podocytes; Microalbumin; Chronic kidney disease*

Introduction

Hypertensive renal disease or hypertensive nephropathy (HN) is the second most common cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD), requiring dialysis or renal transplantation, outnumbered only by diabetic nephropathy. More than 20% of patients with chronic hypertension (CH) have HN[1]. The early course of HN is often asymptomatic, these patients do not regularly undergo a medical check-up, and so at the time of first medical examination, most of them have CKD in the early stage. Once the clinical manifestations or routine testing results have been detected to be irregular, multiple renal lesions might have already formed. Although HN may occur in newly diagnosed hypertension patients, often the clinical manifestations of HN appear after 10- to 15-year duration of hypertensive disease. Therefore, early diagnosis of HN is essential[2]. Screening of high-risk populations, which include individuals with hypertension, diabetes mellitus, and those older than 65 years, is recommended, including two

Correspondence to: *Irena Kostovska, MD, PhD*, Department of Medical and Experimental Biochemistry, Faculty of Medicine, Ss Cyril and Methodius University, 50 Divizija 6, 1000 Skopje, North Macedonia
E-mail: irenakostovska22@yahoo.com

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laboratory tests, glomerular filtration rate (GFR) as a measure of kidney function, and urinary microalbumin to creatinine ratio (UM/CR) as a measure of kidney damage[3]. Microalbuminuria has been proposed as a possible marker of early renal dysfunction and a predictor of ESRD and cardiovascular disease. Microalbuminuria is a non-specific marker often present in other pathologic conditions, thus HN may reflect endothelial dysfunction and be a better predictor of cardiovascular risk than CKD progression[4,5]. HN is characterized by the combination of pathologic changes of the vasculature, glomeruli, tubulointerstitium, capillary endothelial cells, podocytes, and immune cells[6]. Over the last two decades, multiple animal studies have demonstrated that podocyte cell damage is crucial in the pathogenetic mechanisms of HN. A possible explanation is that elevated blood pressure may cause podocyte injury due to the mechanical effects of glomerular hypertension, hyperfiltration, and glomerular hypertrophy, which lead to detachment of podocytes from the glomerular basement membrane and their shedding through urine[7]. Human data are limited, but some studies show that podocyte damage appears in the early course of hypertensive renal injury. Podocytes are highly differentiated cells crucial for maintaining the glomerular filtration barrier, and recently investigated mechanisms showed podocyte effacement and loss, leading to increased protein leakage and decreased GFR. Thus, in HN, podocyturia predicted clinical outcomes[8,9]. A damage and detachment of podocytes from the glomerular basement membrane lead to the presence of podocytes and their specific proteins such as nephrin in urine. Nephrin is a transmembrane glycoprotein located at the slit diaphragm, which serves as a size-selective barrier, preventing plasma protein leakage[10,11]. Thus, the measurement of urinary nephrin (u-nephrin) could be a promising tool in the early detection of podocyte damage in HN. Early detection of podocyte damage will permit early treatment and slow the progression to ESRD. Our study aimed to investigate the association of u-nephrin levels with clinical and laboratory characteristics in patients with HN and to test diagnostic value of u-nephrin as an early biomarker of HN.

Patients and Methods

Subjects

This cross-sectional study was conducted between March 2016 and May 2017 at the Department of Medical and Experimental Biochemistry, Faculty of Medicine in Skopje. The study was performed according to the Declaration of Helsinki, and was approved by the Ethics Committee of the Faculty of Medicine in Skopje, North Macedonia (No. 03-5515/8 as of December 9, 2015). The current study included 84 patients with CH (23 with clinically proven HN and 61 without clinically proven HN) and 30 healthy controls. Patients with clinically proven HN were selected from the Department of Nephrology, Faculty of Medicine in Skopje. Inclusion criteria for patients with CH and HN were clinically diagnosed and manifested HN, characterized by high blood pressure and abnormalities of kidney structure or function (defined by the presence of macroalbuminuria or microalbuminuria or decreased GFR) present for >3 months[12]. Exclusion criteria were diabetes mellitus type 2 and presence of any other kind of kidney disease. Patients with CH without clinically proven HN were recruited from the primary health care offices as new-onset cases with high blood pressure, not reported to the nephrologists for examination of renal function, i.e., without clinically proven HN. Informed consent was obtained from all subjects included in this study.

Patients with CH were divided into three subgroups according to UM/CR, as follows: (1) macroalbuminuric group, UM/CR greater than 300 mg/g (n=4); (2) microalbuminuric group, UM/CR 30-300 mg/g (n=20); and (3) normoalbuminuric group, UM/CR less than 30 mg/g (n=60).

Another classification of all patients with CH was performed according to CKD stage into five subgroups: (1) stage V, eGFR <15 mL/min *per* 1.73 m^2 (n=1); (2) stage IV, eGFR 15-29 mL/min *per* 1.73 m^2 (n=6); (3) stage III a & b, eGFR 30-59 mL/min *per* 1.73 m^2 (n=30); (4) stage II, eGFR 60-89 mL/min *per* 1.73 m^2 (n=38); and (5) stage I, eGFR 90 mL/min *per* 1.73 m^2 and above (n=9).

Both classifications of patients were performed according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines[12,13].

Baseline demographics and clinical data (age, sex, height and weight, duration of disease, comorbidities, laboratory investigations, and blood pressure measurements) were collected on all subjects included in the study.

Urine analysis

First morning urine samples (10 mL) were collected into a plastic sterile tube. Urine samples were divided

into two aliquots, as follows: 5 mL for measurement of urine creatinine and microalbumin, and 5 mL was stored at -80 °C for future measurement of u-nephrin by enzyme-linked immunosorbent assay (ELISA). Urinary microalbumin was measured by the immunoturbidimetric method, and urinary creatinine concentration by Jaffe's reaction on a ChemWell biochemical analyzer (2910® Awareness Technology, Inc., Palm City, FL, USA). The UM/CR was calculated by dividing urinary microalbumin concentration in milligrams by urinary creatinine concentration in grams. The GFR was estimated by the Cocroft and Gault equation[14].

Frozen urine samples were prepared and estimated according to the guidelines set in the u-nephrin ELISA kit manufacturer's instructions (Exocell Inc., Philadelphia, PA, USA). The method for detection of u-nephrin levels was an indirect competitive ELISA with a primary antibody raised in rabbit against the nephrin and an anti-rabbit secondary antibody conjugated to horseradish peroxidase (HRP). As an antigen, u-nephrin competed with nephrin antigens immobilized at the bottom of polystyrene microtiter plates for the added primary antibodies. Detection of primary antibodies bound to u-nephrin was achieved using anti-rabbit HRP secondary antibodies. Several wash steps removed unbound antibodies, and then a chromogenic substrate was added, resulting in color development. The intensity of developed color corresponded inversely to nephrin concentration in the urine sample and was measured at a 450 nm wavelength. The u-nephrin levels were estimated from the standard curve established using commercial standards provided by the ELISA kit. The u-nephrin levels were expressed in ng/mL.

Blood analysis

In all subjects, 5 mL of venous blood samples was collected aseptically into commercial serum tubes and left to clot for 10-15 min. Serum was separated by centrifugation at 3000 rounds *per* minute for 10 min. Blood glucose levels, low-density lipoproteins (LDL), high-density lipoproteins (HDL), total cholesterol, triglycerides, blood urea, serum creatinine, total proteins, and albumin were measured on a ChemWell biochemical analyzer (2910® Awareness Technology, Inc., Palm City, FL, USA). We used commercial reagents and quality control was performed according to the ISO 15189 standard quality assurance program.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, NY, USA) and MedCalc for Windows version 15.0 (MedCalc Software, Ostend, Belgium). Numerical data were presented with median and interquartile range (IQR) (25-75 percentile) due to non-normal distribution of all data detected by the Kolmogorov-Smirnov test. We used the Kruskal-Wallis test to compare differences among more than two groups in terms of clinical and laboratory data, and Mann-Whitney U test to compare differences between two groups in terms of clinical and laboratory data. Mann-Whitney U test was employed as a post-hoc test of Kruskal-Wallis test to detect the groups responding significantly different. Bonferroni corrections were used where multiple comparisons were performed in the same data set. Spearman's correlation coefficient was used to measure the relationship between quantitative variables. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cut-off values of u-nephrin and UM/CR and to measure their diagnostic accuracy in patients with HN. The values of $p<0.05$ were considered to be statistically significant.

Results

Clinical and biochemical characteristics of study subgroups

Statistical analysis showed a significant difference according to age, serum creatinine, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), blood glucose levels, LDL, total cholesterol, triglycerides, urea, UM/CR, total proteins, albumin, and u-nephrin among the subgroups of subjects divided according to UM/CR. Among the subgroups of patients divided according to CKD stage, there was a significant difference according to age, duration of disease, serum creatinine, BMI, SBP, DBP, blood glucose levels, HDL, total cholesterol, triglycerides, urea, UM/CR, total proteins, albumin, and u-nephrin. The results of difference tests on clinical and biochemical data in patients with CH divided according to UM/CR and according to CKD stage, and healthy controls are shown in Tables 1 and 2.

U-nephrin levels in study subgroups

In the subgroups of subjects divided according to UM/CR, the u-nephrin level was significantly elevated compared to healthy controls ($p<0.05$) (Fig. 1). The u-nephrin level was also significantly elevated in the subgroups of patients with CH divided according to CKD stage compared to healthy controls ($p<0.05$) (Fig. 2).

Table 1. Clinical and biochemical characteristics of chronic hypertensive patients divided according to UM/CR and healthy controls

	Macroalbuminuric patients, n=4	Microalbuminuric patients, n=20	Normoalbuminuric patients, n=60	Healthy controls, n=30	Kruskal-Wallis p-value
Age (years)	64.5 (59.5-72.5)	57.5 (46-60.5)	59.5 (54-62)	48 (41-55)	<0.001
Duration of disease (years)	8.5 (5-10)	5 (1-5)	5 (2-5)	/	0.570
BMI (kg/m^2)	32.9 (27.5-35.5)	27.4 (26.2-30.2)	28.5 (26.5-31.8)	25.7(23.1-27.8)	0.001
Blood glucose (mmol/L)	6.06 (4.9-6.56)	5.87 (4.7-8.2)	5.7 (4.6-6.9)	3.9 (3.61-4.87)	<0.001
UM/CR (mg/g)	686.7 (525.6-715.2)	76.9 (52.4-144.6)	11.9 (7.35-18.7)	11 (8.3-16.1)	<0.05
SBP (mm Hg)	155 (145-165)	147.5 (140-155)	140 (140-150)	120 (110-130)	<0.001
DBP (mm Hg)	90 (90-95)	90 (90-100)	90 (90-100)	80 (70-90)	<0.001
Total cholesterol (mmol/L)	6.06 (4.83-7.58)	4.8 (3.64-5.17)	4.42 (3.46-4.96)	3.35 (2.59-4.11)	<0.001
Triglycerides (mmol/L)	2.24 (1.33-4.58)	2.18 (1.55-3.68)	1.36 (0.84-2.22)	1.22 (0.66-1.64)	<0.001
HDL (mmol/L)	1.27 (0.92-1.7)	1.24 (0.93-1.3)	1.25 (0.92-1.56)	1.22 (0.9-1.56)	0.938
LDL (mmol/L)	3.36 (2.89-4.21)	2.51 (1.7-2.76)	2.33 (1.5-3.0)	1.73 (1.0-2.09)	0.005
Total proteins (g/L)	68 (61.5-74.5)	70 (64.5-72)	65.5 (57-74)	74.5 (70-78)	0.001
Albumin (g/L)	39 (36.5-40)	40 (38.5-44.5)	39 (35.5-44)	47 (45-48)	<0.001
Urea (mmol/L)	10.25 (6.03-14.8)	6.12 (5.13-10.0)	6.44 (5.47-8.61)	4.39 (3.47-4.97)	<0.001
Serum creatinine (μmol/L)	171.5 (84.1-243.8)	84.1 (68.6-97.2)	76.7 (69-91.5)	76.8 (65.3-84.1)	0.001
eGFR (mL/min *per* 1.73 m^2)	35.5 (16.9-58.5)	58.4 (40.9-72.5)	63.8 (54.9-73.1)	91.14 (86.6-95.5)	<0.001
u-nephrin (ng/mL)	1291.8 (461.4-2739.8)	403 (308.3-512.5)	444.9 (234.4-797.9)	136.2 (109.4-189.2)	0.002

Results are presented as median and interquartile range (IQR); BMI = body mass index; UM/CR = urinary microalbumin to creatinine ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; eGFR = estimated glomerular filtration rate; u-nephrin = urinary nephrin

Correlation between u-nephrin levels and clinical and biochemical data of subjects

A weak positive correlation was found between u-nephrin levels and SBP (rho=0.252, p=0.01) and between u-nephrin and DBP (rho=0.273, p=0.01), whereas a weak negative correlation was recorded between u-nephrin and eGFR (rho=-0.241, p=0.02).

Diagnostic performance of u-nephrin and UM/CR in patients with CH

To test diagnostic performance of u-nephrin and UM/CR and to detect the optimal cut-off value for u-nephrin and UM/CR, ROC analysis was performed. To determine the best cut-off, we maximized both sensitivity (Se) and specificity (Sp) with their summation (Se + Sp). At this point, Youden index (Se + Sp – 1) was also maximum, so the optimal cut-off was calculated from the maximum Youden index (sensitivity + specificity – 1)[15]. Total accuracy of u-nephrin was 71%, while UM/CR total accuracy was 57.8% in early diagnosis of HN. Results are shown in Table 3.

Percentage of subjects with elevated u-nephrin in study subgroups

In the subgroups of patients divided according to UM/CR, u-nephrin levels were higher than the cut-off value (>210.3 ng/mL) in 78.3% of patients with normoalbuminuria, 95% of patients with microalbuminuria, and 100% of patients with macroalbuminuria (Fig. 3). In the subgroups of patients divided accord-

Table 2. Clinical and biochemical characteristics of chronic hypertensive patients divided according to CKD stage and healthy controls

	Stage I n=9	Stage II n=38	Stage III n=30	Stage IV n=6	Stage V n=1	Healthy controls n=30	Kruskal-Wallis p-value
Age (years)	54 (39-59.5)	59 (53-61)	60 (58-64)	64.5 (59-78)	56	48 (41-55)	<0.001
Duration of disease (years)	4 (1-5)	4.5 (2-5)	5 (2-5)	10 (7-20)	20	/	0.036
BMI (kg/m^2)	30.2 (28.8-37.4)	29.1 (27-32.6)	27.5 (24.8-29.4)	25.5 (23.7-26.9)	26.8	25.7 (23.1-27.8)	<0.001
Blood glucose (mmol/L)	6.7 (4.4-8.3)	5.8 (4.5-6.9)	5.4 (4.73-7)	6.1 (5.7-6.9)	7.6	3.9 (3.61-4.87)	0.002
UM/CR (mg/g)	27.4 (15.4-67.5)	14.6 (7.7-22.6)	17.25 (8.9-30.4)	135.3 (23.2-365.7)	85.8	11 (8.3-16.1)	0.003
SBP (mm Hg)	140 (135-147.5)	140 (140-150)	140 (140-160)	150 (145-160)	150	120 (110-130)	<0.001
DBP (mm Hg)	90 (85-97.5)	90 (90-100)	90 (90-100)	95 (90-100)	90	80 (70-90)	<0.001
Total cholesterol (mmol/L)	4.9 (3.9-6.6)	4.4 (3.4-4.9)	4.8 (4-5.29)	4.7 (3.5-6.9)	4.7	3.35 (2.59-4.11)	0.002
Triglycerides (mmol/L)	3.58 (1.54-6.6)	1.47 (0.9-2.1)	1.23 (0.93-2.89)	2.1 (0.74-4.71)	4.6	1.22 (0.66-1.64)	<0.001
HDL (mmol/L)	1.15 (0.84-1.28)	1.13 (0.8-1.5)	1.27 (1.0-1.5)	1.73 (1.56-1.85)	1.3	1.22 (0.9-1.56)	0.022
LDL (mmol/L)	2.17 (1.61-2.72)	2.27 (1.44-2.84)	2.55 (2.13-3.07)	1.8 (1.2-3.8)	1.3	1.73 (1.0-2.09)	0.086
Total proteins (g/L)	70 (58-78)	65 (56-73)	67 (61-73)	75.5 (70-80)	70	74.5 (70-78)	<0.001
Albumin (g/L)	40 (34.5-44)	38 (35-41)	40 (38-45)	43.5 (40-48)	40	47 (45-48)	<0.001
Urea (mmol/)	7.09 (5.22-8.6)	6.06 (5.1-7.6)	6.56 (5.85-8.77)	12.5 (9.41-16.6)	16.6	4.39 (3.47-4.97)	<0.001
Serum creatinine (μmol/L)	62.1 (51.5-80.5)	71.8 (67.8-83.3)	87 (73.5-103)	247.4 (237-260)	467.1	76.8 (65.3-84.1)	<0.001
eGFR (mL/min *per* 1.73 m^2)	79.2 (93.05-108.5)	69.7 (64.2-73.4)	53.5 (48.5-55.7)	16.9 (16.02-18.12)	12.4	91.14 (86.6-95.5)	<0.001
u-nephrin (ng/mL)	218.3 (196.3-377.7)	451.2 (292.5-789.6)	471.5 (247.3-634.2)	2508.7 (425-3471.8)	425	136.2 (109.4-189.2)	<0.001

Results are presented as median and interquartile range (IQR); CKD = chronic kidney disease; BMI = body mass index; UM/CR = urinary microalbumin to creatinine ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; eGFR = estimated glomerular filtration rate; u-nephrin = urinary nephrin

Table 3. ROC analysis diagnostic performance data of u-nephrin and UM/CR in patients with HN

Diagnostic performance data	u-nephrin	UM/CR
Area under ROC curve (AUC)	0.935	0.626
95% Confidence interval (95% CI)	0.873-0.973	0.531-0.715
Significance level p (area=0.5)	<0.0001	0.0148
Youden index J	0.7863	0.3098
Cut-off value	>210.1 ng/mL	>30.0 mg/g
Sensitivity	89.7%	44.8%
Specificity	88.8%	86.1%
NPV	92.4%	87.5%
PPV	66.6%	41.8%
Diagnostic effectiveness (accuracy)	71%	57.8%

NPV = negative predictive value; PPV = positive predictive value; u-nephrin = urinary nephrin; UM/CR = urinary microalbumin to creatinine ratio; HN = hypertensive nephropathy

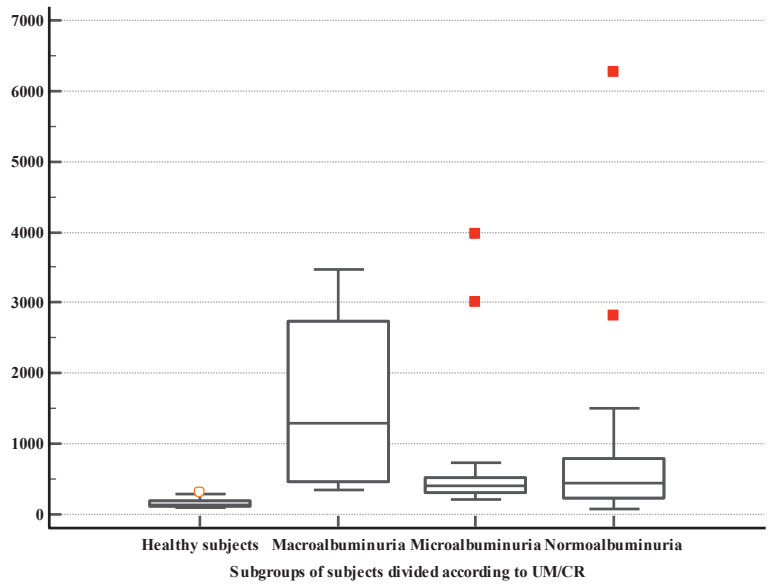

Fig. 1. Comparison of u-nephrin levels among subgroups of patients with CH divided according to UM/CR and healthy subjects.

CH = chronic hypertension; UM/CR = urinary microalbumin to creatinine ratio

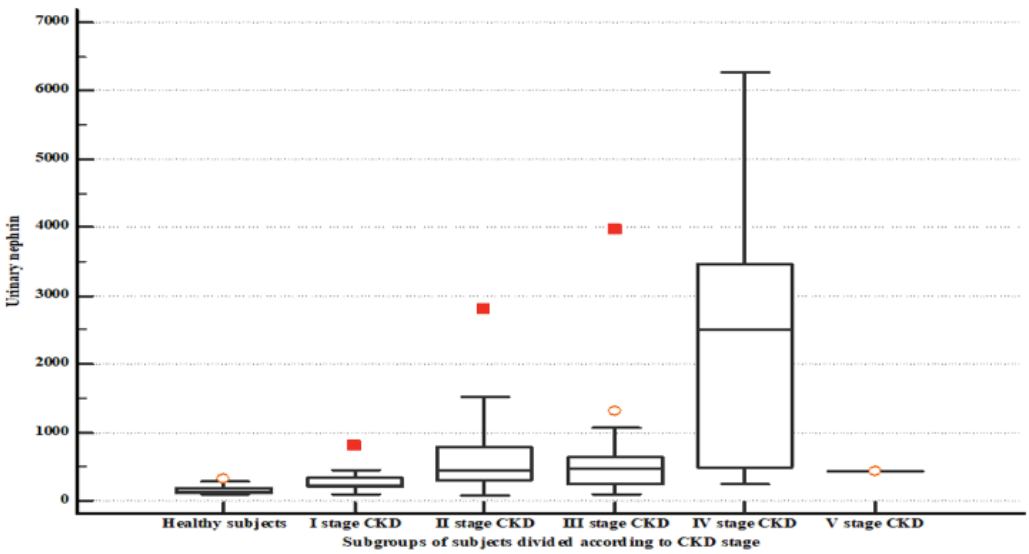

Fig. 2. Comparison of u-nephrin levels among subgroups of patients with CH divided according to CKD stage.

CH = chronic hypertension; CKD = chronic kidney disease

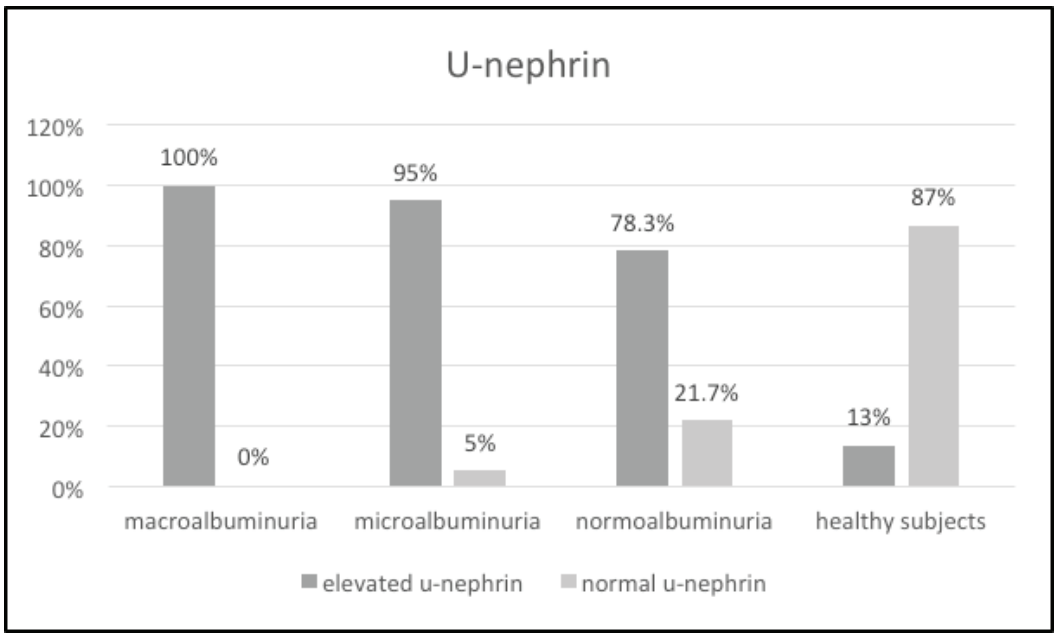

Fig. 3. Elevated u-nephrin in subgroups of patients divided according to UM/CR.

UM/CR = urinary microalbumin to creatinine ratio

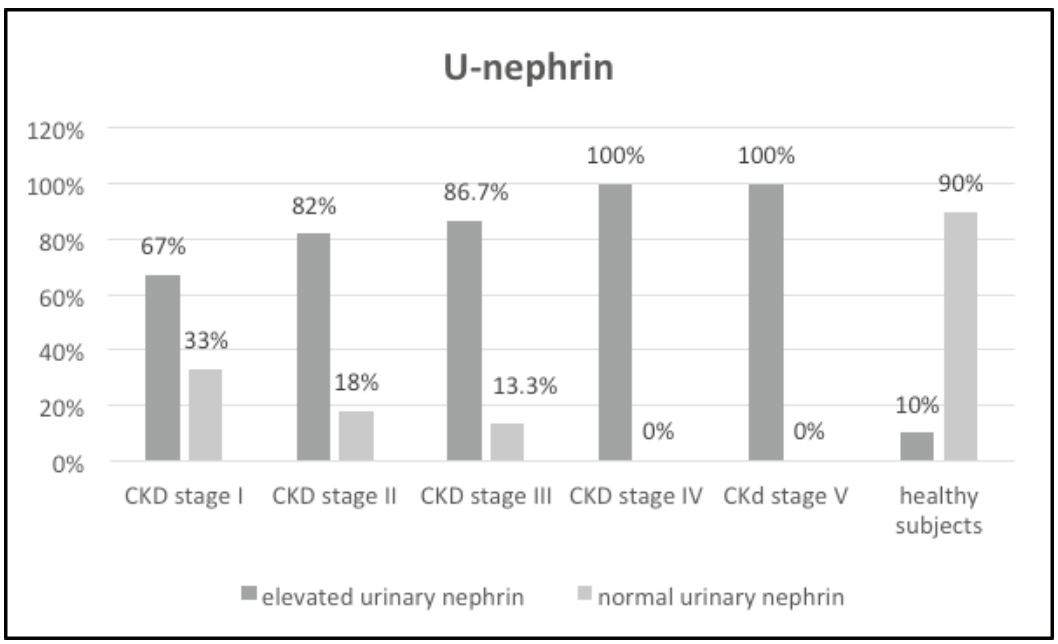

Fig. 4. Elevated u-nephrin in subgroups of patients divided according to CKD stage.

CKD = chronic kidney disease

ing to CKD stage, u-nephrin levels were higher than the cut-off value in all patients in CKD stages IV and V, 86.7% of patients in CKD stage III, 82% of patients in CKD stage II, and 67% of patients in CKD stage I (Fig. 4).

Discussion

Patients with CKD up to stage III and most cases in CKD stage IV usually are asymptomatic, thus CKD is usually named a 'silent killer'. Early detection of CKD in asymptomatic individuals could be done by simple tests such as eGFR and UM/CR, and necessary steps to reduce progression to ESRD should be taken[16]. Microalbuminuria in patients with CH has been described as an early sign of kidney damage and a predictor of ESRD and cardiovascular disease with a prevalence of approximately 50%[5]. However, some patients with CH may have eGFR decline and may progress to ESRD without having significant micro- or macroalbuminuria, while some patients with normoalbuminuria or microalbuminuria have advanced renal pathologic changes indicating that microalbuminuria lacks both sensitivity and specificity to detect HN in early stages[17]. In animal models of hypertension, podocyte injury has been noticed in the early course of nephropathy[18,19]. On the other hand, human data on podocyte damage and their loss through urine in patients with CH are scarce. A recent study suggests that podocyturia occurs in the early stage of hypertensive renal injury and could be a sensitive predictor of HN. In the same study, urinary podocytes were detected following positive staining of nephrin[7]. In our study, u-nephrin levels were estimated in hypertensive patients with and without clinically proven HN to investigate the role of u-nephrin in the early diagnosis of HN. While many studies have focused on various aspects of the subject, none of them deals entirely with this particular idea[1,2,5,7,9,10]. Our study results showed a significant difference regarding almost all clinical and biochemical parameters (presented in Tables 1 and 2). In the study by Poudel *et al*., a significant difference was found according to age, SBP, DBP, serum creatinine, and eGFR among groups of subjects divided according to UM/CR[5]. A significant difference was found regarding u-nephrin among the subgroups of patients with CH divided according to CKD stage and subgroups of hypertensive patients divided according to UM/CR; u-nephrin level also was significantly elevated in the subgroups divided according to UM/CR and subgroups divided according to CKD stage compared to healthy controls ($p<0.05$). Significant differences in u-nephrin levels between normoalbuminuric and microalbuminuric hypertensive patients have been reported in a recent study[5]. Our results showed that u-nephrin levels were increasing gradually along with the stage of CKD and kidney damage, rendering it as a possible early marker in HN. The severity of renal injury is usually related to the degree and duration of elevated blood pressure[16]. ROC analysis showed higher specificity and sensitivity of u-nephrin than microalbuminuria in the prediction of HN. Comparably, the sensitivity, specificity, and positive/negative predictive values of UM/CR ≥30 mg/g detection have been reported as 43.6%, 93.6%, 34.6% and 95.5%, respectively, although urine dipstick was used in a recently reported study[20]. In the literature, we could not find data on diagnostic accuracy (sensitivity and specificity) of u-nephrin in patients with HN. We found that u-nephrin level was elevated in 78.3% of patients with normoalbuminuria and 67% of patients in CKD stage I. These results indicate that nephrin appears in the urine before microalbumin in the early stage of CKD related to CH.

A limitation of our study was the relatively small sample size of the study population and its cross-sectional nature, thus, further follow-up and large research are needed to confirm our results. If further studies could confirm our results, u-nephrin could be used in routine laboratory practice as an early diagnostic marker of HN in patients with CH. The key findings of our study were the high percentage of normoalbuminuric patients with CH and hypertensive patients in CKD stage I who had elevated u-nephrin, gradual increase of u-nephrin levels with CKD stage, and higher sensitivity and specificity of u-nephrin in early detection of HN compared to microalbuminuria. We conclude that u-nephrin could be a significant, highly sensitive, and specific marker for early detection of HN in patients with CH.

References

1. Sharma R, Kamalakar S, McCarthy E, Fields T, Gupta K, Barua R, Savin V. Proteinuria in hypertensive nephropathy: a review. Open J Nephrol. 2014;4:92-9. doi: 10.4236/ojneph.2014.42013.
2. Wang XC, Liu CH, Chen YJ, Wu Y, Yang LS, Liu HM, Liao HL. Clinical and pathological analysis of the kidney in patients with hypertensive nephropathy. Exp Ther Med. 2013;6(5):1243-6. doi: 10.3892/etm.2013.1306.

3. Vaidya SR, Aeddula NR. Chronic renal failure. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020. https://www.ncbi.nlm.nih.gov/books/NBK535404/.
4. Polkinghorne KR. Estimated glomerular filtration rate *versus* albuminuria in the assessment of kidney function: what's more important? Clin Biochem Rev. 2014;35(2):67-73. PMCID: PMC4159781.
5. Poudel B, Yadav BK, Nepal AK, Jha B, Raut KB. Prevalence and association of microalbuminuria in essential hypertensive patients. N Am J Med Sci. 2012;4(8):331-5. doi: 10.4103/1947-2714.99501.
6. Sun D, Wang J, Yao L, Li Z, Ohno S. Research progress in acute hypertensive renal injury by "*in vivo* cryotechnique". J Transl Int Med. 2019;7(4):132-6. doi: 10.2478/jtim-2019-0027.
7. Sun D, Wang JJ, Wang W, Wang J, Wang LN, Yao L, Sun YH, Li ZL. Human podocyte injury in the early course of hypertensive renal injury. World J Clin Cases. 2019;7(22):3698-710. doi: 10.12998/wjcc.v7.i22.3698.
8. Kriz W, Shirato I, Nagata M, LeHir M, Lemley KV. The podocyte's response to stress: the enigma of foot process effacement. Am J Physiol Renal Physiol. 2013;304(4):F333-47. doi: 10.1152/ajprenal.00478.2012.
9. Seccia TM, Caroccia B, Calò LA. Hypertensive nephropathy. Moving from classic to emerging pathogenetic mechanisms. J Hypertens. 2017;35(2):205-12. doi: 10.1097/HJH.0000000000001170.
10. Li X, Chuang PY, D'Agati VD, Dai Y, Yacoub R, Fu J, Xu J, Taku O, Premsrirut PK, Holzman LB, He JC. Nephrin preserves podocyte viability and glomerular structure and function in adult kidneys. J Am Soc Nephrol. 2015;26(10):2361-77. doi: 10.1681/ASN.2014040405.
11. Kandasamy Y, Smith R, Lumbers ER, Rudd D. Nephrin – a biomarker of early glomerular injury. Biomark Res. 2014;2:21. doi: 10.1186/2050-7771-2-21.
12. Chapter 1: Definition and classification of CKD. Kidney Int Suppl (2011). 2013;3(1):19-62. doi: 10.1038/kisup.2012.64
13. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, Kurella Tamura M, Feldman HI. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. Am J Kidney Dis. 2014;63(5):713-35. doi: 10.1053/j.ajkd.2014.01.416.
14. Cocroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16:31-41. doi: 10.1159/000180580.
15. 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. doi: 10.11613/BM.2016.034.
16. Qaseem A, Hopkins RH Jr, Sweet DE, Starkey M, Shekelle P. Clinical Guidelines Committee of the American College of Physicians. Screening, monitoring, and treatment of stage 1 to 3 chronic kidney disease: a clinical practice guideline from the American College of Physicians. Ann Intern Med. 2013;159(12):835-47. doi: 10.7326/0003-4819-159-12-201312170-00726.
17. Koroshi A. Microalbuminuria, is it so important? Hippokratia. 2007;11(3):105-7. PMID: 19582202.
18. Kretzler M, Koeppen-Hagemann I, Kriz W. Podocyte damage is a critical step in the development of glomerulosclerosis in the uninephrectomised-desoxycorticosterone hypertensive rat. Virchows Arch. 1994;425:181-93. doi: 10.1007/BF00230355.
19. Kato T, Mizuguchi N, Ito A. Characteristics of podocyte injury in malignant hypertensive nephropathy of rats (MSHR-SP/Kpo strain). Biomed Res. 2015;36:313-21. doi: 10.2220/biomedres.36.313.
20. Park JI, Baek H, Kim BR, Jung HH. Comparison of urine dipstick and albumin:creatinine ratio for chronic kidney disease screening: a population-based study. PLoS One. 2017;12(2):e017110. doi: 10.1371/journal.pone.0171106.

Sažetak

NEFRIN U MOKRAĆI KAO RANI BIOLOŠKI BILJEG HIPERTENZIVNE NEFROPATIJE

I. Kostovska, K. Tosheska Trajkovska, D. Labudović, O. Kostovski i *G. Spasovski*

Hipertenzivna nefropatija (HN) je obilježena bubrežnim oštećenjem zbog kronično visokog krvnog tlaka. Podociti imaju presudnu ulogu u patogenezi HB pa bi nefrin mogao biti važan u ranoj dijagnostici HN. Cilj ovog istraživanja bio je ispitati povezanost razina nefrina u urinu (u-nefrin) s kliničkim i laboratorijskim značajkama bolesnika s HN, kao i dijagnostičku važnost u-nefrina kao ranog biološkog biljega HN. U ovu poprečnu studiju uključeno je 114 bolesnika, i to 84 bolesnika s kroničnom hipertenzijom (KH) i 30 kontrolnih osoba. Svi bolesnici s KH podijeljeni su prema omjeru mikroalbumina/kreatinina u mokraći (UM/CR) i prema stadiju kronične bubrežne bolesti (KBB). Prikupljeni su uzorci mokraće kako bi se procijenila razina u-nefrina tehnikom ELISA i odredio UM/CR. Uzorci krvi rabili su se za biokemijske analize. Utvrdili smo povišenu razinu u-nefrina u 78,3% osoba s KH i normalbuminurijom. Razine u-nefrina postupno su rasle sa stadijem KBB. Krivulja ROC za u-nefrin pokazala je osjetljivost od 89,7% i specifičnost od 88,8%, dok je UM/CR pokazao osjetljivost od 44,8% i specifičnost od 86,1% u otkrivanju HN u ranom stadiju. Zaključuje se da bi u-nefrin mogao biti koristan kao rani biološki biljeg HN.

Ključne riječi: *Nefrin; Hipertenzivna nefropatija; Podociti; Mikroalbumin; Kronična bolest bubrega*