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DIAGNOSTIC ACCURACY OF MICROALBUMINURIA IN SECONDARY NEPHROPATHIES

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Abstract. Introduction: *Microalbuminuria is an initial indicator of kidney damage in diabetic nephropathy (DN), hypertensive nephropathy (HN), and pre-eclampsia (PE). This study aims to assess the diagnostic accuracy of urinary microalbumin to creatinine ratio (UM/CR) as an early diagnostic tool in patients with DN, HN, and PE.* ***Materials and methods:*** *In this cross-sectional study, we included a total of 143 subjects divided into three groups: first-group patients with DN (n = 30), second-group patients with HN (n = 23), and third-group women with PE (n = 30). Additionally, we included a control group comprising 60 healthy subjects. The study employed fresh urine samples to measure UM/CR by the turbidimetric method and creatinine by the Jaffe reaction. We also estimated the UM/CR and glomerular filtration rate. Some biochemical parameters were measured in blood sera.* ***Results:*** *We determined the cut-off value of UM/CR to be 30.0 mg/g. For patients with DN, UM/CR had a sensitivity of 41.5%, a specificity of 90%, and an overall diagnostic accuracy of 53.1% for detecting DN. Similarly, for patients with HN, UM/CR had a sensitivity of 44.8% and a specificity of 86.1%, with an overall diagnostic accuracy of 57.8% for detecting HN. In contrast, for women with PE, UM/CR had a sensitivity of 100%, a specificity of 93.3%, and a diagnostic accuracy of 96.7% for predicting PE.* ***Conclusion:*** *UM/CR can be used as a good screening tool for early detection of PE, while it has low sensitivity as an early marker for patients with DN and HN.*

Key words: *diagnostic accuracy, ROC, urinary microalbumin to creatinine ratio, diabetic nephropathy, hypertensive nephropathy, pre-eclampsia*

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INTRODUCTION

The excretion of urinary albumin in the range of 30 to 300 mg per day is referred to as microalbuminuria. Earlier, the measurement of urinary microalbumin excretion was carried out by a 24-hour collection method. However, currently, a morning sample of urine is collected to estimate the urinary microalbumin/creatinine ratio (UM/CR) for diagnosing microalbuminuria. This method is more

convenient for adults and strongly correlates with the results obtained through the 24-hour collection method. The UM/CR level is adjusted by creatinine to account for urine concentration and volume. Other factors such as gender, race, blood pressure, muscle mass, time of day, food, water, or salt consumption may influence UM/CR levels [1]. Microalbuminuria is an early indication of potential proteinuria and kidney damage in various diseases, particularly secondary nephropathies, such as diabetic nephropathy (DN), hypertensive nephropathy (HN), and pre-eclampsia (PE). Microalbuminuria has been studied extensively as an early marker of DN in patients with type 2 diabetes mellitus (T2DM). DN is one of the leading causes of chronic kidney disease (CKD) and end-stage renal disease (ESRD). Therefore, the American Diabetic Association (ADA) recommends screening for microalbuminuria once a year for diabetic patients to detect DN at an early stage [2].

High levels of urine albumin excretion are commonly associated with hypertension. Current guidelines recommend regular microalbuminuria screening in hypertensive patients to detect early signs of HN before CKD and ESRD [3]. Microalbuminuria is crucial for predicting mortality and morbidity in patients with cardiovascular and peripheral vascular disorders. It is also an independent risk factor for cardiovascular diseases in patients with DN, chronic hypertension, and the general population. Microalbuminuria may be an early indication of renal dysfunction, chronic hypertension, and an ESRD predictor in patients with T2DM. However, it is essential to note that microalbuminuria is also common in other pathological conditions such as obesity, sickle cell nephropathy, PE, cardiovascular events, and urinary tract infections [4]. Maternal and perinatal morbidity and mortality are significantly affected by PE worldwide. In previously normotensive women, the onset of new conditions such as proteinuria, acute kidney injury, and uteroplacental dysfunction must coexist with hypertension on two separate occasions, at least 4 hours apart, to diagnose PE.

Although there is currently no definitive marker for early PE diagnosis, proteinuria detected in women with PE indicates that detecting microalbuminuria and elevated UM/CR in the 1st trimester could predict the development of PE [5]. Monitoring and evaluating renal function has long relied on measuring microalbuminuria. However, renal function declines before microalbuminuria occurs in about one-third of patients. This means that relying solely on microalbuminuria to detect secondary nephropathies is inadequate for monitoring their incidence and progression. Microalbuminuria is a good predictor for the most common secondary nephropathies and can also be helpful in evaluating the presence of UM/CR. Despite being a low-cost and simple test, numerous studies indicate it lacks sufficient sensitivity and specificity as an early predictor for DN, HN, and PE [6, 7, 8]. Several potential biomarkers have been discovered recently, individually or in combination, that may serve as markers for early diagnosis of DN [9, 10, 11], HN [12, 13], and PE [14, 15, 16].

The main objective of this study is to evaluate UM/CR as an early diagnostic tool in patients with prevalent secondary nephropathies. Sensitivity and specificity are two significant factors that healthcare professionals can rely on to assess the suitability of microalbuminuria as a diagnostic test.

MATERIALS AND METHODS

As part of this cross-sectional study, 143 subjects were included. The study population was divided into three groups: patients with DN (n = 30) formed the first group, patients with HN (n = 23) formed the second group, and women with PE (n = 30) formed the third group. The control group included 60 healthy subjects, comprising non-pregnant individuals (n = 30) and healthy pregnant women (n = 30). The study took place between 2016 and 2017 at the Faculty of Medicine's Department of Medical and Experimental Biochemistry in Skopje, North Macedonia. Patients with DN and HN were recruited from the University Clinic of Nephrology at the Faculty of Medicine in Skopje. The inclusion criteria for DN patients were a progressive increase in microalbuminuria, a decline in estimated glomerular filtration rate (eGFR) to under 60 mL/min/1.73 m^2, elevated serum creatinine levels, and high arterial blood pressure [16]. Individuals with HN were included based on hypertension and impaired kidney structure or function, demonstrated by microalbuminuria and decreased GFR over three months [17]. Patients with any other kidney condition were not eligible for either group. The University Clinic of Gynecology and Obstetrics at the Medical Faculty in Skopje recruited female patients with preeclampsia. The inclusion criteria for this group of patients were the sudden onset of high blood pressure and protein in the urine, usually occurring after 20 weeks of pregnancy or postpartum [18]. Pregnant women under 18 and those with a history of kidney disease were excluded.

Urine samples were collected in plastic tubes without any additives, each containing 10 ml. Urinary strips were used to chemically analyze fresh urine samples. The turbidimetric method was employed to measure microalbumin levels, and creatinine levels were mea-

sured using the Jaffe reaction on the biochemical analyzer ChemWell (2910 Awareness Technology, Inc.). Blood samples were obtained from an antecubital vein in the morning after a night of fasting. After being centrifuged at 3,000 rpm for 10 minutes, the samples were placed in Eppendorf tubes. The UM/CR was calculated as the urinary albumin value divided by the urinary creatinine concentration (mg/g) [19]. Using the Cocroft and Gault formula, the estimated eGFR was determined [20]. Blood samples were obtained and analyzed using the ChemWell biochemical analyzer to estimate several biochemical parameters. Patient questionnaires containing information on medical history, sex, age, height, weight, and blood pressure were also completed.

The ethical principles of the current Declaration of Helsinki were adhered to in the study protocol, which received approval from the Ethical Committee of the Faculty of Medicine in Skopje, North Macedonia (Approval No. 03-5515/8 dated December 9, 2015).

Informed consent was obtained from each participant prior to the commencement of the study.

MedCalc Software (Ostend, Belgium) provided MedCalc for Windows version 15.0, which was utilized to carry out the statistical analysis. Descriptive statistics were conducted in the analysis, and the data distributions were assessed via the Kolmogorov-Smirnov test. We utilized receiver operating characteristic (ROC) analysis to conduct diagnostic statistics [21]. A p-value of less than 0.05 was the threshold used to determine statistical significance.

RESULTS

The baseline characteristics of examined groups of subjects

The study group consisted of 143 participants, with 40 (27.9%) male and 103 (72.1%) female. The clinical and biochemical characteristics of the study participants are presented in Table 1, categorized by groups.

ROC analysis of the diagnostic performance of UM/CR in the study groups

The receiver operating characteristic analysis was used to determine the UM/CR (mg/g) level in response to nephropathy. The estimated cut-off value for UM/CR was 30.0 mg/g. A sensitivity of 41.5% and specificity of 90% were found for patients with DN, with a Youden index J of 0.314 and an area under the curve of 0.636. The accuracy of UM/CR with a cut-off value of > 30.0 mg/g for detecting DN was 53.1%. Patients with HN had a sensitivity of 44.8% and specificity of 86.1%, with a Youden index J of 0.309 and an area under the curve of 0.626. The accuracy of UM/CR with a cut-off value of > 30.0 mg/g for detecting HN was 57.8%. For patients with PE, a sensitivity

Table 1. Baseline characteristics of the study subjects

Variables	DN n = 30	HN n = 23	PE n = 30	Healthy non-pregnant subjects n = 30	Healthy pregnant women n = 30
Male/female n	16/14	11/12	30 females	13/17	30 females
Age (years)	55.8 ± 10.1	56.4 ± 10.3	27.7 ± 4.7	47.8 ± 9.3	29.4 ± 6
BMI (kg/m2)	28.8 ± 4.2	29.1 ± 4.4	29.3 ± 4.6	25.6 ± 3.8	25.7 ± 3.3
Blood glucose (mmol/L)	9.3 ± 3.6	6.7 ± 2.8	5.2 ± 0.5	4.2 ± 1.1	4.6 ± 0.5
UM/CR (mg/g)	209.5 ± 206.1	186.7 ± 212.2	214.3 ± 160.3	15.1 ± 15.4	15.8 ± 11
SBP (mm/Hg)	147 ± 13	151 ± 15	152 ± 14	121 ± 10	119 ± 5
DBP (mm/Hg)	92 ± 8	94 ± 15	95 ± 7	87 ± 6	77 ± 6
Total cholesterol (mmol/L)	5.4 ± 2.1	4.5 ± 1.3	6.6 ± 0.8	3.5 ± 1.2	6.6 ± 1.1
Triglycerides (mmol/L)	2.5 ± 1.8	2.7 ± 1.6	2.1 ± 0.7	1.2 ± 0.6	2.4 ± 0.9
HDL (mmol/L)	1.4 ± 0.5	1.2 ± 0.3	1.5 ± 0.4	1.2 ± 0.4	1.7 ± 0.4
LDL (mmol/L)	2.6 ± 1.3	2.5 ± 0.9	4.1 ± 0.9	1.8 ± 0.9	3.8 ± 1.1
Total proteins (g/L)	69.5 ± 9.9	68.7 ± 7.2	67.6 ± 7.2	73.7 ± 5.8	68.3 ± 5.5
Albumin (g/L)	38.3 ± 9.3	40.2 ± 4.4	34.2 ± 5.1	46.4 ± 2.8	39.1 ± 4.1
Blood urea (mmol/L)	8.3 ± 5.3	8.1 ± 3.7	5.5 ± 1.2	4.3 ± 1.1	5 ± 0.9
Serum creatinine (μmol/L)	94.1 ± 18.1	120.1 ± 94	70.7 ± 9.2	75.1 ± 14.6	56.8 ± 4.6
eGFR (ml/min per 1.73 m^2)	61.1 ± 21.8	54.7 ± 30.2	91.3 ± 15.1	91.3 ± 5.9	95.5 ± 9.6

Results are presented as mean ± SD. Abbreviations: DN – diabetic nephropathy, HN – hypertensive nephropathy, PE – pre-eclampsia, BMI – body mass index, UM/CR – urinary microalbumin to creatinine ratio, SBP – systolic blood pressure, DBP – diastolic blood pressure, HDL – high-density lipoproteins, LDL – low-density lipoproteins, eGFR – estimated Glomerular Filtration Rate

of 100% and specificity of 93.3% were found, with a Youden index J of 0.933 and an area under the curve of 0.997. The accuracy of UM/CR with a cut-off value of > 30.0 mg/g for detecting PE was 96.7%. Table 2 summarizes the ROC analysis of the diagnostic performance of UM/CR in the study groups.

Table 2. ROC analysis of the diagnostic performance of UM/CR in the study groups

Diagnostic performance data of UM/CR	DN	HN	PE
The area under the ROC curve (AUC)	0.636	0.626	0.997
95% Confidence interval (95% CI)	0.540-0.725	0.531 to 0.715	0.934 to 1.00
Significance level p (Area = 0.5)	0.011	0.014	< 0.001
Youden index J	0.314	0.309	0.933
Optimal cut-off	> 30 mg/g	> 30.0 mg/g	> 30.0 mg/g
Sensitivity	41.5%	44.8%	100%
Specificity	90%	86.1%	93.3%
NPV – negative predictive value	35.5%	87.5%	100%
PPV – positive predictive value	91.4%	41.8%	93.75%
Diagnostic effectiveness (accuracy)	53.1%	57.8%	96.67%

Abbreviations: DN – diabetic nephropathy, HN – hypertensive nephropathy, PE – pre-eclampsia, NPV – negative predictive value, PPV – positive predictive value, UM/CR – urinary microalbumin to creatinine ratio

DISCUSSION

Microalbuminuria is the primary diagnostic tool in clinical practice for detecting DN, HN, and predicting PE. However, it has certain limitations, such as increased variability and lower sensitivity [22, 23, 24]. Due to the inadequacy of microalbuminuria as an early biomarker in secondary nephropathies, there is currently a lot of research interest in identifying novel biomarkers for the early detection of DN, HN, and PE. It is crucial to examine the sensitivity and specificity of microalbuminuria as an early marker for detecting DN, HN, and predicting PE. While sensitivity and specificity are essential in diagnostic accuracy test methods, predictive values such as PPV and NPV are also crucial. Sensitivity and specificity can aid physicians in determining whether a patient has a particular condition, while predictive values are more practical and relevant in clinical settings. A reliable diagnostic test is required to confidently diagnose a patient as unfavorable. Even though high sensitivity and specificity are desirable in testing, PPV and NPV can accurately determine whether or not someone has the targeted illness or disease based on the findings [25]. During our research, we tested the accuracy of UM/CR in diagnosing DN, HN, and PE. Our findings indicated the UM/CR cut-off value was 30.0 mg/g. For patients with DN, the sensitivity and specificity were 41.5% and 90%, respectively. The overall diagnostic accuracy of UM/CR in detecting DN was 53.1%, with NPV and PPV values of 35.5% and 91.4%, respectively. These results suggest that microalbuminuria is not a reliable early marker for DN due to its insufficient sensitivity, NPV, and overall diagnostic accuracy. Previous studies have investigated UM/CR and 24-hour albumin urine excretion rate concerning the diagnostic accuracy of microalbuminuria in DN patients. However, the literature data show significant variations in the findings. For instance, in Sampaio et al.'s study, UM/CR had a sensitivity of 79.3% and a specificity of 76.4% in detecting DN [26]. A different research revealed that the specificity of UM/CR is not very high (68.7%), and its positive predictive value is also low (60.3%) [27]. Azam Teimoury and colleagues conducted a study in which they discovered that the UM/CR cut-off value of 30 mg/g had 86% sensitivity and 60% specificity when predicting DN [28].

In our investigation on HN patients, we discovered that UM/CR had a sensitivity of 44.8% and a specificity of 86.1%, with an overall diagnostic accuracy of 57.8% for early detection of HN. Our findings indicated that microalbuminuria had low sensitivity, PPV, and total diagnostic accuracy as an early marker for HN. The reliability of microalbuminuria as a diagnostic tool for HN in patients with essential hypertension needs to be well-established, and limited literature exists on diagnostic accuracy. Our research demonstrated that UM/CR had a sensitivity of 100%, specificity of 93.3%, and total diagnostic accuracy of 96.7%, with a cut-off value of > 30.0 mg/g for identifying PE. These results suggest that microalbuminuria has excellent total diagnostic accuracy as an early marker for PE. According to a study by Salako et al., microalbuminuria could potently predict PE with high sensitivity but poor positive predictive value [23]. The prediction of PE can be statistically significant by measuring elevated UM/CR levels, with the test's overall predictive accuracy of 73.7% [8]. A study has found that UM/CR values more significant than 25.89 mg/gm in asymptomatic pregnant women can predict the onset of PE with an accuracy of 80% sensitivity and 87% specificity. The UM/CR ratio has a high negative predictive value of 99%, which can aid in the precise diagnosis of true negatives [29].

It should be noted that the groups examined in the study were small in number. Nonetheless, it is hoped that larger sample sizes in future prospective studies will confirm whether microalbuminuria can effectively predict secondary nephropathies.

CONCLUSION

Based on our findings, microalbuminuria may not be a sensitive early indicator of DN and HN. However, it is highly effective in diagnosing PE at an early stage. Therefore, using UM/CR as a screening tool can be a valuable strategy for prompt detection of PE.

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