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Urinary Spot Urine Microalbumin/Creatinine Ratio is Not Significant When Compared with β-2 Microglobulin in Defining Tubular Proteinuria

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Abstract

Introduction: β-2 microglobulin is a costly but highly specific biomarker in determination of tubular proteinuria. In this study, we aimed to evaluate differentiation of tubular and glomerular proteinuria with comparing the urine β-2 microglobulin level and the spot urine microalbumin/total protein ratio.

Methods: Patients with proteinuria between the ages of 1 month and 18 years who applied to the Dr. Behçet Uz Children's Hospital, pediatric nephrology clinic were included in our study. Patients with a spot urine total protein/creatinine ratio >0.2 mg/mg were included in the study. The urine microalbumin was studied using the spectrophotometric method in the Architect C-16000 device in the biochemistry laboratory of our clinic. The urine β-2M is measured by photometric method on the Architect C device. Urine samples for determination of the urine β-2M levels can be stored for 2 days at 2℃ to 8℃ and for 2 months frozen (-20°C). Spot urine total protein, micro-albumin, creatinine and urine β-2 microglobulin levels were evaluated. Urine β-2M level >0.32 mg/L was accepted as the upper limit value for tubular proteinuria. Receiver Operating Characteristic (ROC) curve method and pearson correlation analysis was used for comparing spot urine total protein/creatinine ratio, β-2 microglobulin and β-2 microglobulin/creatinine ratio.

Results: Ninety-seven patients included in the study, 39 (40.2%) were female. The mean age was 10.2 ± 5.2 years. Glomerular and tubular proteinuria were found in 57 and 40 patients respectively. In patients with tubular proteinuria were spot urine microalbumin/total protein ratio 3.87 ± 2.80 mg/mg and β-2 microglobulin/creatinine ratio was 14.317 ± 23.787 mcg/g and glomerular proteinuria was 4.7 ± 3.0 mg/mg and β-2 microglobulin/creatinine 22.411 ± 32.231 mcg/g were determined. Pearson correlation analysis was showed no any correlation between values. ROC analysis for differentiation between glomerular and tubular proteinuria does not have any correlation between parameters also (area under curves 0.4).

Conclusion: There was no differences in spot urine microalbumin/creatinine ratio between glomerular and tubular proteinuria. Spot urine microalbumin/creatinine ratio was not useful for differentiation. Urine β-2 microglobulin and other known parameters is necessary for glomerular and tubular proteinuria.

Keywords: Proteinuria; Glomerular proteinuria; Tubular proteinuria; Urine microalbumin; Urine β-2 microglobulin

Introduction

Measurement of protein excretion in urine plays a central role in the identification and classification of renal diseases [1]. Proteinuria may be a marker of serious kidney disease or systemic disorders [2]. Measurement of urinary protein excretion is important in the diagnosis and classification of kidney diseases [1]. Proteinuria is traditionally divided into glomerular and tubular proteinuria groups [3]. For spot urine samples, the urinary concentration of total protein was assessed using the spot urine Total protein/Creatinine (sTp/Cre) ratio. Spot urine Microalbumin/Creatinine ratio (sMa/Cr) and the amount of albumin in urine indicate glomerular proteinuria. Some of the proteins in the urine, other than albumin, include low-molecular-weight proteins and micro-and macroglobulins [4]. Tubular proteinuria occurs when there is increased excretion of normally filtered low-molecular-weight proteins due to impaired reabsorption by the proximal tubules [2]. Low molecular weight proteins are indicators of renal tubular disease [5]. These proteins pass freely through glomerular filtration and molecular weights below 40,000 Da. It consists of β-2 Microglobulin (β-2M), α-1 Macroglobulin (α-1M), Retinol-Binding Protein (RBP) and lysozyme. Increased urinary excretion of β-2M and RBP has high specificity for demonstrating tubular proteinuria [6-8]. Spot urine samples are used in most centers for measurements. Smith et al., revealed that this ratio can be used to differentiate between glomerular and tubular proteinuria by examining the ratio of higher molecular weight proteins (for example, albumin) to the total protein content of urine [5]. The threshold value of the spot urine Microalbumin/Total protein (sMa/Tp) ratio for the differentiation of tubular and glomerular proteinuria was found to be 0.4 mg/mg. A sMa/Tp ratio of <0.4 mg/mg has been shown to have high sensitivity and

specificity for tubulointerstitial diseases [5]. In this study, the β-2M level, which is a highly specific biomarker in tubular proteinuria but is not preferred routinely because it is a difficult and costly procedure, we compared diagnostically with the sMa/Tp. Our aimed to develop a cost-effective method for the diagnosis of tubular proteinuria and glomerular proteinuria differentiation.

Materials and Methods

Samples

Patients between the ages of 1 month and 18 years who were admitted to Izmir Behçet Uz Pediatrics Hospital Pediatric Nephrology Clinic between 2021-2022 were included the study. A total of 97 patients with proteinuria between the ages of 1 month and 18 years who visited the pediatric nephrology clinic were included in the study. Patients with a history of kidney transplantation were excluded from this study. In this study, we performed a diagnostic comparison of the β-2M level, which is a highly specific biomarker in tubular proteinuria and the sMa/Tp ratio. Patients with proteinuria were greater then sTp/Cre ratio >0.2 mg/mg were included to study. Spot urine total protein, microalbumin, creatinine and β-2M levels were measured.

Measurements

Urine samples were collected without preservatives and stored at 4℃ for <48 h or frozen (-20°C) for others. Spot urine total protein, microalbumin and creatinine values were studied in the biochemistry laboratory of our hospital using the Architect C-16000 device and the spectrophotometric method. Spot urine samples also were studied using a photometric method for a β-2M Architect C device. The sTp/Cre, sMa/Cre and sMa/Tp ratios were calculated. The patients were divided into two groups according to their diagnosis of tubular and glomerular proteinuria. The spot urine β-2M concentration in the urine of healthy subjects was 0.098 mg/L, urine β-2M level above 0.32 mg/L were accepted as the tubular proteinuria. The β-2M/Creatine ratio was also calculated for these cases. Studies have shown that a β-2M/Cre ratio above 300 mcg/g is significant for tubular proteinuria [9-16]. In our study, the cutoff values of the cases were re-evaluated to differentiate tubular and glomerular proteinuria for sMa/Tp, spot urine β-2M/Creatine and urine β-2M levels.

Statistical analyses

In the analysis performed by accepting Type I error as 0.01, Type 2 error as 0.2, the area under the curve value ROC detected in previous studies was 0.84, while it was calculated to reach 0.95 in our study and the positive patient group was accepted as 2/1 compared to the other groups. The sample size was calculated as 70 positive and 35 negative for 105 patients. To evaluate the findings, SPSS were used for the statistical analysis. Firstly, all numerical and categorical data were evaluated using by descriptive statistics and normal distribution analyses with Kolmogorov-Smirnov test. Student's t-test was used for the relationship between groups and pearson correlation analysisfor the relationships between parameters and Wilcoxon or Mann-Whitney U tests were used for numerical data that did not show normal distribution. ROC curves and Area Under Curves (AUC) were calculated with urine β-2M level as the gold standard to compare the prognostic value of the urinary markers. We determined cut-off values so that false-positive and false-negative rates were minimal, the proportion of correctly classified patients was maximum and sensitivity, specificity and positive and negative predictive values were calculated.

Results

Glomerular proteinuria was present in 57 of the patients and tubular proteinuria was present in 40 of them. Their mean age was 10.2 ± 5.2 years (58 was male). The sTp/Cre, sMa/Cre, sMa/Tp and β-2M/Cre ratios were compared according to the patients with tubular and glomerular proteinuria **(Table 1)**.

Table 1: The comparison of the spot urine total protein/creatinin ratio, spot urine microalbumin/creatinine ratio, spot urine microalbumin/total protein ratio, β-2 microglobulin/creatinine ratio mean values of patients with tubular and glomerular proteinuria (all of measurements were not statistically significant).

Groups	Tp/Cre* (mg/mg)	Ma/Cre** (mg/g)	Ma/Tp*** (mg/mg)	β-2M/Cre**** (mcg/g)
Tubular proteinuria	0.82 ± 1.00	458 ± 778	3.87 ± 2.80	14.317 ± 23.787
Glomerular proteinuria	1.87 ± 3.34	1.210 ± 2.519	4.65 ± 3.01	22.411 ± 32.231

Note: *: Spot urine total protein/creatinin ratio; **:Spot urine microalbumin/creatinine ratio; ***: Spot urine microalbumin/total protein ratio; ****: β-2 microglobulin/creatinine ratio.

The urine β-2M and sMa/Tp ratio and also β-2M/Cre ratio and sMa/Tp ratio were found not statistically significantly correlated. The ratio of sMa/Tp, β-2M, β-2M/Cre Tp/Cre was evaluated by ROC analysis in patients with and without tubular proteinuria. When the threshold value was accepted as 0.5, no significant value could be determined **(Figure 1)**.

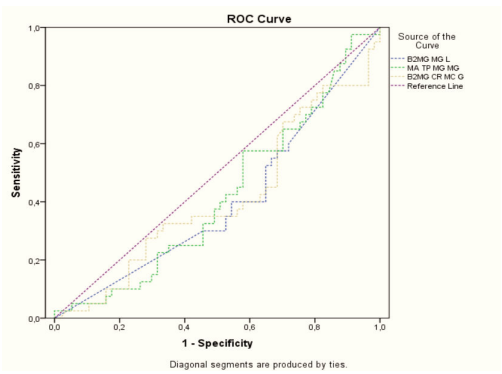

Figure 1: ROC curve analysis of the urine β-2M, Ma/Tp, β-2M/Cre ratio in patients with and without tubular proteinuria. The area under the curve was 0.40 (95% confidence interval 0.28-0.51) for the urine β-2M, 0.42 (95% confidence interval 0.30-0.53) for Ma/Tp, 0.41 (95% confidence interval 0.29-0.52) for β-2M/Cre.

Discussion

Low molecular weight proteins in the urine is used to differentiate glomerular and tubular proteinuria in children with proteinuria. Previous studies have suggested that evaluating the excretion of specific proteins in the diagnosis of tubular proteinuria yields a more objective, rapid and comprehensive result [9,17]. However, this approach is not cost effective and convenient for routine use. In our study, the hypothesis of whether it would be possible to differentiate tubular and glomerular proteinuria by looking at the sMa/Tp ratio as a cost-effective method was evaluated and the β-2M level, β-2M/Cre ratio were compared with the sMa/Tp ratio to predict tubular proteinuria.

Methven et al., in 6842 Chronic Kidney Disease (CKD) patients, it was found that albuminuria did not give an accurate reflection of total proteinuria [18]. We aimed to use this variability in urine total protein by looking at the sMa/TP. In our study, sMa/Tp median value 4.51 mg/mg (IQR: 0.28-9.47) was found. In while in the study of Smith et al., the median value of sMa/Tp median value was 0.49 (IQR: 0.29-0.66), it has also been mentioned that the sMa/Tp ratio was found to be lower in patients with tubular proteinuria [5]. In our study, the rates of sTp/Cre, sMa/Cre and sMa/Tp were compared according to patients with glomerular and tubular proteinuria. The mean value of the sMa/Tp ratio was found to be 3.87 ± 2.80 mg/mg in patients with tubular proteinuria and 4.65 ± 3.01 mg/mg in patients with glomerular proteinuria and similar to the aforementioned study, it was found to be lower in patients with tubular proteinuria. However, the determined values were found to be much higher. We think that this situation may have been caused by the high number of patients with severe proteinuria and albuminuria in our study. In our study, when patients were divided into tubular and glomerular proteinuria according to the diagnostic group, the ROC curve analysis for tubular proteinuria prediction for sMa/Tp revealed an AUC of 0.42 (95% confidence interval 0.30-0.53) and no significant value was found. In the study of Smith et al., the ROC curve analysis for estimating tubular proteinuria for Ma/Tp, the AUC was found to be 0.84 (95% confidence interval 0.82-0.87). In the same study, it was shown that sMa/Tp <0.40 provides high sensitivity and specificity for tubulointerstitial disorders. In the study of Lun et al., sMa/Tp ratio defined glomerular proteinuria in 47/61 (77%) children however, seven cases were attributed to tubular proteinuria and another seven cases to normal proteinuria. Considering the sMa/Tp ratio, 16/19 (84%) children with tubulopathy were detected and three of 19 cases were mistakenly attributed to glomerular proteinuria [17]. In the study of Lun et al., the threshold value for sMa/Tp was accepted as 0.5 mg/mg.

In our study, the β-2M/Cre ratio was calculated by studying the urine β-2M of the cases included in the study and whose sTp/Cre ratio was found to be higher than 0.2 mg/mg. The β-2M level, β-2M/Cre ratio were compared with the sMa/Tp ratio to predict tubular proteinuria using a ROC curve. Area under the curve was 0.40 (95% confidence interval 0.28-0.51) for β-2M, 0.41 (95% confidence interval 0.29-0.52) for β-2M/Cre and that values was not found to be significant for the estimation of tubular proteinuria. In the study of Smith et al., the area under the curve for β-2M/Cre was found 0.85 (95% confidence interval 0.80-0.91) in the ROC curve analysis to predict tubular proteinuria and that was similar and significant with the area under curve for sMa/Tp. Studies have shown that a β-2M/Cre ratio above 300 mcg/g is significant for tubular proteinuria [10-15]. The mean of β-2M/Cre was 4.317 ± 23.787 mcg/g in the patient group with tubular proteinuria and the mean of β-2M/Cre was 22.411 ± 32.231 in the patient group with glomerular proteinuria. We think that the high number of patients with severe proteinuria and albuminuria may have caused the inability to obtain a significant difference. In our study when patients are divided into tubular and glomerular proteinuria according to the diagnosis group, urinary β-2M level and β-2M/Cre ratio were found to be insufficient to show tubular proteinuria. However, comparison with a larger sample group may be instructive. It has also been reported that urinary β-2M is rapidly degraded in urine at pH <5.5. It is thought that similar studies can be done in the future by adding alkaline substance to the patient's urine and keeping the pH between 5.5 and 7.5. When the sMa/Tp ratio was evaluated by ROC curve analysis in the detection of tubular proteinuria, the area under the curve was not found significant. Analytical or biological factors may be responsible for the failure of the sMa/Tp ratio to differentiate between glomerular and tubular proteinuria. Urinary albumin excretion is affected by many factors, such as fever, exercise and infection, which should be excluded when investigating proteinuria in patients with kidney disease. The time of collection of spot urine can also affect this situation.

Conclusion

In our study, we tested the extent to which the sMa/Tp could distinguish between glomerular and tubular proteinuria compared with urinary β-2M levels. When the patients were divided into two groups according to their diagnoses, the sMa/Tp ratio was evaluated with ROC curve analysis in the detection of tubular proteinuria and the area under the curve was not found to be significant, when the sMa/Tp ratio was compared with the urine β-2M and β-2M/Cre ratios.

Analytical or biological factors may be responsible for the failure of the sMa/Tp ratio to distinguish glomerular and tubular proteinuria. Comparison with a larger sample group may be instructive. In addition, it has been reported that urinary β-2M rapidly decomposes in urine pH <5.5. It is thought that similar studies can be conducted in the future by adding alkali substance to the patient's urine and keeping the pH between 5.5 and 7.5.

Urinary albumin excretion is affected by many factors such as fever, exercise and infection, which should be excluded when proteinuria is investigated in patients with kidney disease. The time of collection of spot urine may also affect this situation.

In addition, the ratio of other low molecular weight proteins to Tp other than β-2M used to indicate tubular proteinuria can be calculated in a similar way and their usability in distinguishing tubular and glomerular proteinuria can be investigated.

Considering the importance of distinguishing tubular and glomerular proteinuria in kidney diseases, this subject deserves further study in order to develop a more accessible and cost-effective method as a biomarker.

Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study in the collection, analyses, or interpretation of data in the writing of the manuscript, or in the decision to publish the results.

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Ethical Statement

Approval for the study was obtained from the Health Sciences University Izmir Dr. Behçet Uz Children's Diseases Hospital Clinical Research Ethics Committee (Approval date: 24.06.2021, Protocol number 587, Decision number: 2021/11-07).

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