An unresolved issue: The relationship between spot urine protein-to-creatinine ratio and 24-hour proteinuria

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Abstract

Objective: To investigate the relationship between spot urine protein-to-creatinine (sP/Cr) ratio and 24-h protein excretion in patients with different diagnoses.

Methods: This retrospective study analysed data from the medical records of patients admitted for 24-h proteinuria determination who also had sP/Cr ratio data for the same day.

Results: A total of 1222 urine samples obtained from 694 adult outpatients were analysed. The mean $\pm$ SD age of the patients was 53.6 ± 15.9 years. The mean $\pm$ SD 24-h proteinuria and sP/Cr were 1.7 ± 2.4 g/day and 1.8 ± 2.4, respectively. The correlation between the sP/Cr and 24-h protein excretion was high ($R^2 = 0.89$). The sP/Cr ratio accounted for 72% of the variability in 24-h proteinuria in the entire study population. Areas under the curve for 24-h proteinuria at 0.3 g/day, 1.0 g/day and 3.0 g/day were 0.940, 0.966, and 0.949, respectively. The mean $+ 2SD$ limits of agreement were between $+2.99$ and -2.73 g/day according to the Bland Altman analysis.

Conclusion: This current study found a clinically unacceptable deviation between 24-h proteinuria and sP/Cr ratio. Therefore, the sP/Cr ratio cannot replace 24-h proteinuria. A new method using spot urine protein and creatinine values that is able to minimize under or over estimation is still warranted.

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Introduction

Proteinuria is a well-known biological marker used for the diagnosis and progression of chronic kidney disease (CKD) and a prognostic risk factor for cardiovascular disease and mortality.[1–4] Precise measurement of the amount proteinuria allows the clinician to stage patients with CKD, assess their risk of CKD progression and to monitor their response to treatment.[3,4] Although 24-h urinary protein excretion is considered to be the gold standard method for measuring daily proteinuria, it has several disadvantages, including not being suitable for use in children, over or under collection, inconvenience and the length time taken to undertake the test.[5] Therefore, clinical practice guidelines are now recommending the spot urine protein-to-creatinine ratio (sP/Cr) test for the first-line evaluation of proteinuria.[3,4,6,7] The sP/Cr test is based on the concept of steady levels of creatinine and protein excretion in urine.[5] However, the rate of urinary protein excretion is variable and unfortunately no standard values have been established for timed samples.[5] Research involving patients with various glomerular diseases, which constitute a significant proportion of the patient population in nephrology departments, demonstrated that the random sP/Cr ratio only modestly correlates with 24-h protein excretion.[8,9]

The current study aimed to investigate the relationship between sP/Cr ratio and 24-h protein excretion in a large number of patients with different diagnoses.

Patients and methods

Patient population

This retrospective study analysed the data from the medical records of consecutive patients admitted to the Department of Nephrology, Dicle University Hospital, Diyarbakir, Turkey between January 2007 and December 2010 and the Department of Nephrology, Denizli State Hospital, Diyarbakir, Turkey and the Department of Nephrology, Diyarbakir Memorial Hospital, Diyarbakir, Turkey between January 2011 and July 2015 for 24-h proteinuria determination ho also had an sP/Cr ratio test undertaken on the same day. Exclusion criteria were as follows: (i) patients with missing data; (ii) patients with a daily urine output of < 400 ml; (iii) children; (iv) pregnant women; (v) patients who had undergone transplant surgery. Demographic data and concomitant diseases were recorded from the medical records.

It was not possible to get written informed consent from the patients due to the retrospective nature of the study. The medical records data were recorded by the investigator in such a way that the patients could not be identified and the anonymity of the data was ensured. Ethics review committee approval for this type of retrospective study was not required according regulations in Turkey.

Urine analyses

Fresh blood and urine samples were used immediately and not stored prior to analysis. The blood samples were collected after an overnight fast. Approximately 20 ml urine was obtained from patients for urine chemistry analysis. Serum and urinary creatinine levels were determined using the Jaffe colorimetric method using an automated Abbott Aeroset clinical chemistry analyser (Abbott Diagnostics, Lake Forest, IL, USA). Urinary protein levels were determined using a turbidimetric assay with benzethonium chloride using an automated Abbott Aeroset clinical chemistry analyser. The method used was based on isotope dilution mass spectrometry, which

is an important method for the absolute quantification of peptides and proteins.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA). All quantitative data are expressed as mean ± SD and qualitative data as number of patients (%). A Spearman test was used to analyse the correlation between the methods. The relationship was also analysed by scatter data and the linear regression was expressed by R^2. Kolmogorov–Smirnov test was used to explore the normal distribution. Receiver operating characteristic (ROC) curve analysis was performed to investigate the ability of the sP/Cr ratio to estimate clinically important thresholds of 24-h proteinuria (0.3 g/day, 1.0 g/day and 3.0 g/day). Bland Altman analysis was undertaken to determine the limits of agreement between the two methods. A P-value < 0.05 was considered statistically significant.

Results

This retrospective study analysed a total of 1222 consecutive urinary samples obtained from 694 adult outpatients. The number of patients who provided a single urine sample was 452 (65.1%) and the remaining 242 patients (34.9%) had given multiple urine samples. The number of females and males were 342 (49.3%) and 352 (50.7%), respectively, which were similar. The mean ± SD age of the patients was 53.6 ± 15.9 years. All of the patients were Caucasian. The patients were diagnosed with the following: glomerulonephritis (228 of 694; 32.9%), hypertension (99 of 694; 14.3%), diabetes mellitus (96 of 694; 13.8%), CKD (86 of 694; 12.4%) and other diseases or unknown diseases (185 of 694; 26.7%).

The mean ± SD 24-h proteinuria and sP/Cr ratio were 1.7 ± 2.4 g/day and 1.8 ± 2.4, respectively. The correlation between sP/Cr ratio and 24-h proteinuria was high ($R^2 = 0.89$, $P < 0.0001$). The sP/Cr ratio accounted for 72% of the variability in 24-h proteinuria in the overall study population (Figure 1). In a subgroup analysis based on diagnosis, the correlation was weakest in the diabetes mellitus subgroup ($R^2 = 0.68$, $P < 0.0001$) and stronger in the hypertensive ($R^2 = 0.80$, $P < 0.0001$) and CKD subgroups ($R^2 = 0.75$, $P < 0.0001$) (Figure 1). The correlation coefficient was 0.71 in patients with glomerular disease.

The ROC curve analysis for the clinically important threshold of 24-h proteinuria at 0.3 g/day, 1.0 g/day and 3.0 g/day yielded areas under curve of 0.940, 0.966 and 0.949, respectively (Figure 2).

Bland Altman analysis was used to determine the limits of agreement between the sP/Cr ratio and 24-h proteinuria in the overall study population. The mean + 2SD limits of agreement were between +2.99 and –2.73 g/day. When excluding samples with a spot creatinine < 39 mg/dl (192 of 1222; 15.7%), the mean difference became 0.005 ± 1.298 g/day and the mean + 2SD limits of agreement were between +2.60 and –2.56 g/day.

Discussion

This retrospective study demonstrated a very high correlation between sP/Cr ratio and the gold standard method of 24-h proteinuria ($R^2 = 0.89$, $P < 0.0001$) and the sP/Cr ratio accounted for 72% of the variability in 24-h proteinuria. Similarly, studies comparing sP/Cr ratio and 24-h proteinuria have demonstrated a high correlation coefficient (mostly > 0.90).[9,10]

Unfortunately, a correlation analysis is not adequate to evaluate the utility of a new method against the gold standard. Bland Altman analysis is the most
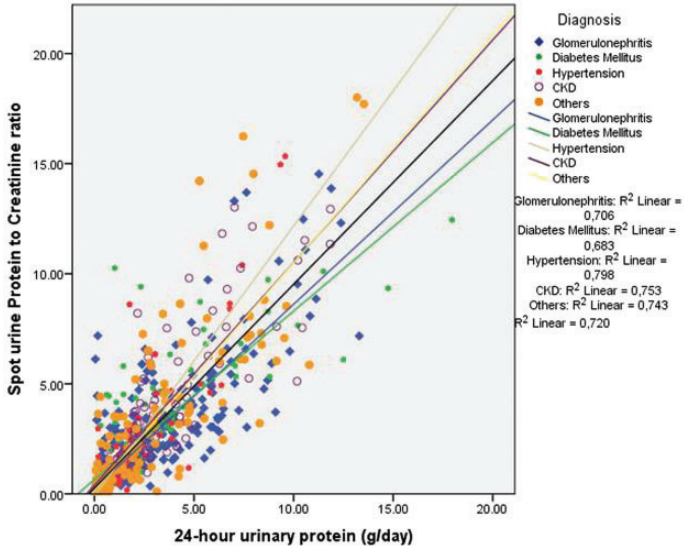
Figure 1. Linear relationship between the spot urine protein-to-creatinine (sP/Cr) ratio test and 24-h proteinuria in the overall study population and in disease subgroups (glomerulonephritis, diabetes mellitus, hypertension and chronic kidney disease [CKD]). The colour version of this figure is available at: http://imr.sagepub.com.
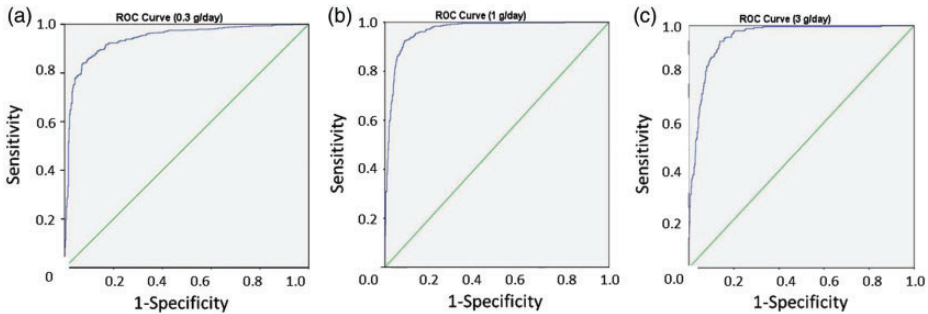

Figure 2. Receiver operating characteristic (ROC) curve analysis was performed to investigate the ability of the spot urine protein-to-creatinine ratio test to estimate clinically important thresholds of 24-h proteinuria (0.3 g/day, 1.0 g/day and 3.0 g/day) to provide a cut-off value to define proteinuria. The areas under the curve for 24-h proteinuria at 0.3 g/day (a), 1.0 g/day (b) and 3.0 g/day (c) were 0.940, 0.966 and 0.949, respectively.

commonly used and accepted test in this regard. The limits of agreement between the sP/Cr ratio and 24-h proteinuria were between +2.99 and –2.73 g/day in the current study according to the Bland Altman analysis. These limits of agreement are too wide to accept in terms of monitoring proteinuric patients and making
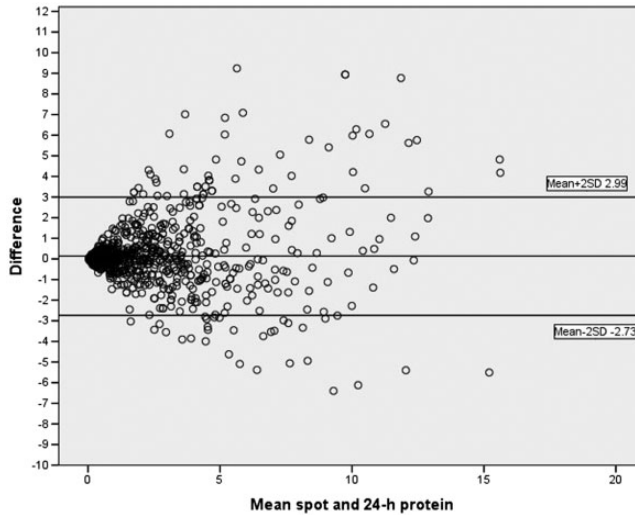
Figure 3. Bland Altman analysis to determine the limits of agreement between the spot urine protein-to-creatinine (sP/Cr) ratio test and 24-h proteinuria in the overall study population. The mean + 2SD limits of agreement were between +2.99 and −2.73 g/day.

decisions about starting or tapering immunosuppressive therapy.

It is well-known that the timing of random urine collection, the amount of proteinuria and kidney function, type of kidney disease and the handling of urine samples may all influence the accuracy of random sP/Cr ratio tests.[11–13] It has been reported that spot urine samples with low or high specific density are more likely to overestimate or underestimate the degree of proteinuria, especially in dilute urine samples with a creatinine level < 38.8 mg/dl or >61.5 mg/dl.[14] Similarly, when excluding the samples with a spot urine creatinine <39 mg/dl (192 of 1222; 15.7%), the mean difference became 0.005 ±1.298 g/day and the mean + 2SD limits of agreement were between +2.60 and –2.56 g/day according to the Bland Altman analysis, which was narrower than that for the overall study population.

Although clinical practice guidelines are now recommending the sP/Cr ratio test for the first-line evaluation of proteinuria,[3,4,6,7] only a modest correlation between random spot and 24-h protein excretion was shown in a study of 302 patients with glomerulonephritis.[8] In a subgroup analysis based on the aetiology of renal disease, the current study demonstrated that the relationship was weakest in the diabetes mellitus subgroup ($R^2 = 0.68$) and stronger in the hypertensive and CKD subgroups ($R^2 = 0.80$ and $R^2 = 0.75$, respectively). The correlation coefficient was 0.71 in the current study for glomerular disease. The difference between the findings might be due to the fact that the current study population was more homogeneous and there were no paediatric patients included in the study.

This current study had several limitations. First, the retrospective nature of the study was the main limitation. Secondly, a high number of samples were from patients with an unknown diagnosis. Thirdly, using random urine samples may also limit the interpretation of the current data.

In conclusion, this current study found a clinically unacceptable deviation between

24-h proteinuria and sP/Cr ratio. Therefore, the sP/Cr ratio cannot replace 24-h proteinuria. Using sP/Cr ratio instead of 24-h proteinuria in subgroups of patients, especially those with diabetes mellitus, should be avoided. A new method using spot urine protein and creatinine values that is able to minimize under or over estimation is still warranted.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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