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Original Research Article

CORRELATION OF 24-HOUR URINARY PROTEIN TO SPOT URINARY PROTEIN TO CREATININE RATIO AT DIFFERENT TIMES OF THE DAY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Abstract: *Spot urine protein to creatinine ratio has become a conventional measure of daily protein excretion in patients with chronic kidney disease (CKD). It is easily obtained and is reproducible. However, the values may vary with the time of the day, and collecting the first-morning void is typical. Twenty-four hours of urinary protein has been the traditional gold standard test for estimating protein excretion. This test is still carried out in centers where spot measurements are unavailable. We conducted our study to determine the correlation between 24-hour urinary protein and spot urinary protein to creatinine ratio at different times of the day (early morning, afternoon, and night). Sixty-seven patients of CKD fulfilling the inclusion criteria were asked to provide 24-hour urine samples according to standard recommendations. According to protocol, small aliquots of urine samples (5 ml each) were taken from the 24-hour sample to be processed for spot urinary protein and creatinine. Data was analyzed on SPSS version 22 and is presented as numbers, means + SD (age, spot urinary protein to creatinine ratio for morning, afternoon, and night), and frequencies (gender, type of kidney disease). Pearson's 'r' was tested for a significant correlation between 24 hours of urine protein and all different samples of spot collections. In contrast, sensitivity and specificity for spot urine protein-to-creatinine ratio predicting 24-hour protein excretion of <1, 1 – 3.5, and >3.5 g/day were determined using receiver operating characteristic curves (ROC Curves). In our cohort of patients across all stages of chronic kidney disease, we observed that there was a strong correlation between all three protein-to-creatinine ratio samples with 24 hours of urinary protein excretion; however, nighttime protein creatinine ratio showed the strongest correlation with 24 hours of urinary protein excretion (Pearson's r: 0.797). Nighttime spot urine protein to creatinine ratio sample was also found to have the highest sensitivity across all 24-hour urinary protein excretion levels.A nighttime spot urinary protein to creatinine ratio sample may reliably predict 24 hours of urinary protein excretion.*

Keywords: Chronic Kidney Disease (CKD), Spot Urine Protein to Creatinine Ratio, 24-Hour Urinary Protein

Introduction

Urinary protein excretion (24 hours of urinary protein > 300 mg/day) is the hallmark of renal disorder. It has been so since the time of Hippocrates, whose astute observation that kidney disease was usually found in patients who noticed "bubbles on the surface of the urine" still holds its position in the first few questions a nephrologist asks for evaluation of kidney disease (Singh, 2016). Proteinuria is independently correlated with the progression of renal disease (Ruggenenti et al., 1997; Tangri et al., 2011) and cardiovascular disease (Hillege et al., 2002; Schmieder et al., 2011). For these reasons, major renal associations have designated this marker to diagnose and follow the progression of kidney disease (Levey et al., 2002).

Different methods for quantifying proteinuria are available, including dipstick analysis, 24-hour urinary protein estimation, spot urinary protein to creatinine ratio, spot urinary albumin to creatinine ratio, and timed collections, which may range from a few hours to whole-day samples. Since it is a screening test, dipstick-positive proteinuria is usually the first indicator of glomerular disease. However, dipstick proteinuria is non-quantitative and, at least in adult populations, cannot be reliably used for follow-up of kidney disease progression or regression. Dipstick analysis tends to be affected by the concentration of the urine, as well as by its pH, resulting in false impressions of adequacy (Waugh et al., 2004). For this reason, and certainly for clinical follow-up, methods to quantify proteinuria are usually required.

A whole day (24 hours) urine collection for estimation of proteinuria has long been the test of choice for diagnosis of kidney disorders. This test is frequently tarnished by patient hesitancy, lack of standardization, and inaccuracies and is cumbersome(Hansen et al., 2002; Miller et al., 2009). The sample must also be refrigerated during collection, a facility that may not be available to a vast majority of patients, especially if the collection is home-based. Owing to the reasons mentioned above, random spot urinary protein to creatinine ratio, which requires a small sample of urine (5 – 10 ml), is currently the preferred investigation in proteinuric patients for diagnosis and follow-up of kidney disease and is known to correlate well with 24 hours urinary protein excretion in various disease states (Biradar et al., 2011; Jan et al., 2017; Yadav et al., 2010). Spot urinary protein to creatinine ratio of 30mg/mg is suggested as the cut-off for further evaluation of proteinuria. Although the first-

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morning sample is conventionally considered to be the most representative of the total daily protein loss contentions, it remains (Abubacker et al., 2016; JMKB et al., 2013; Mohseni et al., 2013; Teo et al., 2015).

We conducted our study to determine the correlation between 24-hour urinary protein and spot urinary protein to creatinine ratio at different times of the day (early morning, afternoon, and night).

Methodology

The study was conducted in the Nephrology Department at our tertiary care hospital in Lahore, Pakistan. The sample size 67 was estimated using a 95% confidence level and 10% margin of error with expected urinary protein loss in 22% of kidney disease patients (Kramer et al., 2003). Sixty-seven (67) consecutive patients aged 18 to 65 years with chronic kidney disease (CKD) stages I to V, irrespective of the underlying cause and positive for proteinuria ($\geq$ +1) by dipstick, were enrolled in this study after obtaining clearance from the Institutional Review Board and informed consents from the participants. Patients with congestive heart failure, pregnant females, patients receiving dialysis, patients with culture-proven untreated urinary tract infections at the time of assessment, those with urinary catheters, and highly febrile patients were excluded. All patients were advised to collect 24 hours of urinary samples per standard recommendations. No restriction was placed on activity; however, adequate rest was also advised. Patients were provided with graduated collection cups and a 24-hour collection jar. After discarding the first voided urine sample on the day of collection, all urine samples were preserved in the specially provided jar for the next 24 hours (refrigerated), including the first void on the next day. None of the urine samples (24 hours or spot samples) were collected via a urinary catheter. Along with this collection, three aliquots (not exceeding 5 ml each) were separated from the collection cup before adding to the 24-hour collection jar at three different periods of the day: Morning: 8 AM – 12 Midday; Evening: 2 PM – 6 PM; Night: 8 PM – 10 PM. These separated aliquots were immediately carried to the central hospital laboratory to be processed for spot urinary protein and spot urinary creatinine. Nephrology residents and staff nurses ensured the appropriate sample collection, preservation, and transport to the lab. The tests were carried out on COBAS e311 (Roche), which uses modified enzymatic techniques for these tests. Proteinuria was defined as total protein excretion of > 0.2 g/day. The normal lower limit for spot urinary protein to creatinine ratio was designated to be < 0.2 mg/mg. The statistical analysis was carried out on SPSS version 25. Age, weight, serum creatinine level, serum albumin, the protein-creatinine ratio for spot samples, and 24-hour urinary protein estimation were described using mean ± SD. Data for gender, underlying kidney disease medications being taken, and stage distribution were described using frequency and percentages. Values were log-transformed to correlate individual spot samples to 24-hour urinary protein to ensure normal distribution. Spearman correlation coefficient was used to check the correlation of all spot samples with 24-hour urinary protein excretion. The sensitivity for spot urine protein-to-creatinine ratio in predicting 24-hour protein "threshold" excretion of $\leq$1.0, $\geq$1.0 to < 3.5, and $\geq$3.5 g/day were determined using receiver operating characteristic (ROC) curves.

Results

We included 67 patients in our final analysis out of 100 patients screened, thus bringing the total number of samples (24-hour urinary collection and three spot samples for each participant) to 268. The rest of the patients were excluded per the exclusion criteria above.

Of the total study population of 67, 39 (58.2%) were males. Demographics are presented in Table 1. The means for spot samples were 3.40±3.13 for the morning sample, 3.75±4.20 for the afternoon sample, and 4.20±4.32 for the night sample. The mean 24-hour urinary protein excretion was recorded as 2024.2 $\pm$ 1524.5 mg/day (Table 1). Serum creatinine had a significant, although weak, negative effect on 24-hour urinary protein excretion (r: -0.377, p: 0.002). (Table 2). This observation was also confirmed on regression analysis (R^2: 0.630, p: 0.008).

Spot urinary protein to creatinine samples were correlated with Spearman's correlation coefficient. All three spot samples correlated significantly with the 24-hour urinary protein estimation. However, the nighttime sample had the strongest correlation (r: 0.797, p < 0.001. Table 2). Night time spot urinary protein to creatinine ratio sample also showed the best sensitivity (100% for protein excretion $\geq$3.5 g/day, 86% for $\leq$1.0 and $\geq$1.0 to < 3.5 g/day) for predicting 24-hour urinary protein loss. (Table 4, Fig 1)

TABLE 1: Baseline characteristics of the study population.

Demographics	
Mean age (years)	32.4 $\pm$ 12.8
Gender	Males 57%, Females 43%
Mean Weight (Kg)	67.5 $\pm$ 15.9
Diabetes	49.2% (32)
Likely/Proven Glomerulonephritis	51.8% (33)
Medications	
ACE Inhibitors /AIIRBs	63% (41)
Diuretics	87% (57)
Steroids/Other Immunosuppressive medications	21.5% (14)
Renal Function Tests and CKD Stage Distribution	
Mean Serum Creatinine (mg/dL)	3.3$\pm$2.5
Mean eGFR (ml/min) CKD-EPI	44 $\pm$ 36.6
Stage 1	16.9% (11)
Stage 2	16.9% (11)
Stage 3	21.5% (14)
Stage 4	15.4% (10)
Stage 5	29.2% (19)
Proteinuria	
Mean 24 hours Urinary Protein (mg)	2024.2 $\pm$ 1524.5
Mean Serum Albumin (g/dL)	2.0 $\pm$ 1.6

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able 2 Correlation of 24-hour Urinary Protein with other variables

Variables	r	p-value
Age	-0.228	0.063
Serum Creatinine	**-0.377**	**0.002**
Serum Albumin	0.055	0.686
Morning P: C Ratio	**0.681**	**< 0.001**
Evening P: C Ratio	**0.587**	**< 0.001**
Night P: C ratio	**0.797**	**< 0.001**

Table.3: Regression Analysis presenting the effect of various variables on 24-hour Urinary Protein

Variables	P-value
Age	0.202
Gender	0.178
Serum Creatinine	**0.008**
R^2 =0.630, R^2-Adj = 0.577, (Dependent variable = 24 hours urinary protein	

Table 4: Sensitivity of three spot samples with 24-hour Urinary Protein at different cut-offs.

24 Hours Urinary Protein	Spot Protein To Creatinine Ratio		
	Morning	Evening	Night
≥3.5	77.8%	66.7%	100%
≤1	81.8%	81.8%	86.4%
>1 to <3.5	72.2%	80.6%	86.1%

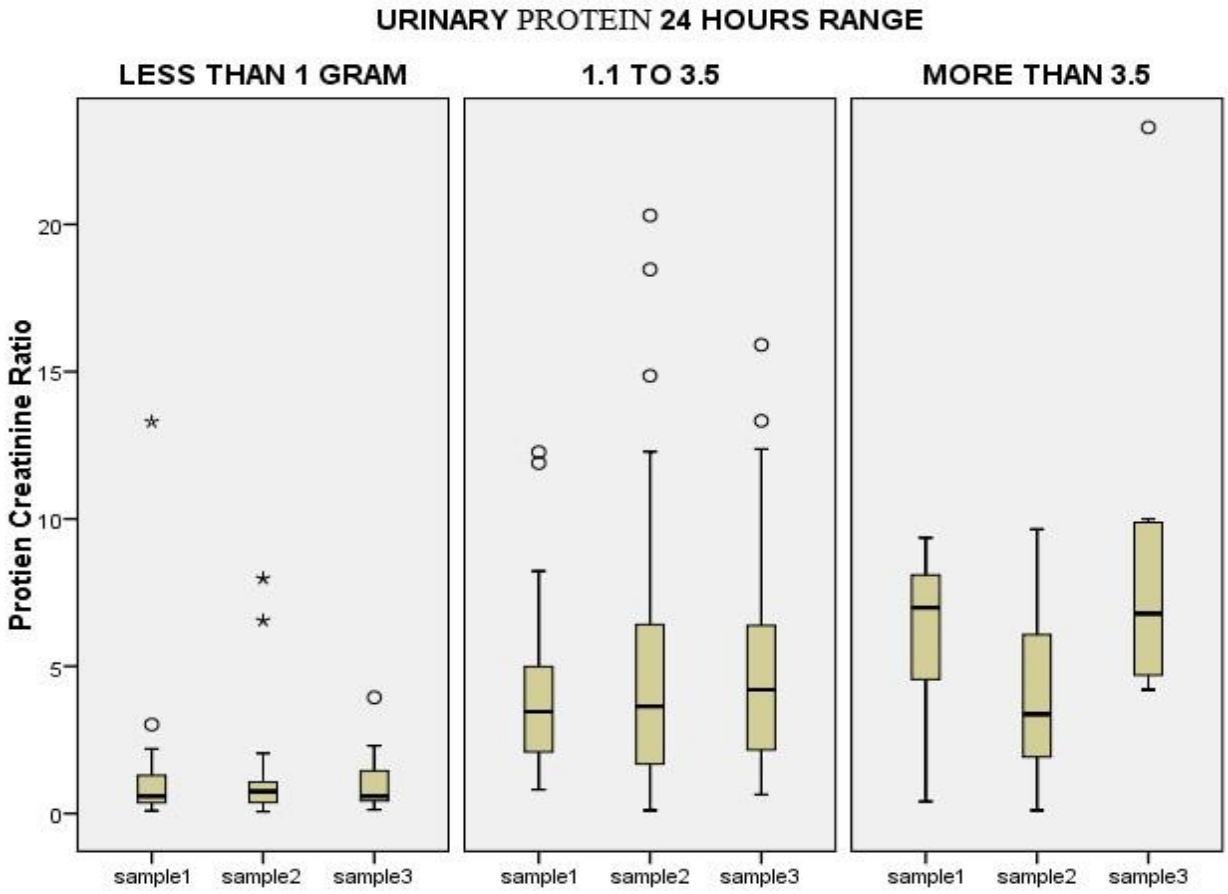

Figure 1: Boxplot presenting the distribution of spot samples for each 24-hour urinary protein category. **(**Samples 1, 2, and 3 denote Morning, Afternoon, and Evening spot urinary protein to creatinine ratio**)**

Discussion

Our study aimed to determine the correlation between 24-hour urinary protein and spot urinary protein to creatinine ratios at different times of the day, considering that urinary protein excretion is variable throughout the day (Agarwal, 2007). Early morning first void sample may miss the orthostatic proteinuria component, thus falsely giving lower values on spot sample in routine clinical practice, hence designating remission/response in patients with various nephropathies as inaccurate. As far as we know, ours is the

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only study that includes patients with all stages of CKD, thus making our study a true representative of the subjects with kidney disease.

It is to be noted that up to 30% of the 24-hour urine collections are considered to be inaccurate or incomplete, thus casting doubt on the findings. However, our nephrology team ensured that none of the patients was dropped from the study for this reason (Côté et al., 2008).

Our study shows that nighttime spot samples correlated best with 24-hour urinary protein excretion. The reason for this finding may be speculative; however, protein excretion throughout the day is influenced by posture, activity level, temperature, emotional factors, and blood pressure fluctuations (Verdonk et al., 2014). Sadjadi et al. described urinary protein excretion variability dependent upon the level of physical activity both for nephrotic and non-nephrotic range proteinuria (Sadjadi and Jaipaul, 2010). Our study enrolled patients without respect to the activity levels, but we surmise that most of our patients were at least semi-active during hospitalization. However, we also recognize that the in-hospital activity level may not represent a normal activity level. Some of our patients had advanced CKD and a normal activity level may not be expected from these patients owing to lethargy and easy fatigability synonymous with a kidney disorder.

Since ours was a hospital-based study in admitted patients, blood pressure recordings were taken according to the normal monitoring protocols. Blood pressures were managed according to standard guidelines, and all efforts were made to achieve the target blood pressure designated for CKD patients. Our study was not designed to study the effect of blood pressure variability on proteinuria. All of the patients remained afebrile during the sample collection period. Our study showed that 24-hour urinary protein excretion rates had a weakly negative correlation with serum creatinine values. Decreasing renal function may reduce protein excretion rate in a similar way that clearances for different toxins are reduced.

Agarwal et al. concluded that nighttime protein excretion rates were higher in patients with CKD and seemed to have lost the dependency upon systolic blood pressure compared to daytime systolic blood pressure. This observation is evident in our study, which shows that mean nighttime protein to-creatinine ratios were higher than the daytime and evening samples (3.40±3.13 for the morning sample, 3.75±4.20 for the afternoon sample, and 4.20±4.32 for the night sample). Agarwal et al. also suggest that orthostatic proteinuria may remain a significant part of proteinuria in patients with CKD. Since our nighttime samples were collected between 8 and 10 PM, we believe that the orthostatic component of proteinuria has been well represented in the afternoon and nighttime samples (Agarwal, 2007).

Certain studies have concluded that non-first morning void samples (random spot samples) correlate better with 24 hours of urine protein excretion, whereas certain other authors have noted that evening samples may not be inferior to the morning samples (Lamontagne et al., 2014; Mohseni et al., 2013; Verdonk et al., 2014). Thus, random protein-to-creatinine ratio samples have been considered accurate.

Our study shows that the nighttime protein creatinine ratio samples had the strongest correlation with total protein excretion during the day and the best sensitivity for the prediction of 24-hour protein excretion across all cut points of proteinuria (<1, >1 – 3.5 and >3.5 grams/day). Most of the literature advocates early morning spot sample as the best predictor. We speculate that the major reason for this inference may be the easy practice of obtaining spot samples in outpatient clinics during morning shifts (Abubacker et al., 2016; Teo et al., 2015). Most of the studies follow this routine. However, certain authors (including authors of this study) have noticed that non-first void samples and even evening samples may be the best predictor of 24 hours of urinary excretion (Chu et al., 1990; JMKB et al., 2013; Mohseni et al., 2013). Hence, nighttime urine samples for protein to creatinine ratio may be confidently and reliably obtained, especially in centers where 24-hour lab services are available. Our findings may also apply to patients admitted out of regular hours for evaluation of proteinuria, thus decreasing the time delay overnight in waiting for a first-morning void.

The strengths of our study are the inclusion of a cohort across the spectrum of renal function and the collection and analysis of a relatively large number of samples. The limitation of our study is the lack of intra-subject variability estimation.

Conclusion

A nighttime spot urinary protein to creatinine ratio sample may reliably predict 24 hours of urinary protein excretion.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department Concerned.

Consent for publication

Approved

Funding

Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

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