

## Assessment of Proteinuria using Protein Creatinine Ratio in Spot Urine Sample Versus 24 Hours Urine Sample

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### ABSTRACT

**Background:** Assessment of proteinuria is used as a diagnostic as well as a prognostic marker for kidney disease. 24 hours urinary protein is a gold standard method to assess proteinuria but collection of 24 hours urine is time consuming, and despite of proper instruction to patients, there may be inevitable chances of error during collection of 24 hours urine sample or inaccuracy in the timing of collection.

**Objective:** The present study was conducted to find the correlation of 24HUP and PCR in spot urine in our setup at various level of proteinuria irrespective of its cause and establish a cutoff PCR value at proteinuria  $\geq 150$ mg/day.

**Material and Methods:** Sixty four patients with clinically suspected cases of proteinuria were recruited after convenient sampling method. 24 hours urine, spot urine and blood sample were collected after obtaining the informed consent.

**Results:** A significant positive correlation (Spearman's correlation  $r = 0.70$ ,  $P < 0.0001$ ) was observed between 24 HUP and PCR in spot urine, and we also observed a stronger correlation with degree of proteinuria from  $\geq 150$  mg/day to  $\geq 1000$  mg/day.

**Conclusion:** A cutoff PCR value  $> 0.2$  was found to be equivalent to proteinuria  $\geq 150$  mg/day as assessed by a standard method.

**Key words:** Kidney disease, proteinuria, specimen collection, urine

### INTRODUCTION

Proteinuria is considered to be a sensitive marker for progressive renal dysfunction and an independent risk factor for cardiovascular morbidity and mortality<sup>[1]</sup> affecting several hundred million people worldwide.<sup>[2]</sup> The key diagnostic and prognostic tools for the effective management of the majority of renal disorders are mostly dependent on the detection and accurate quantification of protein excretion.<sup>[3]</sup> The exact quantification of proteinuria is of considerable value in the assessment of severity and progression of renal disease.<sup>[4,5]</sup> Elevated protein excretion should be used as a screening tool in patients at risk of developing renal diseases as recommended by the National Kidney Foundation.<sup>[6]</sup> Assessment of proteinuria by the measurement of 24 h urine protein (HUP) is one of the imperative investigations of renal disease.<sup>[7]</sup> The 24 HUP excretion also distinguishes between macroalbuminuria and microalbuminuria with microalbuminuria being known as a risk factor for developing overt diabetic nephropathy and cardiovascular disease.<sup>[8]</sup> Although 24 HUP measurement is a gold standard method for the

assessment of proteinuria, the collection of 24-h urine is tedious and despite proper instruction to the patients, an inevitable chance of an improper collection of urine sample or inaccurate time of collection can be incurred.

Twenty-four hours urine collection to measure protein and creatinine is a traditional and a confirmed method of estimation of total protein excretion but still not free of stipulations. The major problem in the respective procedure is the lack of patient compliance and the difficulty in sample collection.<sup>[9-11]</sup> It is inconvenient and time-consuming and deemed to be unreliable in uncooperative patients, and impossible in neonates and young children unless catheterization is used which is invasive and unpleasant. There are risks of under- or over-collection, leading to inaccurate measurements.<sup>[12]</sup> To circumvent this cumbersome method, researchers have discovered a simple and reliable method of single voided spot urine protein/creatinine ratio which can be used as an alternative to 24-h urine collection.<sup>[13]</sup> It has been supported by the findings from numerous researches that a significant positive correlation exist between 24 HUP and spot urine protein creatinine ratio in conditions such as diabetic nephropathy, nephrotic syndrome, and preeclampsia with varied strength.<sup>[10,11,14,15]</sup> For instance, if the spot urine protein is 300 mg/dl and spot urine creatinine is 150 mg/dl, the ratio will be  $300/150=2$  which means 24 HUP excretion is 2 g.<sup>[16]</sup> Use of spot

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urine protein-to-creatinine ratio for the assessment of proteinuria has been strongly acclaimed by US National Kidney Foundation K/DOQI Guidelines in 2000 in lieu of 24-h urine collection.<sup>[6]</sup> Both random and first morning specimens are acceptable with the first morning specimen being more preferable.<sup>[12]</sup> The urinary creatinine excretion is equitably constant; so the ratio of protein to creatinine excretion in single voided samples thus rule out the time factor as well as any dilution effects. Hence, an accurate reflection of quantitative proteinuria is attained.<sup>[15]</sup> Moreover, the guidelines reiterate that it is often unessential for a timed urine specimen collection to assess proteinuria in either children or adults.<sup>[2]</sup> Thus, this study was conducted to find out the correlation of 24 HUP and protein creatinine ratio (PCR) in spot urine in a tertiary care center set up at B.P. Koirala Institute of Health Sciences, Dharan, Nepal, in patients with normal as well as impaired glomerular filtration rate (GFR) at various level of proteinuria irrespective of specific clinical conditions.

## MATERIALS AND METHODS

This is a hospital based cross-sectional study conducted from May 2014 to October 2014 at B.P. Koirala Institute of Health Sciences, Dharan, Nepal, after being approved by Institutional Review Committee. A total of 64 patients were enrolled after fulfilling the inclusion criteria and providing the written informed consent. All patients having proteinuria and advised for assessment of 24 HUP in biochemistry laboratory were included as study participants while patients on dialysis and patients taking Levodopa, methyl dopa, and Na<sup>2</sup>-cefoxitin, having hematuria, urinary tract infection were excluded from the study. 24 h urine samples were collected in a clean container after voiding the first-morning urine up to next first-morning urine. After completion of 24 h urine collection, a random 5 ml urine and 2 ml of blood sample were collected in a sterile vial for estimation of protein and creatinine. Serum and urinary creatinine was estimated by Kinetic Jaffe method based on the colorimetric assay in automated Roche chemistry analyzer of cobas c 311, semi-quantitative estimation of urinary protein was done by dipstick test (Combilizer), and quantitative measurement of urinary protein was done by turbidimetric assay in automated Roche chemistry analyzer of cobas c 311.

Data were entered into Microsoft Excel,<sup>™</sup> and statistical analysis was done by SPSS 11.5 (Chicago Inc.). Normality was tested using Kolmogorov–Smirnov test. Spearman's correlation test was used to observe the correlation between 24 HUP and PCR in spot urine. Receiver operating characteristic curve (ROC) was used to determine the cutoff of PCR in spot urine to detect proteinuria.

## RESULTS

Sixty-four subjects were recruited in the study. Data were not normally distributed, and thus nonparametric tests were applied. The mean age of the study participants was 31.30 ± 13.30 with the range of 8–60 years, respectively. The gender distribution in the present study shows that 69% (*n* = 34) of the study participants were male and 31% (*n* = 20) were female, respectively. Of the total study participants, 53% (*n* = 34) of the patients had impaired GFR and 47% (*n* = 30) had normal GFR. Protein categories and their median 24 HUP showed significant difference among different categories of proteinuria assessed by semi-quantitative dipstick kit in spot urine samples as depicted in Table 1. Spearman's correlation between 24 HUP and PCR in spot urine in subjects having normal and impaired GFR suggests that the 24 HUP and PCR in spot urine were significantly correlated between the normal and impaired GFR as shown in Table 2. The difference in median PCR value in spot urine and 24 HUP showed no significant difference between two groups as shown in Table 3. Median 24 Urinary protein and Median PCR in spot urine have been illustrated in Tables 4 and 5.

A significant positive correlation between 24 HUP and PCR in spot urine in subjects having normal GFR (*r* = 0.79) as well as impaired GFR (*r* = 0.66) as shown in Table 2. A significant positive correlation was found between 24 HUP and PCR at different range of proteinuria, i.e. proteinuria <150 mg/day (*r* = 0.37, *P* < 0.04) and ≥150 mg/day (*r* = 0.68, *P* < 0.0001) as illustrated in Table 4. Correlation of 24 HUP and PCR, we found a significant positive correlation in proteinuria >1000 mg/day (*r* = 0.79, *P* < 0.0001) as depicted in Table 5. ROC curve at various cutoffs for the spot urinary PCR and the sensitivity and specificity has been demonstrated in Figure 1. Considering proteinuria

**Table 1: Median 24 HUP and various protein categories assessed by semi-quantitative dipstick kit in spot urine samples**

Dipstick protein category	Median (IQR)	P value*
Nil ( <i>n</i> =36)	131.6 (75.4, 236.6)	0.04
30 mg/dL ( <i>n</i> =2)	254.8 (134, 514.5)	
100 mg/dL ( <i>n</i> =15)	147.5 (108, 295)	
300 mg/dL ( <i>n</i> =5)	117.8 (72, 195.8)	
2000 mg/dL ( <i>n</i> =6)	1936 (1231.1, 2662.5)	

\*Kruskal–Wallis test *P* < 0.05 considered as statistically significant. HUP: Hour urinary protein, IQR: Interquartile range

**Table 2: Spearman correlation coefficient between 24 HUP and PCR in spot urine in subjects having normal and impaired GFR**

GFR categories	Correlation coefficient ( <i>r</i> )	P value
GFR ≥90 mL/min ( <i>n</i> =30)	0.79	<0.0001*
GFR <90 mL/min ( <i>n</i> =34)	0.66	<0.0001*

\**P* < 0.05 considered as statistically significant. HUP: Hour urinary protein, PCR: Protein creatinine ratio, GFR: Glomerular filtration rate

≥150 mg/day as a cutoff, we measured sensitivity and specificity at different cutoff of PCR. The area under the ROC curve for spot urine PCR at cutoff of 0.20 was found to be 0.85 (95.0% confidence interval [CI]; 0.75–0.95  $P < 0.0001$ ) [Figure 1]. An excellent sensitivity of 83.9% and specificity of 75.8% were achieved to detect proteinuria ≥150 mg/day at the PCR cutoff ≥0.20. With this cutoff, the positive predictive value was found 76.5%, and negative predictive value was found 83.3%. A positive correlation between PCR and 24 HUP (Spearman's correlation  $r = 0.70$ ,  $P < 0.0001$ ) which further improved with degree of proteinuria from ≥150 mg/day (Spearman's correlation  $r = 0.68$ ,  $P < 0.0001$ ) to ≥1000 mg/day (Spearman's correlation  $r = 0.79$ ,  $P < 0.0001$ ). A PCR value ≥0.2 was found to be equivalent to proteinuria ≥150 mg/day as assessed by standard method.

## DISCUSSION

Measurement of urinary protein excretion is a fundamental investigation in renal disease patients. Urinary dipstick test is a common test for the semiquantitative detection of urinary protein. Dipstick test tends to be non-reliable in the detection of urinary protein because the quantification depends not only on the amount of protein but also on the volume of urine at the time of test.<sup>[16]</sup> The use of single voided urine protein/creatinine ratio as an alternative to 24 h urine collection was suggested initially in 1980s.<sup>[15,17]</sup> Thereafter, several researches have

been reported to highlight the importance of spot urine protein creatinine ratio over 24 HUP measurement but with variable results.<sup>[16]</sup> One of the factors was the effect of body mass.<sup>[18]</sup> It is suggested that low muscle mass may overestimate and high muscle mass may underestimate the proteinuria. Thus, the timing of spot urine collection is still a matter of debate.<sup>[15]</sup> Moreover, the protein/creatinine ratio may vary according to ethnicity and race as well.<sup>[19,20]</sup> Furthermore, researches have suggested that orthostatic proteinuria may be missed by protein/creatinine ratio.<sup>[16]</sup>

The present study demonstrates a significant correlation between the PCR in spot urine and the 24 HUP and found that PCR of 0.20 showed maximum sensitivity and specificity at cutoff of proteinuria ≥150 mg/day assessed by gold standard method. This was in accordance to the study done by Yadav *et al.*<sup>[21]</sup> in diabetic patients with proteinuria, where the authors reported a good correlation coefficient ( $r = 0.89$ ) between 24 HUP and PCR in spot urine samples and noticed that protein creatinine ratio of 0.20 has maximum sensitivity and specificity at cutoff of proteinuria ≥150 mg/day.<sup>[21]</sup> Similar study reported by Chitalia *et al.* in renal clinic in adults demonstrated that protein creatinine ratio in a spot urine sample correlates well with the 24 h urine collection ( $r = 0.92$ ) and discerned protein creatinine ratio of 0.26 showed maximum sensitivity and specificity at a cutoff of proteinuria of 250 mg/day.<sup>[14]</sup>

Ginsberg *et al.* conducted a study on 46 patients and demonstrated an excellent correlation between the protein content of a 24 h urine collection and the protein/creatinine ratio in a single urine sample. Among all, the best correlation was obtained when samples were collected after the first voided morning specimen and before the bedtime.<sup>[15]</sup> Protein creatinine ratio at 0.20 was noticed at maximum sensitivity and specificity at

**Table 3: Median PCR in spot urine and 24 HUP at normal and impaired GFR**

GFR categories	Median PCR in spot urine <sup>a</sup>	Median 24 HUP (mg/day) <sup>a</sup>
GFR≥90 mL/min	0.19	145
GFR<90 mL/min	0.31	172
p value	0.067	0.85

<sup>a</sup>Mann–Whitney test. HUP: Hour urinary protein, PCR: Protein creatinine ratio, GFR: Glomerular filtration rate

**Table 4: Spearman's correlation coefficient between 24 HUP and PCR in spot urine at 24 HUP<150 mg/day and≥150 mg/day**

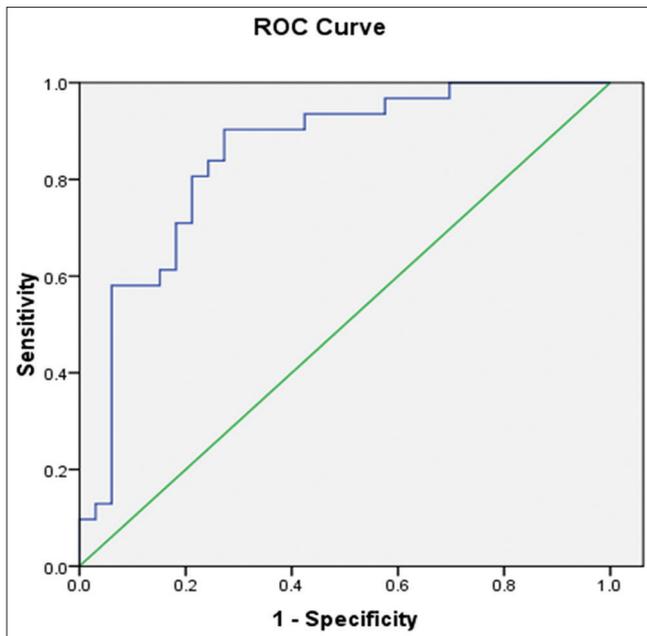
Proteinuria	<150 mg/day (n=32)	≥150 mg/day (n=32)	Total (n=64)
Spearman's correlation coefficient	0.37	0.68	0.7
P value of correlation coefficient	0.04	<0.0001	<0.0001
Median 24 urinary protein	103.5 (66.94, 127.65)	390.02 (232.00, 1611.65)	147.5 (101.25, 390.51)
Median PCR in spot urine	0.12 (0.07, 0.23)	0.52 (0.25, 0.81)	0.24 (0.11, 0.53)

$P < 0.05$  considered as statistically significant. HUP: Hour urinary protein, PCR: Protein creatinine ratio

**Table 5: Spearman's correlation coefficient between 24 HUP and PCR in spot urine at 24 HUP≥1000 mg/day and>1000 mg/day**

Proteinuria	≤1000 mg/day (n=55)	>1000 mg/day (n=9)	Total (n=64)
Spearman's correlation coefficient	0.66	0.79	0.7
P value of correlation coefficient	<0.01*	<0.0001*	<0.0001*
Median 24 urinary protein	17.50 (89.60, 254.8)	2312.33 (1837.25, 4401.00)	147.5 (101.25, 390.51)
Median PCR in spot urine	0.19 (0.19, 0.38)	0.72 (0.43, 0.72)	0.24 (0.11, 0.53)

\* $P < 0.05$  considered as statistically significant. HUP: Hour urinary protein, PCR: Protein creatinine ratio



**Figure 1:** Receiver operating characteristic (ROC) curve to demonstrate the diagnostic sensitivity and specificity of 24 h urinary protein versus protein creatinine ratio (PCR) in spot urine. ROC curve at various cutoffs for the spot urinary PCR the area under the curve was 0.85 (95% confidence interval 0.75–0.95;  $P < 0.0001$ )

cutoff of proteinuria of at 200 mg/day.<sup>[15]</sup> A study done by Dyson *et al.* among renal transplant patients showed similar finding with our study where they reported urinary protein creatinine index to assess proteinuria correlates well with 24 HUP ( $r = 0.77$ ) and at 500 mg/day cutoff with PCR 0.35 have maximum sensitivity and specificity.<sup>[16]</sup>

In the present study, the Spearman's correlation between 24 HUP and PCR in spot urine at proteinuria  $>1000$  mg/day have relatively higher degree of correlation ( $r = 0.79$ ) as compared with correlation with proteinuria  $\geq 150$  mg/day ( $r = 0.68$ ) signifying that the strength of correlation is more at higher range of proteinuria thereby, supporting the use of PCR method in spot urine.

Our finding is in accordance with the study done by Montero *et al.*, in chronic kidney disease cases reported correlation coefficient ( $r = 0.828$ ,  $P < 0.001$ ) at proteinuria range 300–3499 mg/day whereas they found weak correlation ( $r = 0.49$ ,  $P < 0.001$ ) at proteinuria below 300 mg/day.<sup>[22]</sup>

The significant difference in median 24HUP was noticed among a different group of proteinuria assessed semiquantitatively by dipstick method in our study although it did not correlate well when the proteinuria was in the lower range which was similar to the study done by Abitbol *et al.*, in nephrotic patients.<sup>[13]</sup>

Considering proteinuria of  $\geq 150$  mg/day as a cutoff, the present study depicted 83.9% sensitivity and 75.8% specificity at PCR value of 0.20. With this cutoff, the

positive predictive value was found 76.5%, and negative predictive value was found 83.3%. The area under the ROC curve for spot urine PCR at various cutoffs was 0.85 (95.0% CI; 0.75–0.95  $P < 0.0001$ ).

This study was intended on defining the correlation between the protein/creatinine ratio in spot urine and the 24 HUP and showed an excellent correlation when 24 HUP protein values were more than 1000 mg. Thus, the simplification of the collection and subsequent calculation of the ratio in a patient with proteinuria within that range could result in lower health care costs. This finding is supported by the study done reported by Montero *et al.*<sup>[22]</sup> Moreover, the present study is focused on scrutinizing the reduction in health care costs by substituting the 24 HUP for protein/creatinine ratio in spot urine sample will definitely of great use in the future.

## CONCLUSION

The study found a good positive correlation between 24 HUP and PCR in spot urine. The study reports that PCR value of  $\geq 0.20$  represents proteinuria  $\geq 150$  mg/day. Moreover, our study concludes that PCR in spot urine can be used for screening as well as monitoring proteinuria as an alternative to 24 HUP.

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