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Estimation of 24 Hour Protein in CKD Patients by analyzing the Protein/Creatinine Ratio of Four Spot Urine Samples

Jayasekara JMKB[*], Dissanayake DM[*], Gunaratne MDN[**], Amunugama K[***]

[*] Department of Pathology, Faculty of Medicine, University, of Peradeniya, Sri Lanka.
[**] Department of Mathematics, University of Moratuwa, Sri Lanka
[***] Department of Medical Laboratory Science, University, of Peradeniya, Sri Lanka

Abstract- The study was aimed to evaluate whether which spot urine protein to creatinine ratio (PC) can be a reliable alternative to 24-hour urinary total protein (UTP) estimation by analyzing four day time spot urine samples of CKD clinic patients.

We studied 48 CKD patients attending Nephrology unit with different nephritis such as diabetic nephropathy, CKD due to hypertension and unknown etiology (28male and 20 female) with proteinuria over 1g/day (GFR> 45 ml/min/1.73m^2) to determine the correlation between the measures of urine protein excretion by using four spot urine samples namely early morning,7am - 10 am, 10am-4pm and before going to bed. The simple linear regression, central tendency and dispersion were calculated. The Friedman test was done to evaluate difference among urine protein levels of 4 day time urine samples.

The mean 24 hour protein concentration was 3.8g/day$\pm$ 1.6 and the correlation coefficient (*r*) between 24-hour urine total protein and spot urine PC ratio were early morning 0.81 (P < 0.001), 7am - 10 am 0.64 (P < 0.001), 10am-4pm 0.66 (P < 0.001) and before going to bed 0.792 (P < 0.001) in the study population. Early morning spot urine sample showed the highest linear association whereas the 7am-10am and 10am-4 pm shows lower associations compared to other two spot urine samples. Highest and lowest median of PC ratio were 7 am -10am and before going to bed respectively. Highest dispersion of PC ratio was observed in 10am-4 pm and the distribution of before bed is somewhat skewed to right. We conclude that the protein-to-creatinine ratio (PC) in early morning urine sample is an accurate, convenient, and reliable method to estimate the protein excretion in urine in study population in early stages of CKD.

Index Terms- urine protein to creatinine ratio, spot urine sample, 24 hour urine protein estimation, early morning

I. INTRODUCTION

Protein in urine is recognized as an independent risk factor for cardiovascular and renal disease and as a predictor of end organ damage. In particular, detection of an increase in protein excretion is known to have both diagnostic and prognostic value in the initial detection and confirmation of renal disease (1), and the quantification of proteinuria can be of considerable value in assessing the effectiveness of therapy and the progression of the disease (2). The National Kidney Foundation of USA has recommended that an increase in protein excretion be used as a screening tool in patients at risk of developing renal disease (3). An increase in protein excretion has been used in the early detection of several specific conditions, e.g. preeclampsia, diabetic nephropathy, and nephrotoxicity attributable to drugs (1-3). The variation in urine protein excretion from 100 % to 500% throughout the day could be attributed to several factors including variations in water intake, amount of exercise, food pattern and life style of the different populations. The variation may be further exacerbated by pathologic changes in blood pressure and renal architecture. Only some studies concluded that ethnic genetic and unmeasured environmental factors may contribute to proteinuria in patients (4).

Measurement of protein excretion in a 24-hour urinary collection is the gold standard for the quantification of proteinuria. 24-hour urinary collection is used to smooth the fluctuations in proteinuria over the day and gives precise results. However, this method is time consuming, cause inconvenience to patients and often unreliable because of frequent errors in timing the 24 hour sample especially in outpatient setting and for infants and children (5,6,7).

Several authors have studied the relationship between the urine protein to creatinine ratio of different day time spot samples and 24-hour prorein excretion in patients with different types of nephritis which provides a more convenient way to calculate the protein excretion. Kidney Disease Outcomes Quality Initiatives (KDOQI) of United States National Kidney foundation guidelines for chronic kidney disease recommended that assessment of proteinuria in adults and children should be conducted in spot urine sample(2). Some studies show that the protein to creatinine ratio in samples collected in the mornings a reliable estimation of 24 hours protein in patients with glomerulonephritis while second voided urine sample is suggested in another study. Therefore this study was aimed to evaluate which spot urine protein creatinine ratio can be a reliable alternative to 24-hour urinary protein (UTP) estimation by analyzing four day time spot urine samples of patients with different nephritis such as diabetic nephropathy, CKD due to hypertension and unknown etiology attending renal clinics in Sri Lanka.

II. MATERIAL AND METHODS

The ethical clearance was taken from the Ethical committee conducted by the Faculty of Medicine, University of Peradeniya, Sri Lanka and informed consent was taken from each participant. Forty eight (48) CKD patients with different types of nephritis such as diabetic nephropathy, due to hypertension and unknown etiology attending nephrology unit at General Hospital Kandy were selected for the study. The age range of the patients was 18 to 65 years.CKD patients with GFR greater than 45 ml/min/1.73m^2 were chosen for the study including 28 male (age 54$\pm$11) and 20 female (age 47$\pm$8) Patients. Clinical records (files) of the patients were considered and 24 hours protein greater than 1g/24 hours were selected for the study. Although clear instructions were given for all patients regarding urine sample collection, 6 (11%) patients were excluded due to substandard urine collection.Ten milliliters (10 ml) of four (4) spot urine samples namely early morning, spot samples between 7am - 10 am and 10am-4pm and before going to bed were collected, centrifuged and supernatants were frozen immediately at -20^0 C for one day. The 24 hour urine sample was also collected in the same day. The concentrations of total protein in urine in both 24 hour and spot samples were measured by using turbidometric assay (U/CSF protein assay kit, sensitivity-4mg/dl) and the urine creatinine of spot urine samples were measured by using modified Jaffe method(Roche reagent). The PC ratios of all spot samples were calculated by dividing protein concentration (mg/dl) by urine creatinine concentration (mg/dl). Central tendency and data dispersion were observed with a box-plot diagram and Friedman test was done to evaluate whether there are significant differences among evaluated 4 day time spot urine samples. The relationship between 24HUP and PC ratio were evaluated with Pearson correlation coefficient and simple linear regression analysis by using Minitab statistical soft ware.

III. RESULTS

The box plot diagram depicts PC ratio of the four study samples. According to the box plot the highest median was observed during samples collected between 7 am -10am and lowest was before parting to bed. Highest dispersion observed during 10am-4 pm and the distribution was skewed to right in samples collected before going to bed.

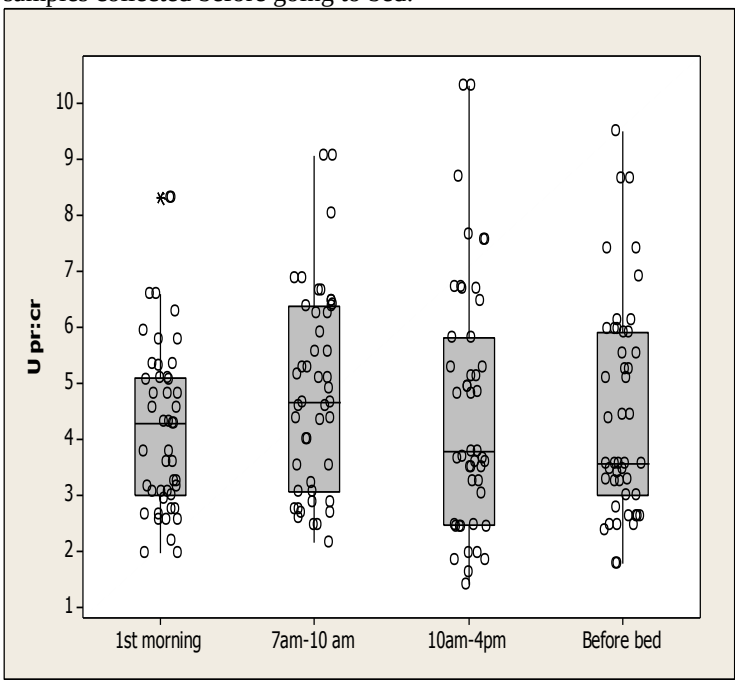

Figure 1- Box plot diagram of PC ratios of four spot urine samples

The relationship between 24-hour urine total protein and spot urine PC ratio were tested with Pearson correlation coefficients. All PC ratios of spot urine samples showed significant linear relationships (<0.0001) with 24-hour urine total protein.

The correlation coefficient (r) between 24-hour urine total protein and spot urine PC ratio are givan in Table 1.

	Early morning	7am to 10am	10am-4pm	Before bed
Correlation cofficient	0.81	0.64	0.66	0.79
P value	<.0001	<.0001	<.0001	<.0001

Table 1. Correlation coefficient between 24 hour urine total protein and spot urine PC ratios

Early morning spot urine sample showed the highest linear relationship whereas the 7am-10am and 10am-4 pm samples showed lower linear relationships compared to other spot urine samples.

Regression models involve total urinary proteins as independent variable and PC ratio as the dependant variable which helps to understand how the typical value of the PC ratio changes when total UTP is varied.

The Friedman test was applied to compare the effect of time on PC ratios. The Friedman test provides the desired test of null hypothesis; all time effects are zero vs. alternative hypothesis: not all time effects are zero. The PC ratio versus time was blocked by patients and the test statistic, had a p-value of 0.446 (P>0.05). Therefore the data do not support that the overall median difference as an effect of time.

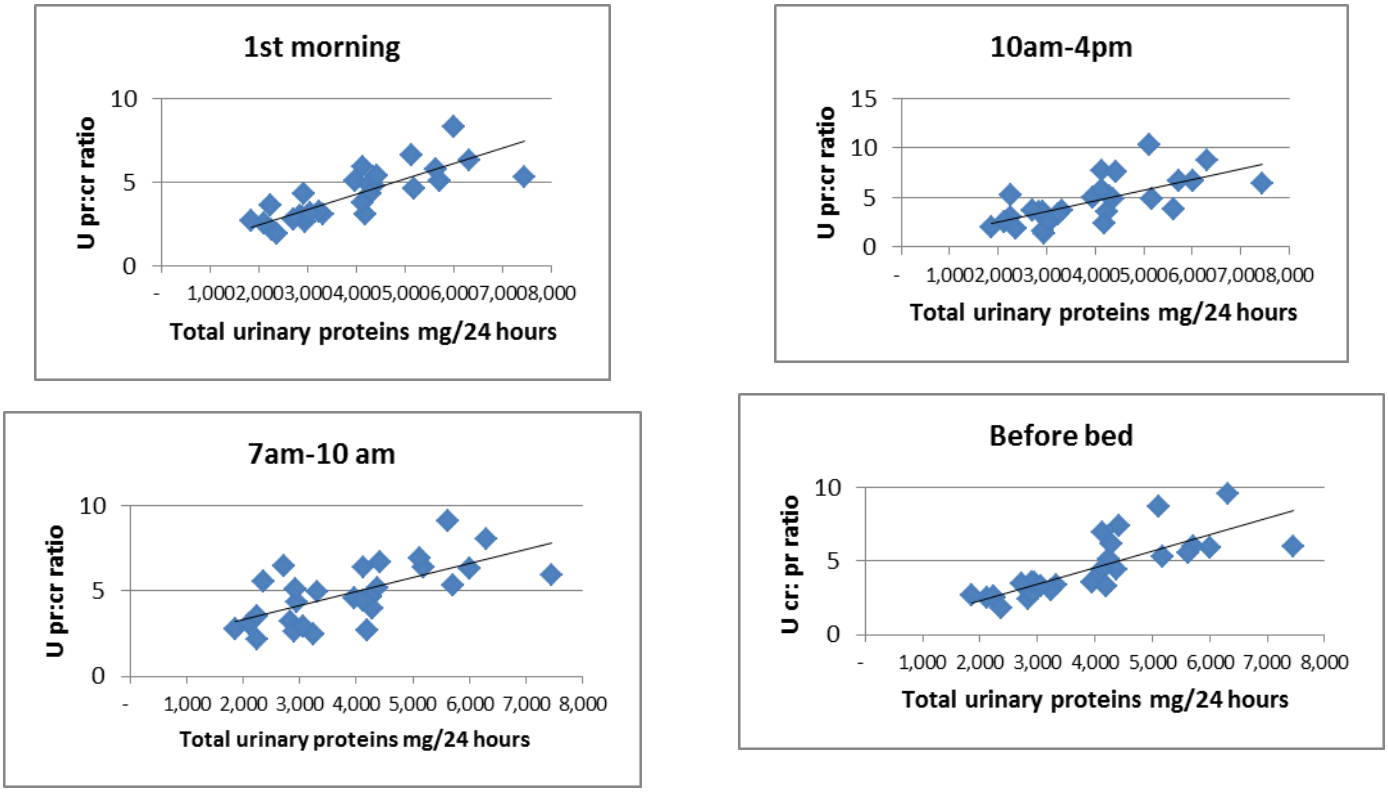

Figure 1 – Relationship between PC ratio in spot urine samples and 24 hour total urine protein

Spot urine sample	Regression equation	R2	P value
1st morning	U pr:cr=0.63192+0.00091971×24HUpr	0.6617	<.0001
7-am-10am	U pr:cr=1.6424+0.00082484×24HUpr	0.4055	<.0001
10am-4pm	U pr:cr=0.36161+0.00107×24HUpr	0.4363	<.0001
Before bed	U pr:cr=0.12113+0.00111×24HUpr	0.6265	<.0001

Table 2- Regression equations for spot samples

IV. DISCUSSION

Quantification of proteinuria is important for monitoring disease progress and the response to therapies in patients with different types of nephritis such as diabetic nephropathy, due to hypertension and unknown etiology. Recently, collecting 24 h urine sample for estimation of total protein has been the standard method(8,9). However, 24 h urinary collections are cumbersome and frequently unreliable due to inadequate collection (11% samples were discarded in this study due to incomplete sample collection) hence a reliable and convenient estimation easy measure like the spot urine PC ratio would be ideal in clinical practice. This study investigated the agreement between 24 h urine total protein and urine PC ratio of four spot urine samples in patients with diabetic nephropathy, CKD due to hypertension and unknown etiology.

Several investigators studied the relationship between the PC ratio and 24-h protein excretion. Ginsberg et al. (10) was investigated a correlation coefficient of 0.972, these authors also studied the variation of this relationship during the course of 24 h

by studying the PC ratio and absolute amount of protein excreted in urine samples 24 hours from 46 patients collected over timed periods throughout the day. They identified that the relationship varied by as much as 30% but that during normal daylight activity when most random samples are likely to be collected the variation was minimal. The greatest differences were seen during the times when the patients were most likely to be recumbent. These authors concluded on the basis of these data that the PC ratio of a spot urine could be used as a reliable estimator of the 24-h urine protein excretion. Further, several investigators have made similar observations and drawn similar conclusions (11), whereas others have stated a preference for the first sample collected after the first morning void (12,13). However, some authors have pointed out that regression analysis and the reporting of a correlation coefficient indicate the degree of linear association between the two variables but do not enable a reliable decision to be made to replace one with the other (14). Thus, the high degree of association between the PC ratio and the 24-h protein excretion does not necessarily give reliable information on whether use of the ratio in a random sample will enable clinicians to reduce their dependence on the 24-h urine collection.

Another study showed that urine protein excretion was influenced by physical activity. They studied 48 patients with proteinuria and varying levels of physical activity to determine the correlation between the measures of urine protein excretion. The correlation coefficient (r) between 24-hour urine total protein and random urine P-C ratio was 0.75 ($P < 0.01$) in the overall study population, but varied according to the level of proteinuria and physical activity in a stratified analysis. Thay conclude that the random urine P-C ratio is a reliable and practical way of estimating and following proteinuria, but its precision and accuracy may be affected by the level of patient physical activity(15).

The present study also reveled that a best correlation coefficient of 0.812 with early morning urine sample and the Friedman test was used to detect differences in 4 day time spot urine samples that not showed any significant difference among them.

According to the kidney disease management guidelines, 24 h urine total protein <0.5 g/day has been used as one of the remission criteria for diagnosing kidney diseases. Proteinuria of >1 g/day is considered as clinically significant or a threshold to recommend renal biopsy, while proteinuria >3.5 g/day is severe in the nephrotic range. In this study, proteinuria > 1g/day were considered for calculation and analysis because renal biopsies are rarely carried out in our health system therefore we can conclude that early morning PC ratio of spot urine sample shows the highest linear relationship with 24 hour protein excretion.

Therefore we conclude that the protein-to-creatinine ratio (PC) in early morning urine sample is an accurate, convenient, and reliable method to estimate the 24 hour protein excretion in urine in study population in early stages of CKD patients (protein excretion >1g/day). Other three urine samples such as 7am-10am, 10am-4pm and before bed will also be used for the estimation.

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AUTHORS

First Author – Jayasekara JMKB, PhD student, Department of Pathology, Faculty of Medicine, University, of Peradeniya, Sri Lanka, kbjayasekara@gmail.com.
Second Author – Dissanayake DM, Professor in Pathology, Department of Pathology, Faculty of Medicine, University, of Peradeniya, Sri Lanka
Third Author – Gunaratne MDN, Msc Student . Department of Mathematics, University of Moratuwa, Sri Lanka

Fourth Author – Amunugama K, , Department of Medical Laboratory Science, University, of Peradeniya, Sri Lanka

Correspondence Author – Jayasekara JMKB, PhD student, Department of Pathology, Faculty of Medicine, University, of Peradeniya, kbjayasekara@gmail.com., Phone: +94776971907