

# Assessment of Proteinuria by using Protein: Creatinine Index in Random Urine Sample

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## Abstract

**Objective:** To assess the quantitative measurement of proteinuria by using random urine protein: creatinine index/ratio in comparison with 24 hours urinary protein excretion in patients of renal diseases having normal glomerular filtration rate.

**Methods:** One hundred and thirty patients, 94 males and 36 females, with an age range of 5 to 60 years; having proteinuria of more than 150 mg/day were included in this study. Qualitative urinary protein estimation was done on random urine specimen by dipstick. Quantitative measurement of protein in the random and 24 hours urine specimens were carried out by a method based on the formation of a red complex of protein with pyrogallol red in acid medium on Micro lab 200 (Merck). Estimation of creatinine was done on Selectra -2 (Merck) by Jaffe's reaction. The urine protein: creatinine index and ratio were calculated by dividing the urine protein concentration (mg/L) by urine creatinine concentration (mmol/L) multiplied by 10 and mg/mg respectively.

**Results:** The protein: creatinine index and ratio of more than 140 and 0.18 respectively in a random urine sample indicated pathological proteinuria. An excellent correlation ( $r=0.96$ ) was found between random urine protein: creatinine index/ratio and standard 24 hours urinary protein excretion in these patients ( $p<.001$ ). Dipsticks showed moderate correlation ( $r=0.52$ ) and error in interpretation of proteinuria.

**Conclusion:** The protein: creatinine index in random urine is a convenient, quick and reliable method of estimation of proteinuria as compared to 24 hours of urinary protein excretion for diagnosis and monitoring of renal diseases in our medical setup (JPMA 55:428;2005).

## Introduction

Proteinuria is one of the determinants of progression of renal diseases as nephrotic syndrome and the amount of protein excretion is a reflection of severity of the disease.<sup>1,2</sup> Assessment of urinary protein excretion is not only diagnostic but also has prognostic value in monitoring of these patients.<sup>3</sup> Traditionally urinary protein excretion assessment has been done in 24 hours urine collection specimens but this approach is time consuming, cumbersome and imprecise.<sup>4</sup> An alternative approach has been advocated by some researchers avoiding 24 hours collection. This is the measurement of protein/creatinine ratio in a random urine sample.<sup>5</sup> This approach is based on the fact that in presence of a stable glomerular filtration rate, urinary creatinine excretion has been reported to be fairly constant in a given individual.<sup>6</sup>

In our population the problems associated with 24 hours urine collection are further highlighted because of lack of education and poor communication between patient and the treating physicians. So far no population - based study for the assessment of protein: creatinine index/ratio has been conducted in Pakistan. The main objective of this study was to compare the accuracy of quantitative measurement of proteinuria by using protein creatinine index/ratio in comparison with 24 hours urinary protein excretion in patients of renal diseases having normal glomerular filtration rate (GFR). The secondary objectives were to establish

the 95% confidence interval (95% CI) of urinary protein excretion index and clinical utility of protein creatinine index/ratio for Pakistani children and adults suffering from kidney diseases.

## Patients and Methods

This cross-sectional descriptive study was carried out in the department of Pathology, Army Medical College, Rawalpindi from January to September 2004.

The patients of nephropathy with persistent proteinuria were referred to the department of pathology Army Medical College for evaluation of 24 hours urinary protein excretion from Military Hospital and civilian nephrologists of Rawalpindi. The patients having urinary tract infection, haematuria, pregnancy, obesity and creatinine clearance less than 70 ml /min were excluded from the study. Thus one hundred and thirty patients consisting of 94 males and 36 females of ages varying from 5 to 60 years having renal diseases with proteinuria of more than 150 mg/day were included in this study.

All the patients were given detailed advice regarding 24 hours urine collection. They were asked to give a 24 hours urine sample starting at 9.00 am for total protein excretion rate. A random morning urine sample was also collected next day for measuring urinary protein and creatinine. Blood sample of 3 ml was also collected for measurement of serum creatinine for estimation of GFR.

Qualitative urinary protein estimation was done on spot urine specimen by dipstick which was graded from negative to 4 +ve. Quantitative measurement of protein in the urine was carried out by a method based on the formation of a red complex of protein with pyrogallol red in acid medium on Micro lab 200 (Merck, Germany).<sup>7</sup> Estimation of creatinine was done on Selectra -2 (Merck, Germany) by Jaffe's reaction. In this reaction creatinine reacts with alkaline picrate forming a red complex.<sup>8</sup> Coefficient of variation of creatinine and urinary protein assay were 4.5 and 3.2%.

Twenty four hours protein excretion was calculated by urine protein (mg/dl) X urine volume (L/24 hours). The urine protein: creatinine index and ratio were calculated by dividing the urine protein concentration (mg/L) by urine creatinine concentration (mmol/L) multiplied by 10 and mg/mg respectively. The 24 hours protein excretion rate, urine protein creatinine ratio and index were compared.

### Statistical Analysis

The data of the different baseline variable was analyzed on SPSS 11 packages. Data of 130 patients was expressed as mean, SD, 2.5<sup>th</sup> and 97.5<sup>th</sup> percentile. The correlation analysis between 24 hour protein excretion rate, dipstick, and protein creatinine index/ratio on spot urine protein was carried out by Pearson correlation coefficient (r). Significance was set at 0.05.

## Results

Over the nine months period, 130 urine samples were analyzed for urinary protein and creatinine. The mean (SD); percentile (95% CI) of different urinary laboratory measurements of these patients are shown in Table-1. The Pakistani children and adult patients who were excreting more than 150 mg urinary protein per day; revealed protein: creatinine index and ratio 141 and 0.18 respectively in random urine samples.

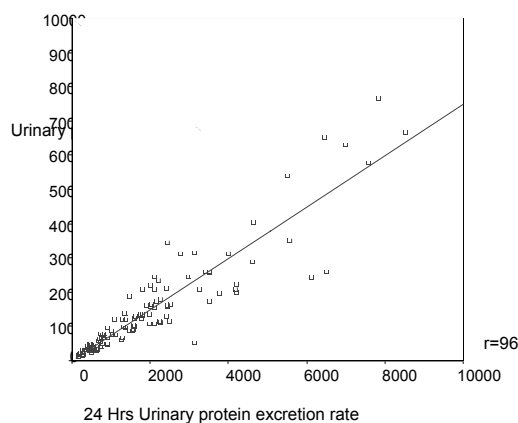
**Table 1. Measurement of biochemical and urinary protein excretion parameters in patients having proteinuria with normal creatinine clearance (n=130).**

Parameters (Units)	Means ± SD	2.5 <sup>th</sup> Percentile	97.5 <sup>th</sup> Percentile
Age (Years)	25.6 ± 11.7	6	51
Urinary Volume (L/24 h)	2.1 ± 0.8	0.9	4.1
Serum Creatinine (umol/L)	85.8 ± 19.6	60.3	134.3
Urinary Creatinine (mmol/L)	6.2 ± 1.6	3.7	12.1
Creatinine clearance ( ml/min)	110.5 ± 30.1	73.2	189.1
Spot Urinary Protein (mg/dl)	87.8 ± 90.2	13.2	417.3
Urinary Protein excretion (g/24h)	2.0 ± 1.9	.15	8.3
Protein: Creatinine Index	1565 ± 144.7	141	7009
Protein: Creatinine Ratio	1.4 ± 1.3	.18	6.5

**Table 2. Comparison of protein: creatinine index and ratio with reference to 24 hours urinary protein excretion rate in patients (Percentile distribution) of renal disease (n=130).**

Percentiles	Urinary Protein excretion (mg/24h)	Protein: Creatinine Index	Protein: Creatinine Ratio
2.5	150	141	.18
5	226	187	.19
10	360	301	.26
15	420	315	.27
20	487	329	.28
25	590	412	.37
30	646	471	.41
35	720	549	.50
40	900	689	.65
45	1099	781	.75
50	1365	954	.85
55	1561	1088	.98
60	1791	1234	1.14
65	1995	1352	1.38
70	2182	1620	1.48
75	2403	1902	1.75
80	2910	2114	1.91
85	3520	2501	2.27
90	4553	3108	2.70
95	6696	6022	5.31

The concentration of protein in the urine is affected by urine volume as well as protein excretion rate. The comparison of the results obtained by random urine protein: creatinine index, ratio and 24 hours proteins excretion (95% CI) are shown in Table-2. It can be observed that the patients of nephrotic syndrome who excreted more than 3.5 g of proteins daily, showed a protein: creatinine index of 2500.



**Figure 1. Correlation of 24 urinary protein excretion rate with spot urinary Protein: creatinine index (n=130).**

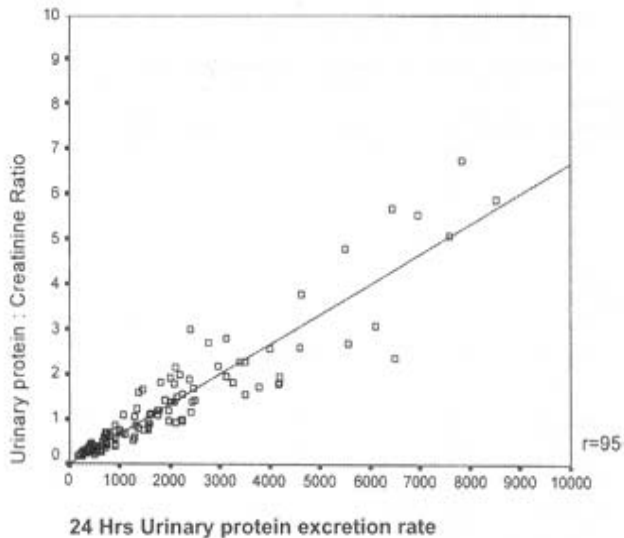


Figure 2. Correlation of 24 urinary protein excretion rate with spot urinary Protein: creatinine ratio (n=130).

An excellent correlation was found for all levels of proteinuria results obtained from the protein: creatinine index/ ratio and standard 24 hours urinary protein excretion in these patients (Figure 1-2). The correlation coefficients between the protein creatinine index ( $r=0.96$ ) and 24 hours urinary protein excretion was significant ( $p<.001$ ). The high correlation suggests that the index/ratio provides an accurate and valid assessment at all level of persistent proteinuria.

Majority of our patients in this series having protein: creatinine index between 300 and 2500 showed traces to 2+ protein on dipstick. Eleven patients who had an index of 300 showed protein trace whereas 20 cases with an index of more than 2500 revealed 3+ results on dipsticks. However the assessment of proteinuria was misclassified in comparison with 24 hours urinary protein excretion by dipsticks due to error in collection of urine volume and interpretation of coloured chart. It showed moderate correlation ( $r=0.52$ ) with 24 hours urinary protein excretion.

## Discussion

Quantitative proteinuria predicts the rate of progression of nephropathy and 24 hours urinary protein excretion rate has been used to assess the severity and monitoring of renal pathology.<sup>9</sup> However our patients having renal diseases are unaware of the importance of the volume and timing of urine collection which is very important for the assessment of total urinary protein excretion. The twenty-four hour urine collection is usually associated with errors in urine volume due to improper timing or missed samples of urine leading to over or under- 24 hours urine volume.

So it was decided to calculate protein: creatinine index/ ratio on random urine samples to overcome the shortcomings

related to 24 hours urine collection.<sup>10</sup> The protein: creatinine index on 130 random urine specimens was found very sensitive and comparable to 24 hour urine excretion. Patients excreting 150 mgs of urinary protein/day, had proteins: creatinine index and ratio 141 and 0.18 respectively (Table-1). Shaw et al (1983) reported slightly less protein: creatinine index (136) of pathological proteinuria as compared to this study.<sup>11</sup> Thus the creatinine correction not only eliminates the need for timed urine collection but also introduces a correction for body size and convenience to patients especially for children.<sup>12</sup> The advantages of this approach are that errors due to improper collection of urine sample or inaccuracy in the timing of collection period are not encountered.<sup>13</sup>

The pattern and magnitude of urinary protein excretion have important clinical implications. The comparison of random urine protein: creatinine index/ratio with 24 hours protein excretion rate is shown in table 2. The index for random urine samples was numerically lower than the protein excretion in mg per 24 hours. The proteins: creatinine index and ratio ranged from 141 to 7009 and 0.18 to 6.5 respectively in comparison with urinary proteins excretion of 150 to 8300 mg/day. Similar findings were reported by Shaw et al (1983).<sup>11</sup> This indicated that spot urine proteins: creatinine index showed same baseline predictor of progression of renal disease as 24 hours urine excretion rate.<sup>14,15</sup>

Patients showed a wide range of proteins-excretion rate in this series. A significant correlation was found between proteins creatinine index/ratio and standard 24 hours urinary proteins excretion in these patients (Figure 1-2) and same has also been concluded by Parag (1986)<sup>16</sup> and chu at el<sup>17</sup> (1990). Proteins and creatinine are highly soluble in water so they will undergo similar changes of dilution or concentration of urine according to the hydrate status of the body. Creatinine excretion varies among individuals according to age, sex and body size but still it shows good accuracy and correlation with urinary proteins.<sup>18</sup> This is probably because the index is independent of errors in urine collection. Thus, the uniformly high correlation coefficients are sufficiently strong evidence to the creatinine proteins index for assessment of persistent proteinuria. The main limitation of proteins: creatinine index is a wide daily variation in the urinary proteins excretion rate associated with changes in posture, physical activity, proteins intake and haemodynamic factors. The proteins: creatinine index and ratio in random urine provide the same information about proteinuria status but the index is more convenient for clinical usage by the treating physicians

The assessment of proteinuria by dipsticks showed moderate correlation ( $r=0.52$ ) and was misclassified in comparison with 24 hours urinary proteins excretion. So the dipsticks should not be used for assessment of proteinuria but it can be used for screening purpose. At the same time it is easier to perform; inexpensive and less time consuming

for the patients. This becomes more relevant where the patient is likely to provide imprecisely collected urine samples as is the case in our population.

### Conclusion

The proteins: creatinine index in random urine is a convenient, quick and reliable method of estimation of proteinuria as compared to 24 hours of urinary proteins excretion for diagnosis and monitoring of renal diseases in our medical environment.

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