



# Comparative Evaluation of Quantitative Protein Measurement of 12-Hour and 24-hour Urine Sample for the Diagnosis of Nephrotic Syndrome and their Correlation with Spot Urinary Protein Creatinine Ratio

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## Key words:

Nephrotic syndrome, urinary total protein, albumin, cholesterol, creatinine, urinary spot protein creatinine ratio

## Abstract:

**Background:** The amount of protein excretion is a reflection of disease activity in Nephrotic Syndrome. 24-hour urine collection has remained as the method of choice to quantify proteinuria. An alternative method for quantitative evaluation of proteinuria is measuring the ratio of protein or albumin to creatinine in an untimed “spot” urine specimen.

**Objective:** To compare 12-hour versus 24-hour proteinuria in nephrotic syndrome and its correlation to spot urinary protein creatinine ratio.

**Materials & Methods:** A comparative cross-sectional study was conducted in the Department of Pediatrics, Sir Salimullah Medical College Mitford Hospital (SSMC & MH), Dhaka over a period from September 2016 to June 2017.

All the babies developing 1st attack of Nephrotic Syndrome and meeting the clinical criteria were included in the study. Complete history was taken from accompanying attendants. Thorough clinical examinations and relevant laboratory investigations (12-hour day urinary protein, 12-hour night urinary protein, 24-hour urinary total protein, S. Albumin, S. Cholesterol, Urinary spot Protein Creatinine ratio) were done.

All the information was recorded in the fixed protocol. Collected data were classified, edited, coded and entered into computer for statistical analyses.

**Results:** Out of 50 cases, the mean age was found 58.3±29.0 months. 12-hour day urinary protein was found 2.02±0.167 gm/m<sup>2</sup>/12 hours, mean; 12-hour night urinary protein was found 2.12±2.18 gm/m<sup>2</sup>/12 hours, mean; 24-hour urinary total protein was found 4.10±3.32 gm/m<sup>2</sup>/24 hours. The mean spot urinary protein creatinine ratio was found 12.58±7.21 with range from 2.02 to 29.85. There was no statistically significant difference between 12-hour day sample and 12-hour night sample. There was also no significant difference between 12-hour day & night sample with 24-hour sample. Results of all 03 samples were comparable to urinary protein creatinine ratio.

**Conclusion:** This study reveals no statistically significant difference in quantitative estimation of 12-hour day and night protein with 24-hour urinary total protein. The 12-hour day and night protein and urinary total protein were comparable with each other and also with urinary spot protein creatinine ratio. So 12-hour day or night urinary total protein may be a diagnostic tool for nephrotic syndrome.

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## Introduction:

Nephrotic syndrome is a common kidney disease in children caused by a variety of glomerular and systemic diseases vary on age, race, geographical locations, but most commonly it is idiopathic.

Estimated annual incidence of nephrotic syndrome is 2-7 per 1,00,000 children and prevalence is 12-16 per 1,00,000 children.<sup>1</sup> There is epidemiological evidence of higher incidence of nephrotic syndrome in a child from south Asia and Africa.<sup>2,3</sup>

Nephrotic syndrome is characterized by massive proteinuria (3 or 4 + protein or >1 gm/m<sup>2</sup>/24 hours), hypoalbuminemia (<2.5 gm/dl), massive edema and hyperlipidaemia (cholesterol >200 mg/dl).<sup>4,5,6</sup>

Urine analysis for proteinuria is an essential investigation for diagnosis of nephrotic syndrome as well as diseased activity. A precise detection of the rate of urine protein is possible only through measuring the 24 hours urine protein hence it is gold standard. Twenty-four-hour urine collections may be associated with significant collection errors, largely due to improper timing and missed samples, leading to over collections and under-collections. Timed overnight collections or shorter timed day time collections may reduce the inconvenience of a 24-hour collection. The 24 hour period required for urine collection often results in a delay in the diagnosis or treatment or prolongation of hospital stay.<sup>8,9,10</sup>

An alternative method for quantitative evaluation of proteinuria is measurement of the ratio of protein or albumin to creatinine in an untimed "spot" urine specimen which overcome the limitation of 24 h urine collections. Recent studies in adult have shown that calculation of protein creatinine ratio in a spot urine sample correlates well with the 24 hour urine sample.<sup>11</sup>

Shortening the period for diagnosis of nephrotic syndrome would be valuable for management purposes as well as decreasing hospital cost and patient inconvenience. If 12 hours sample result is found to be comparable to 24 hours result and urine protein creatinine ratio then 12 hour urine protein measurement can be practiced as an alternative diagnostic method for evaluation of proteinuria in nephrotic syndrome.

To the best of my knowledge there are few studies regarding 12 hours verses 24 hours proteinuria in nephrotic syndrome in our country. For this reason present study intended to compare between 12 hours and 24 hours proteinuria in nephrotic syndrome and their correlation with spot urinary

protein creatinine ratio.

## Materials and Methods

This was Comparative cross sectional study conducted at Department of Pediatrics, Sir Salimullah Medical College Mitford Hospital (SSMC MH), Dhaka September 2016 to June 2017. Total 50 children with 1st attack of nephrotic syndrome were included in our study and Children with congenital nephrotic syndrome, who took steroid for any other causes regular or irregular, severely ill patient or nephrotic syndrome with complication like Spontaneous bacterial peritonitis, thromboembolism etc. were excluded from our study.

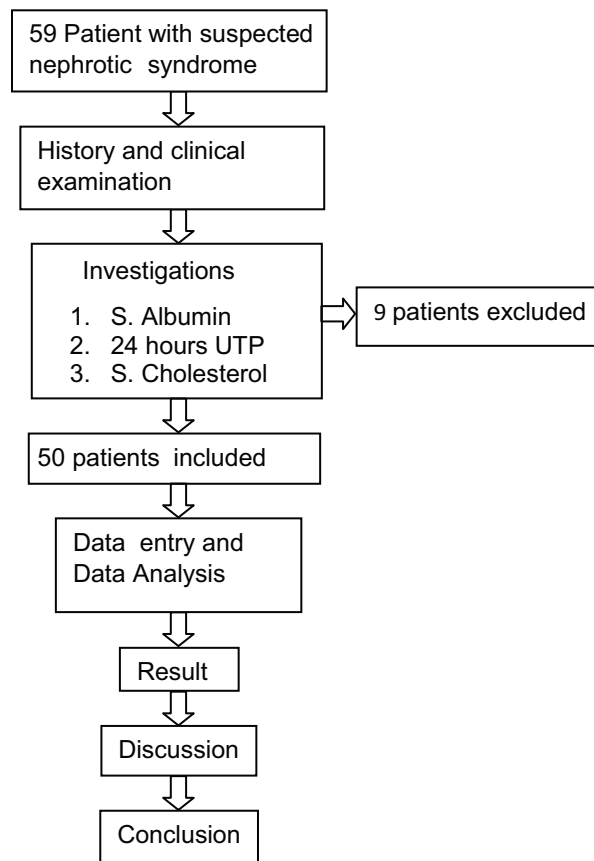
Informed written consent from patient's guardian was taken before enrollment. Detailed history, physical examination and relevant investigations were done.

All patients were provided with 2 containers. Total urine collection time was 24 hours in 2 separate containers. The first container held 1st 12 hour urine starting from 6.00am to 5.59pm and 2nd container held next 12 hour urine from 6.00pm to 5.59am with the idea of taking day time and night time urine sample. Each container was labeled with patient's name, bottle no, collection time and date. Upon completion of the 24 hour urine collection the investigator retrieved the sample. The values of 12 hour total protein and total urine volume of morning and night sample was used for calculation of 24 hours UTP. The 12 hours morning as well as night sample result was compared to 24 hours result. Spot urinary protein creatinine ratio was performed in all patients and its correlation to 12 hours morning and evening urinary protein and 24 hours urinary total protein was evaluated.

Data was processed and analyzed by using computer based software SPSS-23 (Statistical package for Social science). The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages. Paired t-test was used for continuous variables and Pearson's correlation was used for continuous variables. Data was analyzed by employing appropriate statistical test of significance and significance level was  $p=0.05$ .

For this study written approval was taken from the ethical committee of SSMC & MH. The aims and objectives of the study were explained to the parents or caregiver in details and easily understandable language. Informed written consent from the parents was taken.

## Study Flowchart



## Results

Initially 59 clinically suspected patients of nephrotic syndrome were enrolled. Relevant investigations were sent all patients. After getting the investigation reports for 9 patients were excluded from the study as they did not fulfill all criteria for diagnosis of nephrotic syndrome. Finally data of 50 patients were analyzed for study.

**Table-I**  
*Distribution of the study patients by demographic variable (N=50)*

Demographic variable	No. of patients	Percentage	
Age (month)	≤24	9	18.0
	25-48	13	26.0
	49-72	13	26.0
	73-96	12	24.0
	>96	3	6.0
Mean ±SD	58.3 ±29.0		
Range (min-max)	15 -126		
Sex	Male	38	76.0
	Female	12	24.0

Table I shows majority 35(70.0%) patients belonged to age ≤72 months, the mean age was found 58.3±29.0 months. Male were predominant (76.0%) and female were 12(24.0%). Male: female ratio was 3.17:1.

**Table II**  
*Distribution of the study patients by 12 hours day urinary protein (N=50)*

Day urinary protein (gm/m <sup>2</sup> /12 hours)	Mean ±SD
Mean ±SD	2.02±0.1.67
Range (min-max)	0.57 -7.71

Table II shows mean 12 hours day urinary protein was found 2.02±0.167 gm/m<sup>2</sup>/12 hr hours with range from 0.57 to 7.81 gm/m<sup>2</sup>/12 hr.

**Table III**  
*Distribution of the study patients by 12 hours night urinary protein (N=50)*

Night urinary protein (gm/m <sup>2</sup> /12 hours)	Mean ±SD
Mean ±SD	2.12±2.18
Range (min-max)	0.64-9.40

Table III shows mean 12 hours night urinary protein was found 2.12±2.18 gm/m<sup>2</sup>/12 hours with range from 0.64 to 9.40 gm/m<sup>2</sup>/12 hours.

**Table IV**  
*Distribution of the study patients by 24 hours urinary total protein (N=50)*

Urinary total protein (gm/m <sup>2</sup> /24 hours)	Mean ±SD
Mean ±SD	4.10±3.32
Range (min-max)	1.29-13.06

Table IV shows mean 24 hours urinary total protein was found 4.10±3.32 gm/m<sup>2</sup>/24 hours with range from 1.29-13.06 gm/m<sup>2</sup>/24 hours.

**Table V**  
*Comparison between 12 hour day urinary protein per hour with 12 hour night urinary protein per hour (N=50)*

	Mean	±SD	P value
Day urinary protein per hour (gm)	0.17	±0.13	0.75 <sup>ns</sup>
Night urinary protein per hour (gm)	0.17	±0.18	

ns=not significant

P value reached from paired t-test

Table V shows mean day urinary protein per hour was  $0.17 \pm 0.13$  gm and mean night urinary protein per hour was  $0.17 \pm 0.18$  gm. The difference was not statistically significant ( $p > 0.05$ ) when compared between per hour day urinary protein and per hour night urinary protein.

**Table VI**

*Distribution of the study patients by spot urinary protein creatinine ratio (N=50)*

Spot urinary protein creatinine ratio	Number of patients	Percentage
2.0-10.0	19	38.0
>10.0	31	62.0
Mean $\pm$ SD	12.58	$\pm 7.21$
Range (min-max)	2.02	-29.85

Table VI shows mean spot urinary protein creatinine ratio was found  $12.58 \pm 7.21$  with range from 2.02 to 29.85.

**Table VII**

*Comparison between 12 hour day urinary protein per hour with 24 hour urinary total protein per hour (N=50)*

	Mean	$\pm$ SD	P value
Day urinary protein per hour (gm)	0.16	$\pm 0.13$	0.81 <sup>ns</sup>
Urinary total protein per hour (gm)	0.17	$\pm 0.13$	

ns=not significant

P value reached from paired t-test

Table VII shows mean day urinary protein per hour was  $0.16 \pm 0.13$  gm and mean urinary total protein per hour was  $0.17 \pm 0.13$  gm. The difference was not statistically significant ( $p > 0.05$ ) when compared between per hour day urinary protein and per hour urinary total protein.

**Table VIII**

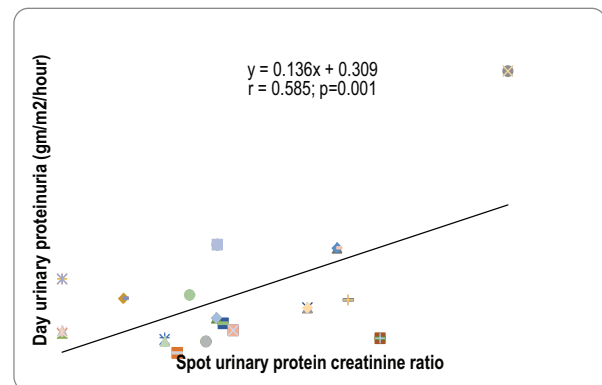
*Comparison between 12 night urinary protein per hour with 24 hour urinary total protein per hour (N=50)*

	Mean	$\pm$ SD	P value
Night urinary protein per hour (gm)	0.17	$\pm 0.18$	0.69 <sup>ns</sup>
Urinary total protein per hour (gm)	0.17	$\pm 0.13$	

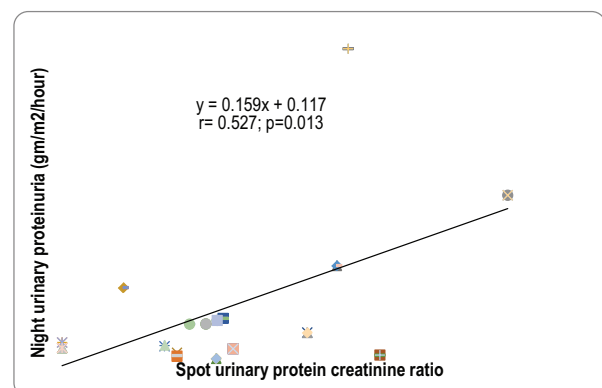
ns=not significant

P value reached from paired t-test

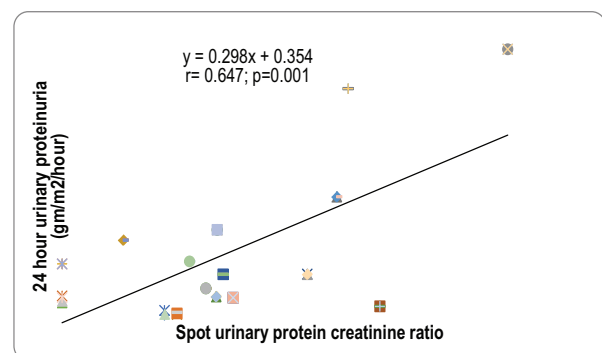
Table VIII shows mean night urinary protein per hour was  $0.17 \pm 0.18$  gm and mean urinary total protein per hour was  $0.17 \pm 0.13$  gm. The difference was not statistically significant ( $p > 0.05$ ) when compared between per hour night urinary protein and per hour urinary total protein.



**Fig.-1:** Scatter diagram showing positive correlation ( $r=0.585$ ;  $p=0.001$ ) between per hour day urinary protein with spot urinary protein creatinine ratio.



**Fig.-2:** Scatter diagram showing positive correlation ( $r=0.527$ ;  $p=0.013$ ) between per hour night protein with spot urinary protein creatinine ratio.



**Fig.-3:** Scatter diagram showing positive correlation ( $r=0.647$ ;  $p=0.001$ ) between 24 hour urinary total protein with spot urinary protein creatinine ratio.

## Discussion

In this present study it was observed that male was predominant (76.0%) and female were 12(24.0%) among them majority 35(70.0%) patients belonged to age  $\leq 72$  months, the mean age was found  $58.3 \pm 29.0$  months which are similar other different study conducted home and abroad<sup>19-26</sup>.

In this study it was observed that mean 12 hours day urinary protein was found  $2.02 \pm 0.167$  gm/m<sup>2</sup>/12 hr hours with range from 0.57 to 7.81 gm/m<sup>2</sup>/12 hr. Mean day urinary protein per hour was found  $0.13 \pm 0.07$  gm/m<sup>2</sup>/1 hr with range from 0.05 to 0.31 gm/m<sup>2</sup>/1 hr. mean 12 hours night urinary protein was found  $2.12 \pm 2.18$  gm/m<sup>2</sup>/12 hours with range from 0.64 to 9.40 gm/m<sup>2</sup>/12 hours. Mean night urinary protein per hour was found  $0.17 \pm 0.18$  gm/m<sup>2</sup>/1 hr with range from 0.06 to 0.94 gm/m<sup>2</sup>/1 hr.

In this study it was mean day urinary protein per hour was  $0.17 \pm 0.13$  gm and mean night urinary protein per hour was  $0.17 \pm 0.18$  gm. The difference was not statistically significant ( $p > 0.05$ ) when compared between per hour day urinary protein and per hour night urinary protein. Mean morning urinary protein per hour was  $0.16 \pm 0.13$  gm and mean urinary total protein per hour was  $0.17 \pm 0.13$  gm. The difference was not statistically significant ( $p > 0.05$ ) when compared between per hour day urinary protein and per hour urinary total protein. Mean night urinary protein per hour was  $0.17 \pm 0.18$  gm and mean urinary total protein per hour was  $0.17 \pm 0.13$  gm. The difference was not statistically significant ( $p > 0.05$ ) when compared between per hour night urinary protein and per hour urinary total protein. In Asgharnia et al.<sup>8</sup> study there was a high correlation of 12-h urine protein  $> 165$  mg with a 24-hour urine protein  $> 300$  mg.

In present study observed in 12 hours in morning nephrotic range proteinuria was found all cases, 12 evening nephrotic range proteinuria was 50(100.0%), and 24 hours nephrotic range proteinuria also 100% cases and urine protein creatinine ratio  $> 2$  was in all cases.

In present study observed that positive correlation ( $r = 0.585$ ;  $p = 0.001$ ) between per hour day urinary protein with spot urinary protein creatinine ratio. Positive correlation ( $r = 0.527$ ;  $p = 0.013$ ) between per hour night protein with spot urinary protein creatinine ratio. Positive correlation ( $r = 0.647$ ;

$p = 0.001$ ) between 24 hours urinary total protein with spot urinary protein creatinine ratio. In a study on 46 patients by Ginsberg et al.<sup>28</sup> an excellent correlation between the protein content of a 24 hour urine collection and the protein/creatinine ratio. Asgharnia et al.<sup>8</sup> the 4-hour urine protein results correlated positively with the 24-hour results for diagnosis of preeclampsia ( $r = 0.9$ ,  $p < 0.001$ ). Amirabiet al.<sup>29</sup> divided their patients into three groups for proteinuria (no proteinuria, mild and severe proteinuria), they showed that value of 4-h sample period did correlate with that of 24-hour samples for mild and severe proteinuria, with a significant correlation between 4- and 24-hour urine protein concentration ( $p < 0.001$ ,  $r = 0.97$ ), in which sensitivity and specificity of 4-h urine test were 93.2% and 90.2% respectively. Also Rabiee<sup>30</sup> indicated protein value for the first 8 or 12=h of 24-hour urine samples correlated with 24-hour samples for patients with proteinuria. Kieler et al.<sup>13</sup> study reported albumin concentrations in the day and night samples agreed well with concentrations of the 24-hour samples. The median differences between the 24-hour and the day and night albumin concentrations were -3 mg/L (interquartile range -264 to 116 mg/L), and 17 mg/L (interquartile range -186 to 210 mg/L), respectively. In some studies the urinary albumin/creatinine ratio is a valid estimate of albumin excretion rate, whereas others<sup>31-32</sup> find that adding analyses of creatinine is of limited use and only increases the costs. As the increase in albumin excretion in severe pre-eclampsia occurs rapidly and suddenly<sup>39</sup> frequent urine analyses are required. These analyses should be valid and easy to perform, and should be as inexpensive as possible. A night urine sample, starting at 2000 h and ending at 0800 h fulfils these requirements and ought to be more acceptable to the woman than a 24-hour collection. A shorter period should reduce the risk of incomplete collection. Hogan et al.<sup>14</sup> strong correlation ( $r = 0.87$ ) and modest predictability ( $R^2 = 0.65$ ) was observed between random UPCr and 24hUP in the validation sample. Ananthakumar et al.<sup>27</sup> spot PCR was compared with 24-HUP, 86.7% (26/30) of samples with spot PCR  $< 0.5 / < 0.2$  had a protein excretion of  $< 4$  mg/m<sup>2</sup>/h. 82.4% (28/34) of samples with spot PCR 0.5-2/0.2-2 had a protein excretion of 4-40 mg/m<sup>2</sup>/h. Biswas et al.<sup>11</sup> studies where

dipstick correlates well with 24-HUP irrespective of the degree of proteinuria.

### Limitations of the study

1. The study population was selected from one selected hospital in Dhaka city, so that the results of the study may not reflect the exact picture of the country.
2. Small sample size was also a limitation of the present study. Therefore, in future further study may be undertaken with large sample size.

### Conclusion

It was concluded from this study that no statistically significant difference was found in quantitative estimation of 12 hours day and night protein with 24 hours urinary total protein. The 12 hours day and night protein and UTP were comparable with each other and also with urinary spot protein creatinine ratio. So, 12 hours day or night UTP may be a diagnostic tool for nephrotic syndrome.

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