Original Article

Correlation between 24-hour Urinary Total Protein Excretion and Single Urinary Protein Creatinine ratio in the Diagnosis of Childhood Nephrotic Syndrome : Study in a Tertiary Level Hospital

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Abstract:

Introduction: *Protein excretion varies in the course of the day, for this reason 24-hours urinary total protein excretion has been considered as the classic reference method for protein determination in nephotic syndrome. As the collection of urine for 24 hours is tedious and errors may occur during the process, so the protein:creatinine ratio was developed as a diagnostic alterative for Nephrotic syndrome.The main aim of this study is to determine the correlation between 24-hour urinary total protein excretion and single urinary protein creatinine ratio in childhood Nephrotic syndrome.*

Methods: *This cross-sectional study was conducted in the department of Paediatrics,Shaheed Suhrawardy Medical College & Hospital from June 2018 to May 2019. After taking valid consent total 60 cases of Nephrotic syndrome were selected purposively according to inclusion and exclusion criteria. Here we tried to determine the correlation between 24-hour urinary total protein excretion and single urinary protein creatinine ratio in the diagnosis of childhood Nephrotic syndrome by measuring the spot urinary protein creatinine ratio and the 24 hours urinary total protein excretion of the patients.*

Results : *Among total 60(N) children of Nephrotic syndrome 42 (70%) patients were male and 18 (30%) patients were female with M:F = 2.16:1.The mean age was 4.57 ± 2.01SD years. Most of the patients were presented with some associated diseases like urinary tract infection (30%), upper respiratory tract infection (28.3%), viral illness(8.3%), atopic dermatitis(3.3%) and nonspecific illnesses(30%). The main physical findings were only puffy face(100%), with ascites (86.6%) and swelling of genitalia (38.3%).The mean 24 hours urinary total protein excretion was 3114.45 ± 1627.89 mg/24 hours and spot urinary protein creatinine ratio was 5.57 ± 3.13 SD. There was a very significant correlation between 24 hour total urinary protein excretion and spot urinary protein creatinine ratio. The sensitivity of the study was 80.49 – 100% and specificity of 91.78 - 100% at ≤2000 mg /sqm body surface area/ day protein excretion. The p value was .002 with correlation coefficient was 0.39.*

Conclusion: *The protein creatinine ratio of a random urine sample might be used as an alternative method for the diagnosis of Nephrotic syndrome in children in view of the obvious advantages in terms of cost, time and patient convenience.*

Key Words:
24-hour, Urinary Total Protein, Urinary Protein Creatinine ratio

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Introduction:

Proteinuria is one of the earliest sign of almost all renal diseases.[1-2] It is also a major determinant of progression of renal disease.[3] Proteinuria occurs due to damage to glomerular apparatus (Glomerular proteinuria) or due to failure of reabsorption of the filtered protein by tubular cell (Tubular proteinuria).[1,4] Estimation of proteinuria helps to differentiate tubulointerstitial and glomerular disease, to follow the progression of renal disease and to assess the response to therapy.[1,5]

Nephrotic syndrome, a manifestation of glomerular disease, is characterized by nephrotic range proteinuria and the triad of clinical findings hypoalbuminemia, edema , hyperlipidemia associated with large urinary losses of protein. Nephrotic range proteinuria is defined as protein excretion of > 40mg/m^2/ hour or a first morning protein: creatinine ratio of >2:1.[6]

The term 'Nephrotic syndrome' was first introduced by Leiter in 1931 to differentiate the association of massive proteinuria, oedema, hypoproteinaemia and hypercholesterolaemia due to what may be minimal change disease from glomerulonephritis (from 'lipoid nephrosis)'. Nephrotic syndrome was considered to have the same features without hypertension, nitrogen retention or an excess of red blood cells in the urine.[7]

Protein excretion varies in the course of the day. For this reason 24 hour proteinuria has been considered as the classic reference method for protein excretion determination.[8,9] It is also the gold standard for the quantitative evaluation of proteinuria.[10] However, the collection of 24 hours urine sample is inconvenient and it is subject to errors since it is difficult to collect a complete 24 hour urine sample accurately, specially in children.[10,11] Studies have shown that more than 25% of the samples have to be discarded because an incomplete collection is suspected.[11] The protein creatinine ratio in spot urine was developed as a diagnostic alternative.[8-10,12,13] It provides a more convenient method to assess protein excretion and is recommended by NKFK/DOQI guidelines.[10,14]

Spot urine is collected during the second urination of the morning and the first 20-25 ml is discarded. Without disrupting urination, the middle urine is collected in the container and the last portion is discarded.[8,15] The potential error in determining proteins in a spot urine sample as a result of daily variation does not exceed the error in collecting a 24 hour urine sample.[8,16] Although some studies suggests moderate correlation between spot urine protein creatinine ratio and 24 hour urinary protein excretion ,[17-18] but the results of those studies are variable in accordance with different levels of proteinuria.[8,15,19-20]

This study was carried out to determine whether the protein creatinine ratio in random urine sample, is a convenient, quick and reliable method for the estimation of proteinuria in diagnosing and monitoring Nephrotic syndrome in children, as it avoids collection error and may give more relevant information.

Materials and Method:

This cross sectional study was conducted in the Department of Paediatrics, Shaheed Suhrawardy Medical College Hospital, Dhaka from June 2019 to May 2019. All the admitted children with Nephrotic syndrome were included in this study. Of them 60 children were selected purposively. Valid informed consent was taken from their parents. Renal function was stable in all of the patients measured by normal serum creatinine levels. All the patients were subjected to a detailed history and clinical examination. Routine haematological tests serum urea, creatinine, cholesterol, complete blood count, urine for albumin, routine and microscopic examination, culture and sensitivity of urine for urinary infection were performed. Special investigations like protein content in 24 hour urine sample and single urinary protein creatinine ratio were done in each subject. Nephrotic syndrome with renal impairment, patients on treatment of Nephrotic syndrome,age <1 year >12 year and presence of any systemic disease were excluded from this study. The main Outcome variables were age, sex, socioeonomic condition, mode of presentation, biochemical parameters of blood, serum and urine, 24 hour urinary total protein & spot urinary protein creatinine ratio.

Sample collection

The procedure of collecting the sample was explained to the parents. On the test day (of 24 hours UTP) in the morning at the commencement of collection period patients were asked to void urine and discard this sample as it contains the overnight urine present in the bladder. Subsequently 24 hours urine sample from 8 a.m to 8 a. m of the following day was collected in a clean container according to laboratory manual. After each void, the cap of the container was tightly closed. The 24 hour urine sample was labeled as TUV from which UTP (Urinary total Protein) was measured as mg/dl by VITROS urine protein slide method. After completion of collection of 24 hour urinary sample, on the same sitting, urine was collected in another clean container with tight lid, one hour later at 9.00 am. This 9.00 am sample was labeled as SUV (Spot Urine Volume), from which SUTP (Spot Urine Total Protein) and SUC (Spot Urine Creatinine) was measured by VITROS urine creatinine slide method.Finally protein creatinine ratio was calculated for each voided random urine sample. At last the values of 24 hour urinary total protein and random urinary protein creatinine ratio were compared to find a correlation between two.

Estimation of protein in urine by VITROS urine protein slide method

Principles of the procedure

The VITROS UPRO Slide method is performed using the VITROS UPRO Slides and the VITROS Chemistry Products Calibrator Kit 10 on VITROS 250/350/950/5,1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated Systems. The VITROS UPRO Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers.

The binding of protein to a pyrocatechol violet-molybdate complex in the presence of oxalate causes a shift of the absorption maximum from 450 to 670 nm. After a fixed incubation period, the reflection density of the dye is measured spectrophotometrically. The amount of colored complex is proportional to the protein concentration.

Reaction scheme

Mo^{+6} - pyrocatechol violet dye + oxalate + protein $\rightarrow$ colored complex dye

Test Type and Conditions

Test Type	Colorimetric
VITROS System	250
ApproximateIncubation Time	5 minutes
Temperature	37 °C (98.6 °F)
Wavelength	670 nm
Reaction SampleVolume	10 iL

Measurement of spot urine creatinine by VITROS urine creatinine slide method

Principles of the Procedure

The VITROS CREA Slide method is performed using the VITROS CREA Slides and the VITROS chemistry products calibrator Kit 1 on VITROS 250/350/950/5,1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System. The VITROS CREA Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Creatinine diffuses to the reagent layer, where it is hydrolyzed to creatine in the rate-determining step. The creatine is converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine, in the presence of sarcosine oxidase, is oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product. Following addition of the sample, the slide is incubated. During the initial reaction phase, endogenous creatine in the sample is oxidized. The resulting change in reflection density is measured at 2 time points. The difference in reflection density is proportional to the concentration of creatinine present in the sample.

Reaction Scheme

creatinine + H2O —(creatinine amidohydrolase)—> creatine

creatine + H2O —(creatine amidinohydrolase)—> sarcosine + urea

sarcosine + O2 + H2O —(sarcosine oxidase)—> glycine + formaldehyde + H2O2

H2O2 + leuco dye —(peroxidase)—> dye + 2H2O

Test Type and Conditions

Test Type	VITROS System	Approximate Incubation Time	Temperatur	Wavelength	Reaction SampleVolume
Two-point rate	250	5.0 minutes	37 °C (98.6 °F)	670 nm	6 µL

Data collection and analysis

All the relevant data were recorded systematically in preformed data collection form and quantitative data were expressed as mean and standard deviation and quantitative data as frequency distribution and percentage. Statistical analysis was performed by standard statistical method. Pearson's correlation was done (0.39) and probability value (p=0.002) was <0.05 was considered as level of significance.

Results:

Among total 60 (N) patients 42 (70%) were below 5 years and 18 (30%) were above 5 years. Mean±SD age was 4.57 ± 2.01 yrs and the age range was 1-12 yrs (shown inTable 1), 42(70%) were male and 18(30%) were female. Male gfemale ratio was 2.16:1 (Table-II). 24 hrs UTP excretion was shown in table 3. Among the children 24 hours UTP ≤2000 mg was in 17 (28.33%) children, 2000-4000 mg was in 28 (46.67%) children, 4000-6000 mg was in 9 (15%) children and >6000 mg was in 6 (10%) children. Protein creatinine ratio was shown in Table IV. Among the children PCR 1-2.5 were in 5(8.33) , PCR 2.5- 4 was in 21 (35%) children and PCR >4 in 34 (56.67%) children. The cut off value of PCR was 2.5. linear relationship between the two variables (24 hour urinary total protein and spot urinary proten creatinine ratio was shown in figure 1.

Table-I

Age distribution of the studied children (N=60)

Age in years	No of patients	Percentage	Mean±SD
≤5	42	70%	
>5	18	30%	4.57 ± 2.01
Total	60	100	

Table-II

Sex distribution of studied children (N=60)

Sex	No of patients	Percentage	Male : Female
Male	42	70%	
Female	18	30%	2.16:1
Total	60	100%	

Table-III

Distribution of 24 hours UTP of the studied children (N=60)

UTP (mg/sqm body surface area)	Frequency	Percentage
≤2000	17	28.33%
2000-4000	28	46.67%
4000-6000	9	15%
>6000	6	10%
Total	60	100%

UTP= Urinary Total Protein

Table-IV

Distribution of protein creatinine ratio of the studied children (N=60)

PCR	Frequency	Percentage
1-2.5	5	8.33%
2.5-4	21	35%
>4	34	56.67%
Total	60	100%

PCR= Protein creatinine ratio

Figure 1 Linear Relationship between protein creatinine ratio and 24 hours urinary total protein of studied children (N=60)

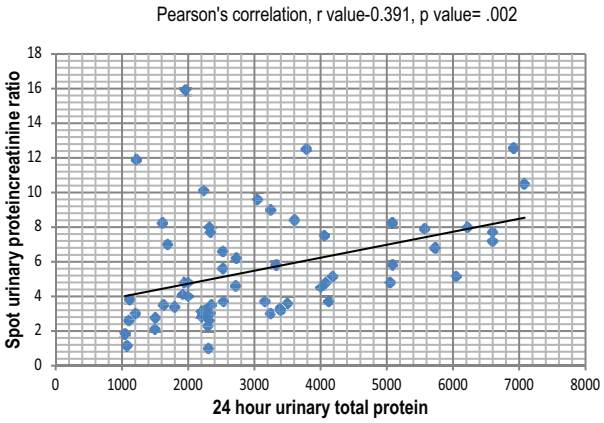

Fig.-1: *linear relationship between the two variables(24 hour urinary total protein and spot urinary proten creatinine ratio).*

Discussion:

This cross sectional study was done with the objective to find out the correlation of 24 hour urinary total protein with the spot urinary protein creatinine ratio of childhood Nephrotic syndrome patients. In this study, sixty children with first attack of Nephrotic syndrome without renal impairment were taken and the patients' ages were within 1-12 years.

Quantitative assessment of proteinuria from 24 hours urine collection still remains a gold standard for urinary protein estimation.[21] But 24 hours collection are often inaccurate , time consuming, cumbersome, inconvenient and unreliable one. It is also very difficult to collect urine from infant and young children. This problems suggest and need for a more convenient and accurate method of urinary protein estimation particularly in the outpatient department. A first morning urinary protein creatinine ratio is superior to random urinary protein creatinine ratio in the assessment of proteinuria.[23]

There are several studies about the spot urine protein creatinine ratio to see the nephrotic range proteinuria. Sandeep Garg and his colleagues in their study demonstrated the protein creatinine ratio >3.5 as a nephrotic range proteinuria.[1] Morales et al. reported in their study the best cut-off protein creatinine ratio to define Nephrotic range proteinuria is 3.0.[15] Other study shows this ratio ≥2.5 as a case of nephrotic range proteinuria.[24] In this study spot urinary protein creatinine ratio ≥2.5 was taken to see the correlation with the 24 hours urinary total protein.

The result of this study showed that there was no significant difference between 24 hours urinary total protein and spot urinary protein creatinine ratio. Spot urinary protein creatinine ratio was increased with the increasing of 24 hours urinary total protein. The study also showed that the average spot urinary protein creatinine ratio was 5.57 ± 3.13 SD. In 5 patients it was within the range of 1-2.5, in 21 patients it was within 2.5-4 and in 34 patients it was more than 4. In their study, Kelsch RC and Sedman found that normal level of spot urinary protein creatinine ratio was less than 0.2 and >1 in a suspicious range for nephrotic syndrome.[24] In our study, spot urinary protein creatinine ratio of all cases were more than 1 and majority of cases were more than 2.5. Only 5 cases were less than 2.5. So, in this regard the study correlates with the study of Kelsch RC and Sedman.[24]

In my study I also have found a good correlation between 24 UTP and SPCR with a sensitivity of 80.49 – 100% and specificity of 91.78 - 100% at ≤2000 mg /sqm body surface area/day protein excretion. A prospective study showed a good correlation between 24 UTP and SPCR with a sensitivity of 68.5 – 100% and specificity of 77.0 - 91.9% at 500 to 1500 mg /day protein excretion. Thus my study also showed similar result with that study.[22]

When the relationship between 24 hours urinary total protein and spot urinary protein creatinine ratio was graphically represented it showd the relationship line was reasonably straight. The regression line showed moderately positive correlation. The Pearson's correlation co-efficient (r) value =0.391, p value= 0.002 which were statistically significant . Sandeep Garg, Alok kumar Gupta in their study found the correlation co-efficient (r) =.375 and p value= .012.[1] In another study, it was found that the correlation co-efficient (r) = 0.96 and p value was Â.001. Thus the result of this study correlates with the results of both the studies.[1,25]

It is evident from the results of this study that spot urinary protein creatinine ratio of childhood Nephrotic syndrome patients accurately reflects the 24 hours urinary total protein excretion of those patients. Therefore, spot urinary protein creatinine ratio provides a very useful ,simple and convenient method for quantitative assessment of protein for the diagnosis of Nephrotic syndrome which can replace 24 hours urinary total protein estimation in indoor, outpatients and follow up clinics as it gives quick and reliable results.

Conclusion:

From the present study it can be concluded that there is a strong correlation between 24 hours urinary total protein excretion and spot urinary protein: creatinine ratio in childhood Nephrotic syndrome patients. The protein: creatinine ratio of a spot urine sample might be used as an alternative of significant proteinuria as defined by a quantitative measure of the 24-h protein excretion for the diagnosis of nephrotic syndrome in children.

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