Section: Paediatric

Original Research Article

QUANTITATION OF PROTEINURIA BY DIPSTICK, SPOT URINE PROTEIN TO CREATININE RATIO (UPCR) IN CORRELATION WITH 24-HOUR URINE PROTEIN IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

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Received *: 25/08/2025*
Received in revised form *: 13/10/2025*
Accepted *: 01/11/2025*

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DOI: 10.70034/ijmedph.2025.4.182

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (4); 1019-1024

ABSTRACT

Background: Nephrotic syndrome, characterized by edema, heavy proteinuria (>1g/m2 daily;>40 mg/m2/hr) and hypoalbuminemia (serum albumin < 3g/dL) is among the most common kidney diseases in childhood. The condition has an annual incidence ranging from 1.2 to 16.9 per 100,000 children. Protein excretion varies in the course of the day, for this reason estimation of 24-hour proteinuria has traditionally been considered as the gold standard to determine the degree of urinary protein excretion. However, this method is time consuming, cumbersome for patients and influenced by urine collection process, circadian rhythm and handling methods. Sample collection is associated with several technical problems such as inaccurate collection. For this reason, the protein/creatinine ratio (P/C) in spot urine was developed as a diagnostic alternative. The aim is to quantification of spot urine protein: creatinine ratio in random urine samples, timed sample, urine dip stick and their correlation with 24-hour urine protein excretion in children with primary(idiopathic) nephrotic syndrome. The objective is to quantify proteinuria by dipstick, spot urine protein, creatinine ratio (UPCR) timed sample, urine in children with idiopathic nephrotic syndrome.

Materials and Methods: This hospital based observational study was done from December 2023- November 2024 in the Department of Paediatrics, SVPPGIP, SCB MCH, Cuttack, 100 number of patients between 1 yr to 14 yrs of age with primary nephrotic syndrome were taken into the study.

Results: The estimation of proteinuria by UPCR in spot sample came out to be significant as compared to the gold standard test (24-hour urine protein), with a p- value of 0.008. Nephrotic range Proteinuria estimation by urine dipstick method did not came out to be significant as 24-hour urine protein.

Conclusion: The protein-creatinine ratio in spot urine specimens is an accurate, convenient, and reliable method to estimate the protein excretion in urine and can be used as a diagnostic method in primary nephritic syndrome patients.

Keywords: proteinuria, children, nephrotic syndrome

INTRODUCTION

Nephrotic syndrome, characterized by edema, heavy proteinuria (>1g/m2 daily;>40 mg/m2/hr) and hypoalbuminemia (serum albumin < 3g/dL) is among the most common kidney diseases in childhood. The condition has an annual incidence ranging from 1.2 to 16.9 per 100,000 children. (Sinha et al.,2021).[1] The proteinuria and low albumin levels in nephrotic syndrome is due to increase in permeability of

glomerular capillary wall due to podocyte food process effacement. Proteinuria represents an early and essential element for diagnosis, assessment of disease severity and monitoring of treatment response in severe renal diseases (Singh et al., 2019).[1]

A reasonable upper limit of normal protein excretion in healthy children is 150mg/24 hr (0.15 g/24 hr). More specifically, normal protein excretion in children is defined as <4 mg/m2/hr; abnormal proteinuria is defined as 4-40 mg/m2/hr; and nephritic range proteinuria is defined as >40 mg/m2/hr. (Chen et al.,2019).[2]

Protein excretion varies in the course of the day, for this reason estimation of 24-hour proteinuria has traditionally been considered as the gold standard to determine the degree of urinary protein excretion. (Montero et al.,2012), (Kobayashi et al.,2019).[3,4] However this method is time consuming, cumbersome for patients and influenced by urine collection process, circadian rhythm and handling methods. (Montero et al.,2012).[3] Sample collection is associated with several technical problems such as inaccurate collection. For this reason, the protein/creatinine ratio (P/C) in spot urine was developed as a diagnostic alternative.

Hypoalbuminemia results from urinary losses of albumin during proteinuria, insufficient compensation by hepatic synthesis and increased albumin catabolism. (Mace et al.,2014).[5]

AIM: Quantification of spot urine protein: creatinine ratio in random urine samples, timed sample, urine dip stick and their correlation with 24-hour urine protein excretion in children with primary(idiopathic) nephrotic syndrome.

Objective:

Primary- To quantify proteinuria by dipstick, spot urine protein: creatinine ratio (UPCR) timed sample, urine in children with idiopathic nephrotic syndrome.

Secondary-

1. To determine the correlation with 24-hour urine protein.
2. To determine the correlation with serum albumin.

MATERIALS AND METHODS

After obtaining clearance from the institutional ethical committee this Hospital based observational study was conducted in the Department of Paediatrics, SVPPGIP, SCB MCH, CUTTACK from December 2023- November 2024. 100 patients between 1 yr to 14 yrs of age with primary nephrotic syndrome were taken into study fulfilling the inclusion criteria.

Inclusion Criteria: All nephrotic children between 1 -14 years of age, irrespective of clinical state (relapse or remission).

Exclusion Criteria

1. Children < 1 year of age,
2. Secondary nephrotic syndrome,
3. Nephrotic syndrome with AKI,
4. Refusal of consent by parents or caregivers for the study

Method of Data Collection: 100 children both OPD and indoor cases, who fulfil the inclusion criteria to be enrolled in the study. A predesigned and pretested proforma used to collect information. Informed consent to be obtained from parents or guardians for enrolment of their children in the study.

A complete history related to the onset, duration of illness and associated symptoms to be obtained. Clinical examinations including general physical examination, systemic examination to be done. All the relevant investigations to be carried out. 24-hour urine sample to be collected for protein estimation. A random urine sample to be collected either before starting or after completion of the 24-hour urine collection.

Blood sample to be collected for serum albumin estimation. Urine samples to be carried to laboratory of Department of Biochemistry, SCB Medical College for estimation of proteinuria 24 hr collection and urine spot protein/creatinine ratio estimation.

Sampling Technique: Convenient sampling

Statistical Analysis: Statistical analysis was being done by using software MS office and SPSS version 20.0.1.

RESULTS

The current study was conducted in the Department of Paediatrics, SVPPGIP, PG Department of Bio-chemistry SCB MCH, CUTTACK, a tertiary care hospital in Odisha. One hundred cases of nephrotic syndrome admitted to the pediatric ward during December 2023 to November 2024 were studied. The observations are illustrated below by following tables.

Table 1: Age and sex distribution in nephrotic syndrome

Age group	Male (N=60)		Female (N=40)		Total
	N	%	N	%	
<2	12	52.12	11	47.8	23 (100)
2-5	30	69.8	13	30.2	43 (100)
5-10	15	53.6	13	46.4	28 (100)
11-14	3	50	3	50	6 (100)

In less than 2 years of age group 12 cases (52.12 %) were males and 11 cases (47.8%) were females. Most of the study population come under age group of 2-5 years (43 %), out of which 30 were males (69.8%) and 13 cases were females (30.2%).

Table 2: Clinical Presentation

Clinical findings	Frequency	Percentage
Edema	100	100
Ascites	44	44
Fever	43	43
Decreased Urination	34	34
HTN	1	1
Pain abdomen	29	29
Hematuria	1	1
Cough	33	33

The edema was present in all cases. Besides ascites (44%), fever (43%) and decreased urination (34%), cough (33 %) were other common manifestations. These presenting symptoms were according to the nature of associated infections.

Table 3: Distribution of Associated Infections In Nephrotic Syndrome

Associated infection	Frequency	Percentage
0	26	26
1	29	29
2	4	4
2,3	2	2
2,4	1	1
2,6	1	1
3	32	32
5	5	5

The common infections were UTI (32 %), URTI (29%). No infection was associated with 26 cases (26 %) of nephrotic syndrome

Table 4: course of nephrotic state on admission

Attack	Frequency	Percentage
1st	88	88
2nd	12	12

Out of 100 cases 88 (88%) cases were first episode. 12 cases were first relapse.

Table 5: comparison of urinary spot protein: creatinine ratio (UPCR) with 24 hour urinary protein

UPCR	24hr Protein Urine Protein		p-value
	>1 n (%)	<1 n (%)	
>2	93 (94.9)	5 (5.1)	0.008
<2	1 (50)	1 (50)	

The urinary spot protein: creatinine ratio was correlated with 24-hour urinary protein. Out of 100 study cases 98 cases showed UPCR >2 and in 2 cases UPCR values were < 2.

Sensitivity: 98.94% (95%CI: 94.21% - 99.97%)
Specificity: 16.67% (95%CI: 0.42% - 64.12%)
Accuracy: 94% (95%CI: 87.40% - 97.77%).

Table 6: comparison of urine protein by dipstick method with 24-hour urinary protein

DIPSTICK	24hr Protein Urine Protein		p-value
	>1 n (%)	<1 n (%)	
3+,4+	87 (94.5)	5 (5.5)	0.974
2+	7 (87.5)	1 (12.5)	

Among 100 study subjects 92 cases (92%) showed nephrotic range proteinuria by dipstick method (3+, 4+). 8 cases showed 2+ in urine dipstick method but among those 8 cases, 7 cases showed nephrotic range proteinuria by 24-hour urine protein estimation.

Sensitivity: 92.55% (95%CI: 85.26% - 96.95%)
Specificity: 14.29% (95%CI: 0.36% - 57.87%)
Accuracy: 87.13% (95%CI: 79.00% - 92.96%)

Table 7: level of serum albumin of nephrotic patients on admission and its correlation with 24 hour urine protein

Serum Albumin	24hr Protein Urine Protein (>1)	
	Frequency	Percentage
<1	27	28.7
1 – 2.50	62	66.0
2.51 – 3.50	3	3.2
>3.50	2	2.1

P value = 0.853

The serum albumin levels of nephrotic patients. Among the cases showing nephrotic range proteinuria by 24-hour urine protein estimation, 62 cases (66%) have serum albumin levels between 1 to 2.5 gm% and 27 cases (28.7%) have serum albumin levels below 1gm%. However, by chi square test, it is found out that serum albumin level has no correlation with proteinuria by 24-hour urinary protein estimation, with a p value of 0.853.

Table 8: level of serum albumin of nephrotic patients on admission and its correlation with UPCR

Serum Albumin	UPCR (>2)	
	Frequency	Percentage
<1	28	28.6
1 – 2.50	65	66.3
2.51 – 3.50	3	3.1
>3.50	2	2.0

P value = 0.800

The serum albumin levels of nephrotic patients. Among the cases showing nephrotic range proteinuria by UPCR (>2), 65 cases (66.3 %) have serum albumin levels between 1 to 2.5 gm% and 28 cases (28.6%) have serum albumin levels below 1gm%.%. However, by chi square test, it is found out that serum albumin level has no correlation with proteinuria by spot urine protein: creatinine ratio estimation, with a p value of 0.800.

Table 9: blood urea levels on admission

Serum Urea	>1 g/m2		<1 g/m2	
	n	%	N	%
10.0 – 20.0	20	21.3	2	33.3
20.1 – 40.0	58	61.7	3	50.0
40.1 – 60.0	15	16.0	1	16.7
60.1 – 80.0	0	0	0	0
80.1 – 100.0	0	0	0	0
>100	1	1.1	0	0

83% cases had normal range of blood urea (<40 mg%) among nephrotic patients. Only 1.1 % cases had higher blood urea level.

Table 10: mean value of blood urea and serum creatinine levels

Renal Function Test	>1 g/m2		<1 g/m2	
	Mean	SD	Mean	SD
Blood. Urea	29.183	12.40	29.233	9.27
S. creatinine	0.486	0.14	0.555	0.20

Mean blood urea was 29.18 ±12.4 and mean serum creatinine was 0.48 ± 0.14 in patients having nephrotic range proteinuria.

DISCUSSION

Diagnosis of nephrotic syndrome is based on clinical sign and symptoms and estimation of massive proteinuria. Nephrotic range proteinuria is defined as proteinuria ≥ 1000 mg/m2 per day in a 24 -hour urine sample. This method has traditionally been considered as the gold standard to determine the degree of urinary protein excretion. In view of several limitations, other methods of urine protein estimation such as spot UPCR and urine dipstick method were developed as diagnostic alternative. In this study we want to find the correlation of these later methods as compared to the gold standard test i.e 24-hour urine protein estimation.

Urinary protein creatinine ratio(UPCR) has already been used to quantitate proteinuria in children with HIV nephropathy,[6] microalbuminuria in IDDM,[7] follow up proteinuria in kidney transplant patients,[8] follow up patients with Wilms tumour after nephrectomy,[9] monitoring intravenous methyl prednisolone and oral alkylating agent therapy of prednisolone resistant paediatric focal segmental glomerulosclerosis,[10] and monitoring the progress of patients with diarrhea associated hemolytic uremic syndrome.[11] It is expected that in children with nephrotic syndrome estimation of UPCR is likely to be of same significance as 24 hour urinary protein measurement.

It is evident that 43 cases (43%) of NS patients were in the age group of 2- 5 years. This patient observation is an agreement with the work of Agarwal et al,[12] and Srivastava et al 1975.[13] Mean age of study population was 4.76 years ranging from 1 to 14 years, which is different from Sinha et al,[1] where mean age of study population was 6.31 ± 2.99 years ranging from 1.5 to 12 years.

The male to female ratio was 3:2, which is similar as figure quoted in ISKDC.[14] Schlesinger ER,[15] postulated that as the age advances the male preponderance gradually diminishes and after 13 years of age no sex predilection is seen, which is also a fact in our present study.

The present study shows that edema (100 %) in various degrees is the hallmark of all the clinical presentations. Next to this are ascites (44%), fever

(43%), oliguria (34%), cough (33%) and pain abdomen (29%). Hematuria, HTN, anorexia, scalp edema and skin infection were present to lesser extent. Presence of edema as an important presenting feature has been documented by various authors like White 1971,[16] Srivastava et al 1975.[13]

That 26 % cases out of 100 did not have any accompanying infection whereas rest 74 % had various type of associated infections on admission. UTI was the most common (33 %), followed by URTI (29%), LRTI (7%), gastroenteritis (5%), cellulitis and acute suppurative otitis media (ASOM) 1 case each. The rare accompanying illness like ASOM, cellulitis in the present study could be due to the association of poor environmental sanitation, with superadded infection due to lowering of immunoglobulin level. The infection in our study is in agreement with the reports from Brazil59 and also from Alwadhi RK et al 2004.[17]

Out of 100 cases, there were 88% cases with first episode and rest 12% cases were with first relapse.

Laboratory investigations were carried out in all the patients. Among all study cases in 93% cases UPCR value was >2 and 24-hour urine protein estimation was >1000 mg/m2 per day. The correlation of UPCR with 24-hour urine protein estimation. The sensitivity was found out to be 98.94% (95% CI: 94.21 % - 99.97%). Specificity of the UPCR test was found out to be 16.67% (95% CI: 0.42% - 64.12%), with an accuracy of 94% (95% CI : 87.40% - 97.77%). On statistical analysis by using chi sq test for correlation of UPCR versus 24-hour proteinuria, P value of 0.008 was observed and found to be statistically significant. Similar observations were also made by Sinha et al.,2019.[1]

Mean urinary protein creatinine ratio in our study was 6.85 according to a study done by Iyer et al 64, the ratio was found to be 5.55. Study done by Navale et al,[18] Chahar et al,[19] showed a slightly lower ratio of 3.28 and 2.52 respectively.

The correlation of urine proteinuria estimation by dipstick method with 24-hour urine protein estimation. The sensitivity of the dipstick test was found out to be 92.55% (95% CI : 85.26% - 96.95%), specificity was 16.67% (95% CI : 0.42% - 64.12%) and with an accuracy of 88% (95% CI : 79.98% - 93.64%). On statistical analysis by using pearson's correlation of coefficient for correlation of proteinuria by dipstick method versus 24-hour proteinuria, P value was observed to be 0.974, which is not statistically significant.

In a similar study done by Thanyaluk Chotayaporn et al, 2011,[20] comparison of proteinuria determination urine dipstick, spot UPCR and urine protein 24 hours but on lupus patients, they found out that a ≥2+ dipstick test is relatively sensitive to detect significant proteinuria, but is poorly correlated with quantitative 24-hour urine protein. The UPCR and 24- hour urine protein can be used interchangeably for follow up in lupus nephritis as it was statistically significant. These findings are quite similar to our study results.

In children Abitbol et al 1988,[6] studied the relative feasibility of using random urinary dipstick testing and urinary protein: creatinine ratio in the quantitation of proteinuria.64 cases with relapsing nephrotic syndrome from 1.5 to 16 years were selected as study population. The log regression analysis of the UPCR and 24-hour urinary protein excretion was highly significant ($r = 0.97$; $p < 0.001$). The validity of these tests was assessed by sensitivity, specificity and predictive value and compared with that of random testing by dipstick. The UPCR offered good reliability as a test for classifying degrees of proteinuria and accurately predicted nephrotic and physiologic proteinuria. The random dipstick testing was reliable only when the results were distinctly positive and when specificity and sensitivity were low. With dipstick, positive predictive value was high (89%), though negative predictive value was low (60%).

The serum albumin levels of nephrotic patients. Among the cases showing nephrotic range proteinuria by 24-hour urine protein estimation, 62 cases (66%) have serum albumin levels between 1 to 2.5 gm% and 27 cases (28.7%) have serum albumin levels below 1gm%. Thus, it is evident that in majority of cases there was a low serum albumin level. Barratt and Rubin 1975,[21] had pointed out that due to loss of albumin selectively hypoalbuminemia occurs and globulin levels rise altering A:G ratio. Studies in PGI, Chandigarh, India showed similar results.

The serum albumin levels of nephrotic patients. Among the cases showing nephrotic range proteinuria by UPCR (>2), 65 cases (66.3 %) have serum albumin levels between 1 to 2.5 gm% and 28 cases (28.6%) have serum albumin levels below 1gm%. It is evident from the table that majority of cases with massive proteinuria have serum albumin levels in the range of 1 to 2.5 gm%. However, by chi square test, it is found out that serum albumin level has no correlation with proteinuria by 24-hour urinary protein estimation, and by spot UPCR.

On admission most cases had blood urea levels within normal limits (10- 40 mg%). 16 cases had blood urea levels (40 -60 mg%) and only 1 case had levels >100 mg%. This present observation is in proximity with reports of Srivastava et al, 1975,[13] and Agarwal et al,[12] 1975 who had shown raised blood urea in 9.2% and 12.1% of cases respectively. According to them this rise in blood urea level is transient in nature and does not give diagnostic clue. The cause of this rise was due to hypovolemia. The mean blood urea was 29.18 ±12.4 and mean serum creatinine was 0.48 ± 0.14 in patients having nephrotic range proteinuria, which is very similar to a study done by Sinha et al,[1] where the mean blood urea level was 31.72 ±10.17 and mean serum creatinine was 0.63 ± 0.23.

The urinary protein creatinine ratio (UPCR) has already been used to follow proteinuria in various renal disorder like HIV nephropathy, IDDM, HUS. It has also been used to assess response to therapy to

oral alkylating agents and intravenous methylprednisolone in prednisolone resistant FSGS. However, in these cases it has been used to quantitate proteinuria which is small and qualitatively does not change significantly over several months or even years. Hence high sensitivity of UPCR is utilized in such cases as compared to other method.

Thus, UPCR is probably more suitable to monitor proteinuria in renal disorders including nephrotic syndrome. However, study involving larger number of patients will further elucidate its significance.

Summary: The most vulnerable age group was 2 to 5 years and males dominated over females in all age groups except over 11 years where both male to female ratio were same. Edema was a presenting feature in almost all the patients followed by ascites, fever and decreased urination. UTI was the most common (33%) associated /preceding infection followed by URTI (29%), LRTI (7%), gastroenteritis (5%). Cellulitis and acute suppurative otitis media (ASOM) were rare. 26 % cases presented without any infection.88 % cases were presented with first attack and the rest 12% were presented with first relapse. The estimation of proteinuria by UPCR in spot sample came out to be significant as compared to the gold standard test (24-hour urine protein), with a p-value of 0.008. Nephrotic range Proteinuria estimation by urine dipstick method did not came out to be significant as 24-hour urine protein. Majority of cases with nephrotic range proteinuria have serum albumin levels in the range of 1 to 2.5 gm%.

CONCLUSION

24-hour urine collection which is the gold standard used to estimation proteinuria is cumbersome. This method is time consuming, influenced by urine collection process, circadian rhythm and handling methods. Sample collection is associated with several technical problems such as inaccurate collection. Hence, a spot urine examination would be more acceptable and less time consuming. It would also help school going children from missing an extra day of school. Therefore, the ratio will be of great value to primary care physicians in early diagnosis of nephrotic syndrome in children thereby helping in early initiation of treatment. The usefulness of spot urine sampling using the protein/creatinine ratio and protein dipstick was thus tested against the 24, hour urine protein excretion, which is the gold standard for estimation of protein excretion. Serum albumin level does not correlate with proteinuria by 24-hour urinary protein estimation and by spot UPCR. We conclude that the protein-creatinine ratio in spot urine specimens is an accurate, convenient, and reliable method to estimate the protein excretion in urine and can be used as a diagnostic method in primary nephrotic patients.

Limitation: Limitations of our study is that this is a single centered study and further multicenter studies are needed for results to be generalized.

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