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Assessing the Accuracy of Diagnostic Tests to Quantify Proteinuria in Nephrotic Children: A Cross-Sectional Study

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ABSTRACT

Objective: To assess the diagnostic accuracy of 24-hour proteinuria estimation and urine dipstick-taking spot urine protein creatinine ratio as the gold standard in children with steroid-sensitive nephrotic syndrome.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Paediatric Nephrology, Sindh Institute of Urology and Transplantation, Karachi Pakistan, from Oct 2020 to Mar 2021.

Methodology: Proteinuric children were enrolled to quantify proteinuria by 24-hour urine protein estimation, spot urine protein creatinine ratio and urine dipstick. Sensitivity analysis was performed, and receiver operating curves were plotted to assess the diagnostic accuracies of 24-hour proteinuria and urine dipstick against spot urinary protein creatinine ratio. Scatter plots compared the correlation of serum albumin and cholesterol with 24-hour urine protein estimation and spot urine protein creatinine ratio.

Results: Forty-two children with a median age of 8 years (IQR 6–10) were included. Nephrotic range proteinuria was detected in 39(93%) children with spot ratio, 16(38%) cases using 24-hour proteinuria estimation and 50% with a urine dipstick. Twenty-four-hour protein estimation showed a sensitivity of 63.4%, and urine dipstick had a sensitivity of 53.8% in detecting nephrotic range proteinuria compared to spot ratio with a negative predictive value of 6.3% and 14.3%, respectively. Hypoalbuminemia and cholesterol correlated better with spot ratio than 24-hour proteinuria with r- values 0.0143 and 0.0713, respectively.

Conclusion: Twenty-four-hour urine protein estimation and dipstick correlate with spot urine protein creatinine ratio in detecting nephrotic proteinuria with no statistical difference in diagnostic accuracy.

Keywords: Diagnostic test, Nephrotic syndrome, Urine, Proteinuria.

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INTRODUCTION

Idiopathic Nephrotic syndrome (INS) is children's most common acquired glomerular disease, with an annual incidence of 1.15-2.10/100,000 children.[1] The diagnosis of nephrotic syndrome and its relapse is based on a constellation of symptoms and diagnostic tests. The primary pathology is the filtration barrier in the glomeruli, resulting in heavy proteinuria, which leads to hypoalbuminemia, oedema, & hypercholesterolemia.[2,3]

The most recent KDIGO guidelines 2021 have set variable cut-off values for nephrotic range proteinuria for three different tests that are commonly used.[4] For 24-hour urine protein estimation (UPE), the diagnostic cut-off is set at more than 1g/m^2/day for spot urinary protein creatinine ratio (UPC) >2mg/mg and protein on urine dipstick (Udip) +3 to +4. Along with proteinuria, serum albumin of <3g/L, generalized oedema, and serum cholesterol of more than 200mg/dl may or may not be present at the presentation time.[5,6]

The choice of test to quantify proteinuria in clinical practice can be challenging. UPE is considered a gold standard, but collecting and interpreting the sample is time-consuming and difficult and requires validation through multiple complex and indirect calculations based on the collected sample's volume and quantity of creatinine.[7,8] Proteinuria detected by dipstick is a rapid and inexpensive bedside method commonly employed to screen proteinuria at home and in an outpatient setting. Since it is observer-dependent, semi-quantitative, and affected by multiple factors, its application is also limited in the treatment of INS.[9,10]

This study aimed to assess the diagnostic accuracy for quantifying proteinuria in children with INS using all three methods and to assess their correlation with clinical and laboratory parameters (cholesterol and albumin). The results of this study would help clinicians to compare and select the most accurate and convenient method to detect nephrotic

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range proteinuria according to the recently revised guidelines.

METHODOLOGY

The cross-sectional analytical study was conducted at the Outpatient Paediatric Nephrology Department at Sindh Institute of Urology and Transplantation, Karachi Pakistan, from October 2020 to March 2021. The study was approved by the Institutional Ethical Review Committee (ERC certificate number 2020/A-237).

Inclusion Criteria: Patients with either the first episode or relapse of steroid-sensitive nephrotic syndrome were included in the study.

Exclusion Criteria: Patients having deranged renal function with estimated glomerular filtration rate (eGFR) <90 ml/min/1.73 m^2 and urinary tract infections were excluded.

Since participation in research required additional visits to the hospital and no monetary compensation was offered, only those patients whose parents could conveniently arrange extra visits and gave written consent after full disclosure. Verbal assent was obtained from children more than 12 years of age.

Based on the previous estimate of the correlation coefficient between UPC and 24-h proteinuria observed as (0.833) with the power of test 80% and 5% significance level, a sample of 40 was required for the study.[11]

Detailed proforma was filled to record demographic and clinical data. Blood samples for serum creatinine, albumin, and cholesterol were taken at enrollment. Urine for protein was checked using a DIRUI dipstick. After immersing the colour-coded pads into the urine for 30 seconds, results were interpreted using a semi-automated analyzer based on urinary protein quantity as follows: trace (0.1-0.3 g/L), +1(0.3-1 g/L), +2(1-3 g/L), +3(3-10 g/L), +4(>10g/L).[12] The same urine sample was sent for spot UPC and urine culture. Clear instructions and jars were provided for 24-hour urine collection.

Parents were advised to collect urine throughout the day after discarding the first-morning void till the first urine of the next day. Urinary creatinine was calculated by kinetic Jaffe's method, and urinary protein quantification was determined by photometric colour test through auto analyzer Beckman Coulter (Japan). Nephrotic range proteinuria in children on 24-hour collection, UPC and Udip was defined per the KDIGO 2021 clinical practice guidelines on glomerular disease.

All the data was entered and analyzed in Statistical Package for Social Sciences (SPSS) version 20.00 and STATA-17. The quantitative variables (age, weight, height, serum albumin, serum cholesterol and serum creatinine) were expressed as mean with standard deviation or median with interquartile range (IQR) for age. For UPC, the values were obtained by dividing the quantity of protein in milligrams by the quantity of creatinine. Edema was expressed only as either present or absent. The receiver operating characteristics (ROC) curves between the UPE and Udip were generated to predict nephrotic range proteinuria, keeping UPC the reference standard with a 95% confidence interval. Scatter plots were used to correlate the serum cholesterol and serum albumin results with UPC and UPE. At the same time, sensitivity analysis was performed to compare the 24-hour urinary estimate and urine dipstick against the set gold standard of spot urinary protein creatinine ratio. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of both diagnostic tests against spot UPC were then reported in the results.

RESULTS

This study enrolled 42 children: 23(55%) boys and 19(45%) girls. The median (IQR) age was 8(6-10) years, and a mean body surface area of 0.94±0.17m^2. All children had nephrotic syndrome, out of which 31(74%) had presented with relapse, while the rest were diagnosed with first episode. None of the children had evidence of urinary tract infection. Table-I summarizes the distribution of the participants for clinical and laboratory parameters. Table-II summarizes the sensitivity analysis parameters (specificity, positive predictive value and negative predictive value) when the results of two methods (UPE and Udip) were compared against spot UPC. While evaluating collected urine samples for adequate collection, almost two-thirds of the samples of the 24-hour collection were inadequate for both the volume and quantity of protein expected in a nephrotic child. Udip detected nephrotic range proteinuria in 50%, whereas UPE in only 16(38%). However, 39 (93%) UPC samples were consistent with nephrotic range proteinuria. Based on these results, as estimated by UPE and Udip, urinary protein quantity was plotted against the UPC as the standard of reference to estimate the receiver operating characteristic (ROC) curve. The Area under the curve (AUC) of UPE with 95% CI in our cohort was found to be 0.81 for UPE, representing a sensitivity of 81% in detecting nephrotic range

proteinuria compared to UPC. Similarly, AUC for Udip was 0.77, representing a sensitivity of 77% in detecting nephrotic range proteinuria when compared to UPC.

Table-I: Distribution of Clinical and Laboratory parameters of Study Participants (n=42)

Clinical & Laboratory Diagnostic Parameters		n(%)
Serum Albumin (mg/dL)	< or =2.5 (mg/dL)	30(71%)
	>2.5 (mg/dL)	12(29%)
Serum Cholesterol (mg/dL)	< or =200 (mg/dL)	6(14%)
	>200 (mg/dL)	36(84%)
Edema	Present	35(83%)
	Absent	7(17%)
24 Hour Urinary Protein	Adequate volume collection (0.5-2 ml/kg/hour)	14(33%)
Volume Collection (ml/kg/hour)	Under collection (<0.5 ml/kg/hour)	18(43%)
	Over collection (>2 ml/kg/hour)	10(24%)
24 Hour Urinary creatinine (mg/kg/day)	<10 (mg/kg/day)	22(52%)
	11–20 (mg/kg/day)	15(36%)
	>20 (mg/kg/day)	5(12%)
24 Hour Proteinuria (gm/m²/day)	≤1 gm/m²/day	26(62%)
	>1 gm/m²/day	16(38%)
Spot Urinary Protein Creatinine Ratio (Spot U PCR)	≤2 (gm/gm)	3(7%)
	>2 (gm/gm)	39(93%)
Urinary Dipstick Test	≤+3	21(50%)
	≥+3	21(50%)

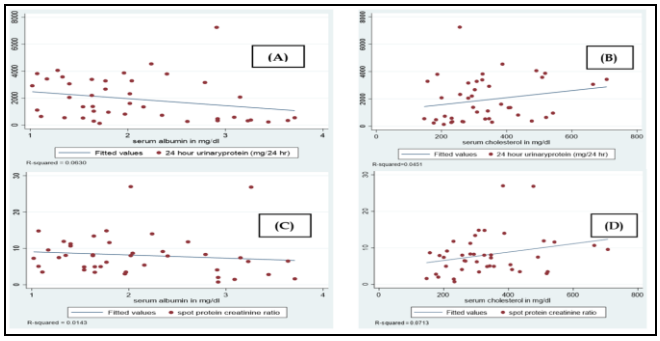

Figure: Serum Albumin and Serum Cholesterol plotted against UPE and UPC as Scatter Plots

(A)-Serum albumin versus 24 hour UPE

Interpretation: The R-Squared value of this scatter plot shows that there is a moderately positive correlation between the 24 hour UP and serum albumin levels.

(B)-Serum cholestrol versus 24 hour UPE

Interpretation: The R-Squared value of this scatter plot shows that there is moderately positive correlation b/w the 24 hour UP and serum cholesterol levels.

(C)-Serum albumin versus UPC

Interpretation: The R-Squared value of this scatter plot shows that there is moderately positive correlation b/w UPC and serum albumin levels.

(D)-Serum cholestrol versus UPC

Interpretation: The R-Squared value of this scatter plot shows that there is an almost strongly positive correlation b/w the SPOT URINE PCR and serum cholesterol levels

DISCUSSION

The utility and diagnostic accuracy of all the methods to quantify proteinuria in children with nephrotic syndrome still need to be determined. The diagnostic cut-off values recommended by recent guidelines are variable for each test. For UPE, it is 1gm/m²/day; for UPC, it is 2gm/gm, which, according to published literature, translates into 2grams/24 hours; for Udip, it is 3+, which is estimated to be ≥3 grams/day.[12,13]

Table-II: Sensitivity Analysis of two diagnostic methods vs. Spot urine PCR (n=42)

Diagnostic Methods	Area under the curve (AUC)	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
24-hour urinary protein	0.82	63.4%	100%	100%	6.3%
Urinary Dipstick	0.77	53.8%	100%	100%	14.3%

For further analysis, serum albumin and serum cholesterol were plotted against UPE and UPC as scatter plots (Figure). There were a significant number of children with serum albumin levels of <2mg/dl and serum cholesterol of >200mg/dl who did not show nephrotic range proteinuria in the 24-hour proteinuria estimation (Figure-a, b). Similarly, some patients with high-grade proteinuria had lower serum cholesterol levels. In contrast, there were very few outliers when the same was plotted against UPC (Figure-c, d) and most children with ≥+3 on urine dipstick had serum albumin <3mg/dl and serum cholesterol greater than 200mg/dl.

Our study has demonstrated the difficulty in collecting and validating the 24-hour urine samples in nephrotic children. In our cohort, despite detailed instructions, the collection needed to be improved in about two-thirds of cases. Wahbeh *et al*. have also addressed this problem. About one-third of the cases needed an adequate collection in their cohort.[14] This

difference could be because there were also many non-nephrotic patients in their cohort. Due to the oliguria in nephrotic children and increased weight due to oedema, the volume of urine and muscle mass of the person is lower than the estimated value. This effect is demonstrated in our results when clinical parameters were plotted against UPE.[15,16]

UPC in our cohort was the most reliable to diagnose nephrotic range proteinuria. However, in our cohort, some of the results showed very high values of proteinuria that were not consistent with the child's clinical status. Our results show that UPC may be a good test to diagnose nephrotic range proteinuria; however, it may not estimate the exact quantity in children with heavy proteinuria.[17] Witte *et al*. and Naufal *et al*. have shown that the first-morning void predicts proteinuria more reliably than any other sample. Further studies on UPC on the first-morning void samples may help clarify this issue.[18,19]

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LIMITATIONS OF STUDY

Our study has many limitations. The UPE samples with over or under-collection could not be repeated as it was already very difficult for parents to submit one sample. Since there was no monetary compensation for participating in the study, asking them for repeat samples would be unfair. Further studies collecting such samples as in-patients under direct observation may establish this test as the gold standard for quantifying proteinuria in nephrotic children.

CONCLUSION

The urine protein creatinine ratio was a better method of quantifying proteinuria when compared to UPE and Udip to estimate nephrotic range proteinuria in children with nephrotic syndrome. However, further studies are required to establish its accuracy to quantify heavy proteinuria. Collecting a 24-hour urine sample in children is difficult and may need other methods to validate the adequacy of collection in nephrotic children. Udip may be a good screening tool to detect any proteinuria, but its use as a diagnostic test for nephrotic range proteinuria is limited.

Conflict of Interest: None

Authors Contribution:

Following authors have made substantial contributions to the manuscript as under:

HFY & AZ: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

ISB & SK: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

MA & ASL: Concept, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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