QUANTITATION OF PROTEINURIA BY SPOT URINE SAMPLING

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ABSTRACT

Few studies have shown that calculation of protein/creatinine ratio in a spot urine sample correlates well with the 24-hour urine collection. A study was conducted to compare the accuracy of a spot urinary protein/creatinine ratio (P/C ratio) and urinary dipstick (albustix) with the 24-hour urine protein (24-HUP). Fifty samples from 26 patients were collected. This included a 24-hour urine sample followed by the next voided spot sample. The protein/creatinine ratio was calculated and dipstick (albustix) was performed on the spot sample. This was compared with the 24-hour urine protein excretion. The correlation between the three samples was statistically highly significant ($p < 0.001$) for all levels of proteinuria. The normal value of protein/creatinine ratio in Indian children was also estimated on 100 normal children attending the OPD and was calculated to be 0.053 (S.E of mean $\pm$ 0.003).

KEY WORDS

Proteinuria, spot urine sampling, nephrotic syndrome, mean.

INTRODUCTION

Proteinuria is a major determinant of progression of renal disease. Recent studies have shown that proteinuria itself causes further tubular injury and thus can perpetuate further damage (1). In certain conditions like Nephrotic Syndrome the amount of protein excretion is a reflection of activity of disease. Quantitative measurement of proteinuria by a 24-hour urine collection has been the accepted method of evaluation. However the process is tedious and there is a high possibility of error in the absence of a reliable collection. Recent studies in adults have shown that calculation of protein creatinine ratio in a spot urine sample correlates well with the 24- hour urine collection. (2)

AIMS of STUDY

To compare the accuracy of spot urinary protein/creatinine ratio (P/C ratio) and urinary dipstick (albustix), markers of proteinuria in children with renal disease, with the 24-hour urine protein (24-HUP) excretion.

Secondary objective was to estimate the normal values of protein/creatinine ratio in Indian children.

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MATERIAL AND METHODS

This was a collaborative study between the Child Health Unit & Clinical Biochemistry Departments of Christian Medical College and Hospital, Vellore. Children 12 years of age and below, attending the Child Health OPD with underlying Nephrotic Syndrome formed the study group. They were asked to give in a 24-hour urine sample with collection starting at 8.00 am on the first day and completing by 8.00 am the following day. The next random urine sample after this collection was then submitted for protein (mg/dl) and creatinine (mg/dl) estimation and was also subjected to the dipstick test. The protein/creatinine ratio was calculated. The protein/creatinine ratio, dipstick (albustix) and 24-hour protein excretion were then compared.

Reference ranges for protein/creatinine ratio was taken as < 0.2 , $0.2-3.5$, > 3.5 (2). 24 HUP was graded into 3 groups: < 4 mg/m²/hr, $4-40$ mg/ m²/ hr and > 40 mg/ m²/ hr (3). Dipstick was graded as negative, trace, 1+ (closest to 30 mg/dl), 2+ (closest to 100 mg/dl), 3+ (closest to 300 mg/dl) and 4+ (> 2000 mg/dl) (4). Hundred children attending the OPD for other disorders or health check up with no historical or clinical evidence of renal disease were selected for protein/creatinine ratio estimation. The normal ranges for children were estimated from the results of the 100 control children.

RESULTS

Over a six-month period 50 urine samples from a total of 26 patients were analyzed. Each patient gave a 24-hour urine collection followed by the

next voided sample which was simultaneously checked for protein/ creatinine ratio (P/C ratio) and albumin levels by a dipstick.

Table I shows the results obtained in the different groups. A comparison was then made between the 3 groups. Eighty percent (8/10) of samples with a P/C ratio of > 3.5 also had nephrotic range proteinuria (excretion > 40 mg/24 h). Also 91% (32/37) samples with P/C ratios of < 0.2 had protein excretion of < 4 mg/ 24 h. This was statistically significant ($p = < 0.001$) (Table II) When compared with the dipstick, 35/37 (95%) of samples with P/ C ratio of < 0.2 also had a negative dipstick (Negative or 1+). Seventy percent (7/10) of samples with P/C ratio > 3.5 had a dipstick positivity of 3+ or 4+. This was also statistically significant ($p = < 0.001$) (Table III).

A similar correlation was found when the 24-HUP was compared against the dipstick (Table II). 8/10 samples (80%) had a protein excretion of > 40 mg/ 24 h as well as $> 3+$ proteinuria on the dipstick. All samples (100%) with a 24-HUP of < 4 mg/ 24 h had a negative dipstick (1+ or negative). This was also statistically highly significant ($p = < 0.001$). These tests thus showed that all the 3 tests are reliable and correlated significantly with each other.

The reliability of the dipstick is widely accepted except in situations where the urine is very concentrated or there is hematuria (5). Nine patients had a urine volume of < 500 ml. Four negative dipsticks and 3 positive dipsticks (3+) correlated well with the corresponding 24HUP values. In the remaining two, one had a urine dipstick of 2+ but the corresponding 24 UP was in the nephrotic range (> 40 mg/ 24 h) and in the other the dipstick showed 1+ when the 24-HUP was in the intermediate range (4-40 mg/ 24 h range). Children with hematuria were excluded from the study hence the association with proteinuria could not be assessed.

The mean protein/creatinine ratio for the population of children who were analyzed was 0.053 (S.E of mean $\pm$ 0.003). Male: Female ratio was 65:35

Table II

	24 HUP			Total
	> 40 mg/24 h	4-40 mg/24 h	< 4 mg/24 h	
P/C Ratio*				
> 3.50	8	1	1	10
0.2-3.5	0	1	2	3
<0.2	2	3	32	37
Dipstick**				
Neg/1+	1	5	35	41
2+	1	-	-	1
3+/4+	8	-	-	8
*p = < 0.001 **p = < 0.001				
Comparison of Protein Creatinine Ratio and urinary dipstick results against 24 HUP showed highly significant correlation between the tests.				

Table III

P/C ratio	DIPSTICK			
	Neg or 1+	2+	3+/4+	Total
< 0.2	35	1	1	37
0.2-3.5	3			3
> 3.5	3		7	10
Total	41	1	8	50
* p = < 0.001				
Comparison of protein/creatinine ratio with dipstick showed a significant correlation between the two.				

Table I

No.	Protein Creatinine Ratio			Urine Dipstick					24 H urine mg/ 24 h		
	< 0.2	0.2-3.0	> 3.5	Neg	1+	2+	3+	4+	< 4.0	4-40	> 40
No	37	3	10	34	7	1	7	1	35	5	10
%	74	6	20	68	14	2	14	2	70	10	20
The above table shows the results obtained by the three different methods of estimation of urinary protein used in the study											

Patients were distributed in the age groups of 0 - 3 years (26), 3-5 years (32) and 6-12 years (39). The protein/creatinine ratio in these age groups was comparable being 0.045 in the first group, 0.05 in the second and 0.06 in the third group.

DISCUSSION

24 hour urine collection, which is the gold standard used to estimate proteinuria is cumbersome, and in older children, aesthetically unacceptable. 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. The usefulness of spot urine sampling using the protein/creatinine ratio and albutix was thus tested against the commonly used 24-hour urine protein excretion.

The protein/ creatinine ratio takes into account the fact that creatinine excretion remains fairly constant in the presence of a stable GFR. The protein excretion would also likewise be fairly stable. Hence the ratio of the two in a single voided sample would reflect the cumulative protein excretion over the day, as the two stable rates would cancel out the time factor (2).

In a study on 46 patients by Ginsberg *et al.* (2) an excellent correlation between the protein content of a 24 hour urine collection and the protein / creatinine ratio in a single urine sample was found. The best correlation was found when samples were collected after the first voided morning specimen and before bedtime. Our study took the first sample soon after the first voided sample. The correlation between the protein/ creatinine ratio and the corresponding dipstick value for albumin was statistically highly significant ($p = <0.001$) for all levels of proteinuria.

The protein /creatinine ratio is also a method of rapid quantitative assessment of proteinuria. In children with nephrosis it would be particularly advantageous in determining response to treatment. Abitbol *et al.* (5) studied 64 children with nephrosis from 18 months to 16 years of age. 24-hour urine collection and random urine specimens were compared with each other as well as with a dipstick. All patients had normal creatinine clearance as estimated by serum creatinine and height index. The urinary protein/creatinine ratio offered good reliability as a test for classifying degrees of proteinuria and accurately predicting nephrotic and physiological range proteinuria. However the random dipstick test was reliable only when definite proteinuria was present. It did not correlate well when the proteinuria was in the lower range. Our study however showed an excellent correlation with P/C ratio at all levels. 35/37 samples with negative or trace dipstick values had a P/C ratio of < 0.2 and this was statistically highly significant. Correlation with creatinine clearance was

not looked at.

Schwab *et al.* (6) did a similar quantitation of proteinuria by use of the P/C ratio in a single urine sample in a group of patients with a broad spectrum of renal disease, wide range of proteinuria and various degrees of reduction in GFR. Here also it was found to correlate very well with 24-hour protein excretion in 80% of patients ($p = <0.001$). Our study was however restricted to patients with Nephrotic Syndrome.

In the presence of stable renal function a protein/ creatinine ratio of 3.5 or above can be taken to represent nephrotic range proteinuria and a value of less than 0.2 mg /mg can be taken to be within normal limits (1). The mean value of protein/ creatinine ratio in normal children as analyzed by this study was representative of physiological levels of protein excretion in our population.

CONCLUSION

Spot urine sampling is a reliable method of testing in children with proteinuria. Protein /Creatinine ratio correlates well with urine dipstick in a spot sample and the 24 hour urinary excretion of protein irrespective of the degree of proteinuria. Dipstick test has the advantage of giving an instant reliable result. However, protein /creatinine ratio, which is a quantitative test would be useful additionally in monitoring progression of renal disease.

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