

Original Article

Assessment of the validity of protein-osmolality ratio in a randomly collected urine specimen in the estimation of proteinuria in children

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Abstract

Background

Proteinuria is an important marker of kidney disease. Protein-creatinine ratio and protein-osmolality ratio in a spot urine sample have been proposed as alternatives to estimation of 24-hour urinary protein excretion in order to simplify sample collection and minimize errors due to incorrect sample collection.

Objective

The study aimed to estimate the validity of urine protein-osmolality ratio and urine protein-creatinine ratio in a random urine sample as predictive measures of 24-hour urinary protein excretion, in a paediatric population.

Methods

A cross sectional descriptive study was conducted among 85 children with kidney disease from the medical wards and nephrology clinic of the Lady Ridgeway Hospital and 56 healthy children aged 3-12 years. Twenty four-hour urine samples and spot urine samples were collected from each participant. Urine protein-osmolality ratio and urine protein-creatinine ratio in the spot urine sample were determined and compared with 24-hour urinary protein excretion. Data was analysed using SPSS statistical package. Standard descriptive methods (mean, median, standard deviation etc.) were used to describe the measured parameters. Receiver operator characteristic (ROC) curves were created using protein-osmolality ratio and protein-creatinine ratio as test variables, and 24-hour urinary protein excretion as the 'gold standard' variable. Optimum cut-off values, maximizing sensitivity and specificity, for protein-osmolality ratio and protein-creatinine ratio were determined using ROC curves.

Results

The optimal value discriminating normal from abnormal protein excretion was determined to be a protein-osmolality ratio of 0.38mg/L:mOsm/kgH₂O (sensitivity 85.7%, specificity 100%) and a protein-creatinine ratio of 28mg:mmol (sensitivity 100%, specificity 94%). The cut-off value for discriminating mild from nephrotic proteinuria was a protein-osmolality ratio of 2.00 mg/L:mOsm/kgH₂O (sensitivity 91.5%, specificity 100%) and a protein-creatinine ratio of 186 mg:mmol (sensitivity 93%, specificity 98.5%).

Conclusions

Both the protein-creatinine ratio and the protein-osmolality ratio in a spot urine sample can be used to determine nephrotic proteinuria. Urine protein-creatinine ratio was more sensitive than urine protein-osmolality ratio in detecting patients with mild proteinuria.

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Introduction

Proteinuria is an early and sensitive marker of kidney damage in many types of chronic kidney disease¹. Estimates of proteinuria in monitoring kidney disease are also useful in predicting the progression of renal damage and evaluating response to therapy. Hence quantification of urinary protein excretion is an important part of a nephrological evaluation¹. Most definitive and reliable method for quantification of proteinuria is by assessing 24-hour urinary protein excretion¹. However, obtaining an accurate timed urine collection is difficult and cumbersome, especially in younger children and infants and in those suffering from urinary incontinence or enuresis. Incorrect volume of urine collection may be associated with over or underestimation of proteinuria. A 24-hour collection of urine may need admission to a ward and it is time consuming.

Ratios of urine protein-osmolality and urine protein-creatinine in a single voided urine sample have been suggested as alternative methods to overcome difficulties in collection of a timed urine volume for quantitative assessment of 24-hour urinary protein excretion. Each uses the denominator of the ratio (osmolality or creatinine respectively) to correct for degree of concentration and /or dilution of a given urine sample. Several studies have reported a good correlation between urine protein-creatinine ratio in a spot urine sample and 24-hour urinary protein excretion². However, patterns of excretion of creatinine may vary according to the severity and type of glomerular disease. Protein-creatinine ratio in a spot urine sample is affected by age, gender, body size (muscle mass) and hydration³.

Compared to urine protein-creatinine ratio, urine protein-osmolality ratio in a spot urine sample is easy to perform, less time consuming, cost effective. Urinary osmolality, the direct measure of degree of concentration of urine, may be a better factor against which to standardize urinary protein excretion⁴.

Several studies have reported urine protein-osmolality ratio in a random urine sample, as a valuable predictor for the assessment of quantitative proteinuria in children as well as in adults⁵⁻¹⁰. Normative data for urine protein-creatinine and urine protein-osmolality ratio have been published for adults and paediatric population^{4,7,10}. Few studies on direct statistical comparisons between the two ratios have been published⁴.

This study was performed to determine cut off-values for urine protein-osmolality ratio to distinguish between normal and elevated urinary protein levels and to compare the validity of urine protein-osmolality and protein-creatinine ratio in a spot urine sample, as predictive measures of 24-hour urinary protein excretion.

Methods

Children with proteinuria secondarily to kidney disease (chronic renal failure or nephrotic syndrome) who were admitted to medical wards and attended the nephrology clinic at Lady Ridgeway Hospital for Children, Colombo were selected as the study group. Healthy children with no past history of urinary tract infections, renal disease or abnormal urine analysis results and a negative family history of

renal disease were recruited as the control group. Controls were selected from those who were admitted to wards for an acute illness other than a kidney disease and were willing to participate. Once they were discharged after recovering from their acute illness they were reviewed 2 weeks later, and children who fulfilled the inclusion criteria to be in the control group were recruited. The study was approved by the Ethics Review Committee of the Lady Ridgeway Hospital for Children.

Informed written consent was obtained from the parents of the participants and a random urine sample was obtained for microscopic (urinary full report) and biochemical evaluation (dipstick testing for urine glucose). Children with urinary tract infections, haematuria, glycosuria and who were on recent treatment with cephalosporins were excluded.

During the first interview demographic data was collected. Anthropometric measurements of body weight and height were measured adopting standard protocol. Body weight was measured to the nearest 0.1 kg by a standard beam balance. Body height was measured to the nearest 0.5 cm without shoes using a wall-mounted stadiometer.

A timed urine sample for 24-hour protein estimation was collected. Within the period of urine collection for 24-hour urinary protein excretion, a random early morning urine sample was collected for urine protein, creatinine and osmolality measurement. All the samples were analysed without delay on the same day of collection, in the Department of Chemical Pathology, Lady Ridgeway Hospital for Children. Urine protein was measured by a dye binding method (pyrogallol red method) with a between run coefficient variation of 5% and 4% at 15 mg/dL and 101 mg/dL levels of concentration respectively.

Urine creatinine was measured by kinetic Jaffe method. For urine creatinine, between-run coefficient variation was 5.8% and 4.7% at 3004 $\mu\text{mol/L}$ and 11200 $\mu\text{mol/L}$ level of concentrations respectively. All were analysed by konelab Prime 30® fully automated analyser (Thermo Scientific, Finland). Calibration was done using standards provided by the manufacturer and with each analytical run, quality control samples at 2 levels (high and low) were analysed.

In spot urine samples osmolality was measured using the principle of freezing point depression by AdvancedTMMicrosmolar Model 3300 Osmometer (Advanced Instruments, USA).

Protein-osmolality ratios were expressed as mg/L:mOsmoles/kgH₂O. Protein:creatinine ratios were expressed as mg:mmol. Twenty four hour urinary protein excretion was determined as mg/m²/hour. The adequacy of the volume of collected urine was assessed using normal creatinine excretion rates. The reference value of 24-hour urinary creatinine for a child was taken >71 $\mu\text{mol/kg/day}$. If the above creatinine excretion rates were not achieved it was considered that the urine volume was inadequate and another 24-hour urine sample was collected on a subsequent day.

Data was analysed using SPSS statistical package. Standard descriptive methods (mean, median, standard deviation etc.) were used to describe the measured parameters. Receiver operator characteristic (ROC) curves were created using protein-osmolality ratio and protein-creatinine ratio as test variables, and 24-hour urinary protein excretion as the 'gold standard' variable. Optimum cut-off values, maximizing sensitivity and specificity, for protein-osmolality ratio and protein-creatinine ratio were determined using ROC curves.

Results

Fifty four children were enrolled as healthy subjects in the study. Eighty five children with chronic kidney disease were selected as having mild or nephrotic range proteinuria. A normal proteinuria level was defined as $<4 \text{ mg/m}^2/\text{hour}$ and nephrotic range proteinuria as $> 40 \text{ mg/m}^2/\text{hour}$ in the protein excretion of 24-hour collection. Children who had their 24-hour urinary protein excretion between those values ($4 - 40 \text{ mg/m}^2/\text{hour}$) were categorized as children having mild proteinuria. Demographic data concerning these children are listed in Table 1.

Table 1: Demographic and anthropometric data for healthy children and children with kidney disease

	Healthy children (54) Mean (SD)	Children with kidney disease (85) Mean (SD)
Sex (male/female)	37/17	48/37
Age (years)	8.2 (2.5)	6.9 (2.7)
Height (cm)	127.6 (16.4)	115.5(16.9)
Weight (kg)	28.7 (12.5)	22.4 (8.9)

The difference in mean height and weight between patients with proteinuria and normal healthy children may be due to the difference in age distribution in the two groups.

The median protein-creatinine and protein–osmolality ratios in a spot urine sample and the median value of protein excretion in a 24-hour urine collection for the healthy subjects and the children with kidney disease are listed in Table 2.

Table 2: Urinary protein excretion assessment data of healthy children and children with kidney disease

	Healthy children (54)	Children with kidney disease (85)
P-CR (mg:mmol)	11.0 (7-16)	824 (283-1603)
P-OR (mg/L: mOsmoles/ kgH ₂ O)	0.13 (0.09-0.22)	6.3 (2.12-18.93)
24-protein excretion ($\text{mg/m}^2/\text{hour}$)	2.64 (1.69-3.29)	155 (68-281)

Data are expressed as median (range)

P-CR - Protein-creatinine ratio; P-OR - Protein–osmolality ratio

Twenty-four hour urinary protein excretion strongly correlated with both protein-creatinine and protein-osmolality ratios in a random urine sample in proteinuric group (mild+nephrotic) [Figures 1(c) and 1(d)]. However, there was a statistically significant but weak correlation between urine protein-creatinine and urine protein-osmolality ratios with 24-h protein excretion in non-proteinuric group (healthy children) [Figures 1(a) and 1(b)], (Table 3).

Table 3: Spearman’s Correlation coefficients between 24-hour urine protein excretion and protein-osmolality ratio and protein-creatinine ratio in an early morning urine specimen.

Group	Correlation between protein-osmolality ratio and 24-hour protein excretion		Correlation between protein-creatinine ratio and 24-hour protein excretion	
	r	P	r	P
Healthy children	0.297	0.029	0.378	0.005
Children with proteinuria	0.826	<0.001	0.827	<0.001
Entire group	0.914	<0.001	0.924	<0.001

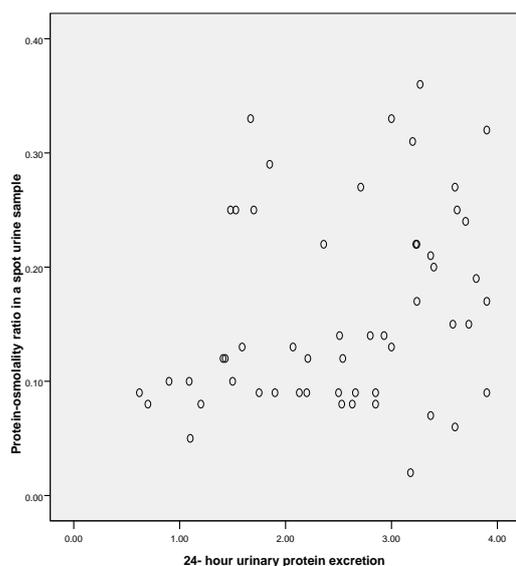


Figure 1(a)

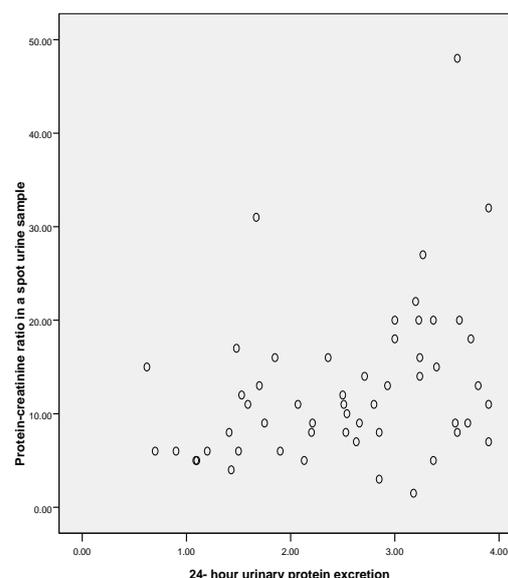


Figure 1(b)

Figure 1(a) - Scatter diagram showing the relationship between protein-osmolality ratio in a random urine sample (mg/L: mOsm/kgH₂O) and 24-hour urinary protein excretion (mg/m²/hour) in healthy children

Figure 1(b) - Scatter diagram showing the relationship between protein-creatinine ratio in a random urine sample (mg/mmol) and 24-hour urinary protein excretion (mg/m²/hour) in healthy children

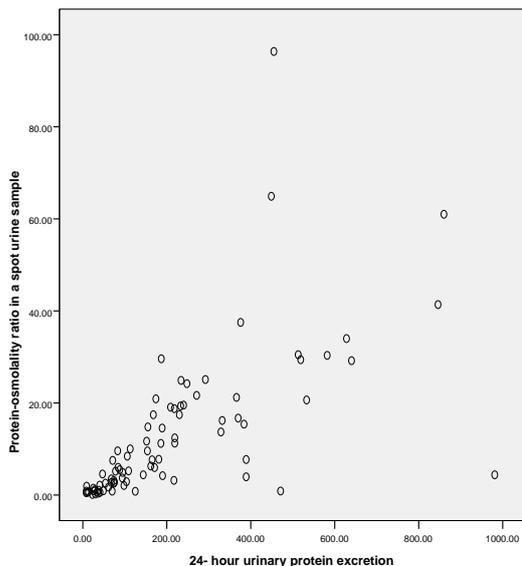


Figure 1(c)

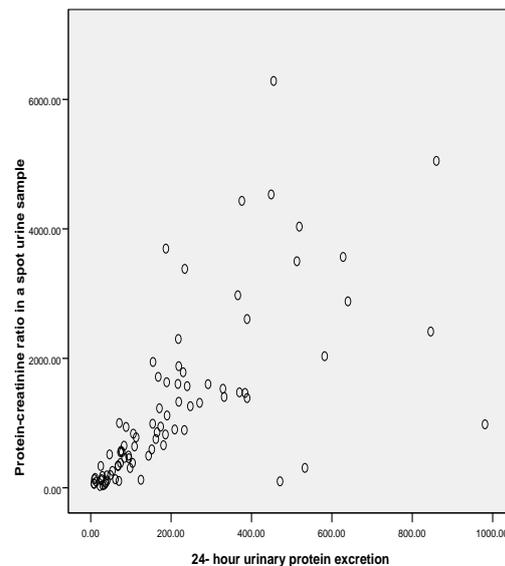


Figure 1(d)

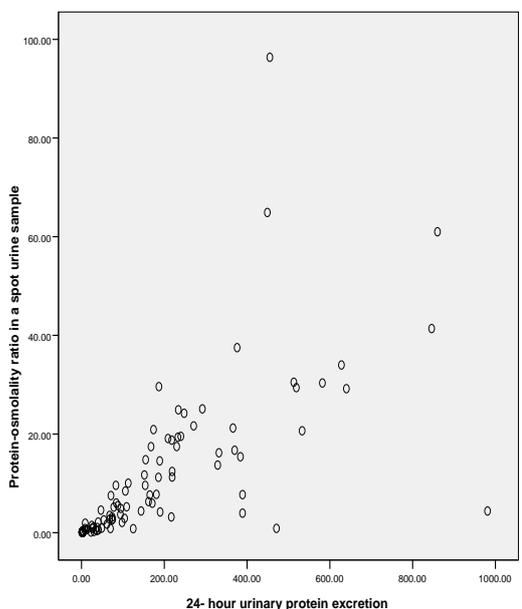


Figure 1(e)

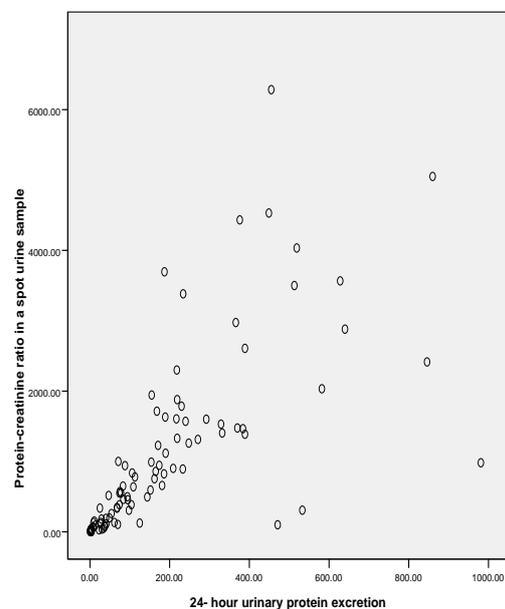


Figure 1(f)

Figure 1(c) - Scatter diagram showing the relationship between protein-osmolality ratio in a random urine sample (mg/L: mOsm/kgH₂O) and 24-hour urinary protein excretion (mg/m²/hour) in children with proteinuria

Figure 1(d) – Scatter diagram showing the relationship between protein-creatinine ratio in a random urine sample (mg:mmol) and 24-hour urinary protein excretion (mg/m²/hour) in children with proteinuria

Figure 1(e) - Scatter diagram showing the relationship between protein-osmolality ratio in a random urine sample (mg/L: mOsm/kgH₂O) and 24-hour urinary protein excretion (mg/m²/hour) in the study population

Figure 1(f) - Scatter diagram showing the relationship between protein-creatinine ratio in a random urine sample (mg:mmol) and 24-hour urinary protein excretion (mg/m²/hour) in the study population

Based on ROC curve analysis for healthy children, the optimal value discriminating normal from abnormal protein excretion and the optimal value discriminating mild protein excretion and nephrotic protein excretion were determined (Figure 2, 3).

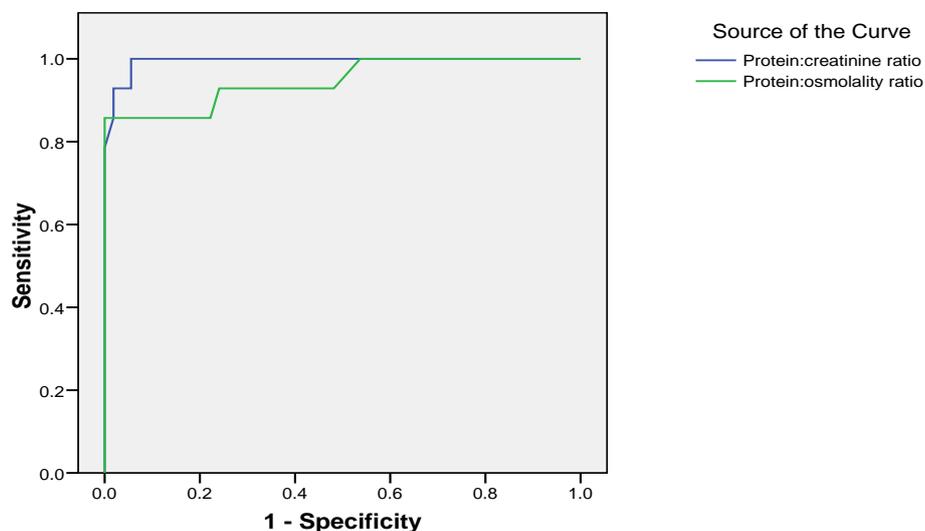


Figure 2: Comparison of receiver operator characteristic curves for protein-osmolality ratio and protein-creatinine ratio in healthy children

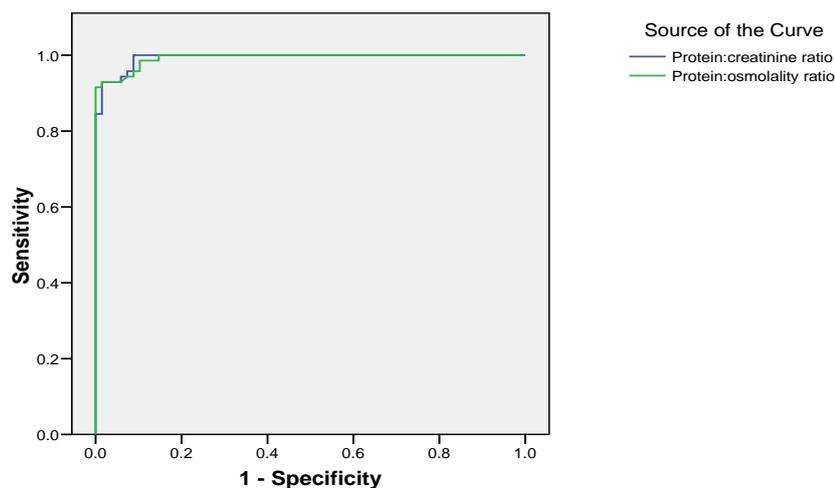


Figure 3. Comparison of receiver operator characteristic curves for protein-osmolality ratio and protein-creatinine ratio in patients with heavy proteinuria

Based on analysis of ROC curves for healthy children, the optimal value discriminating normal from abnormal protein excretion was determined to be a P-OR of 0.38 mg/L: mOsm/kgH₂O and a P-CR of 28 mg:mmol (Table 4).

The optimal value discriminating mild from heavy urinary protein excretion was determined to be a protein-osmolality ratio of 2.00 mg/L: mOsm/kgH₂O (Figure 4) and a protein-creatinine ratio of 186 mg:mmol (Table 4).

Table 4: Validity indicators of protein-creatinine and protein-osmolality ratios in spot urine samples in predicting mild and nephrotic proteinuria

Ratio	Cut-off point	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)
*P-CR	Mild (28 mg:mmol)	100	94.4 (90.58 - 98.22)	94.6	100
	Nephrotic (186 mg:mmol)	93.0 (88.77 - 97.23)	98.5 (96.48 -100.52)	98.4	93.3 0
**P-OR	Mild (0.38 mg/L: mOsm/kgH ₂ O)	85.7 (79.9 - 91.5)	100	100.	87.4 0
	Nephrotic (2.00 mg/L: mOsm/kgH ₂ O	91.5 (86.99 - 96.01)	100	100	92.1 6

*P-CR - Protein-Creatinine Ratio; P-OR - Protein –Osmolality Ratio

The direct comparison between the protein-creatinine ratio and protein-osmolality ratio in early morning spot urine samples revealed following correlations (Figure 4, Table 5)

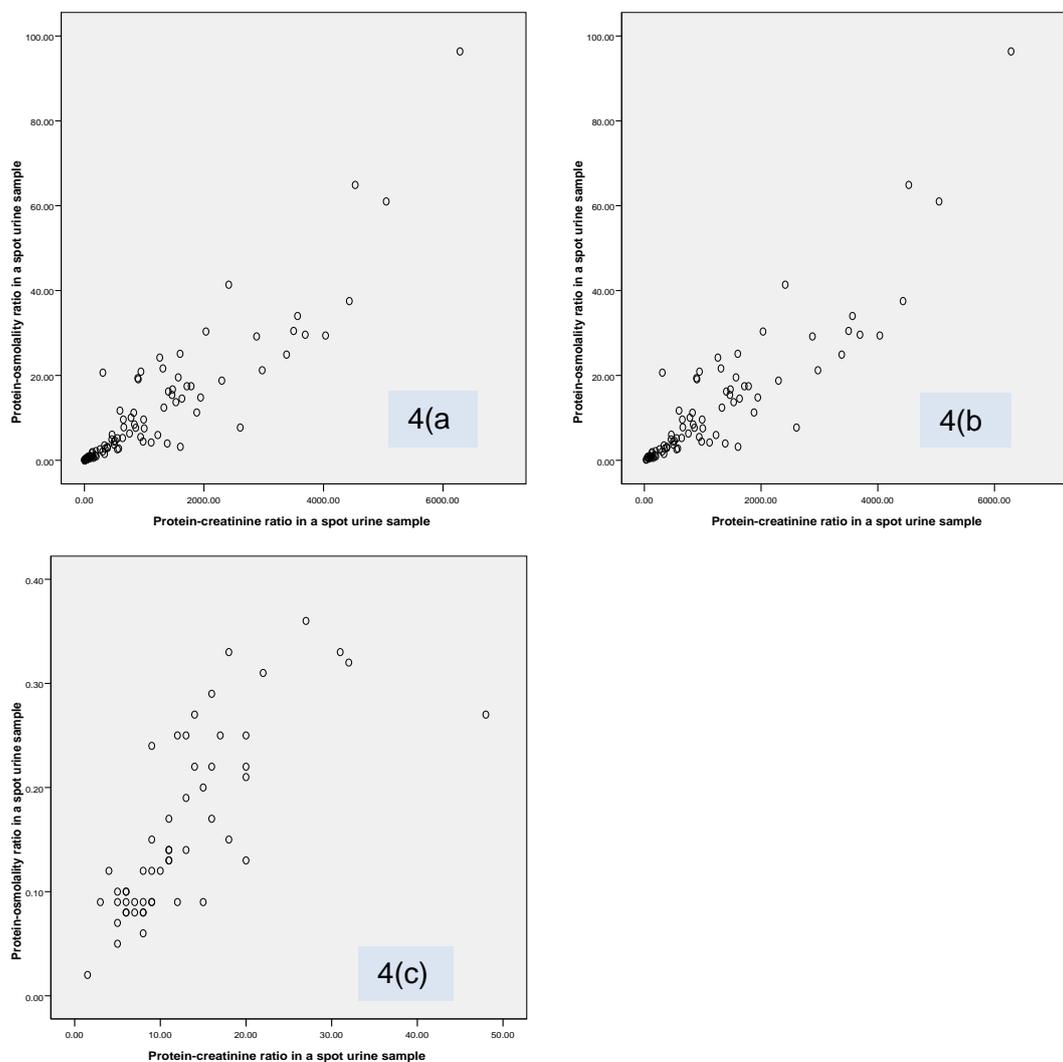


Figure 4. Scatter diagrams showing the relationship between protein-creatinine ratio in a random urine sample (mg:mmol) and protein-osmolality ratio in a random urine sample (mg/L: mOsm/kgH₂O)
 (4a) in the entire population (4b) in children with proteinuria (4c) in healthy children

Table 5. Spearman's Correlation coefficients between protein-osmolality ratio and protein-creatinine ratio in an early morning urine specimen.

	r	p
Healthy children	0.825	<0.001
Children with proteinuria	0.903	<0.001
Entire group	0.965	<0.001

Discussion

The recent National Kidney Foundation Kidney Disease Outcome Quality Initiative (K/DOQI) clinical practice guideline for kidney disease has recommended the use of protein-creatinine ratio in an untimed spot urine specimen, for the assessment of kidney damage and monitoring of proteinuria, in patients with renal disease except in post pubertal children with diabetes mellitus¹. In addition, protein-osmolality ratio in a random urine sample has been suggested as another tool for the assessment of renal disease. Compared to urinary creatinine concentration, urinary osmolality, a direct measure of degree of concentration of urine, may be a better factor against which the urinary protein excretion could be standardized⁴.

Data describing the protein-osmolality ratios of normal population and in patients with proteinuria have been published for adults as well as for children. According to Morgenstern et al the Mayo Clinic has been using this ratio as a tool to screen for significant proteinuria in adults since 1993 on all routine urinalysis⁴. Few studies have been done to statistically compare these two ratios.

Studies have been performed in view of assessing the correlation between 24-hour protein excretion and protein-osmolality ratio in children. Twenty-four hour urinary protein excretion in our population of healthy children had a weak, but statistically significant correlation with protein-creatinine ratio or protein-osmolality ratio in a random urine sample. ($r = 0.378$, $P < 0.05$ and $r = 0.297$, $P < 0.05$, respectively). In patients with proteinuria this correlation was stronger for both protein-creatinine ratio and protein-osmolality ratio, $r = 0.827$, $P < 0.001$ and $r = 0.826$, $P < 0.001$ respectively. Similarly Morgenstern et al. reported a significant correlation with 24-hour urinary protein excretion and both urinary protein-creatinine ratio and protein-osmolality ratio for healthy children ($r=0.35$, $P<0.0001$ and $r=0.29$, $P<0.0001$ respectively). In the same study correlations between the considered parameters for children with kidney disease were also significantly similar to current study ($r=0.99$, $P<0.0001$ for protein-creatinine ratio and $r=0.91$, $P<0.0001$ for protein-osmolality ratio)⁴. According to Kim et al., in a group of healthy children twenty-four hour urinary protein excretion had no significant correlation with urine protein-creatinine ratio or urine protein-osmolality ratio. However they revealed a highly significant correlation of 24-hour urinary protein excretion with both protein creatinine ratio and protein-osmolality ratio in the group of children with proteinuria ($r = 0.88$, $P < 0.001$ and $r = 0.87$, $P < 0.001$ respectively)⁸.

The cut-off value of protein-osmolality ratio to diagnose abnormal proteinuria was 0.38 mg/L: mOsmoles/kgH₂O. This figure is close to the values reported by Kim et al. and Hooman et al. who used the turbidimetric method for measuring urinary protein (0.23 and 0.27 mg/L: mOsmoles/kgH₂O respectively)^{7,8}. Cut-off value to detect abnormal urinary protein excretion obtained in our study is higher than the value obtained by Morgenstern et al. (0.15 mg/L: mOsmoles/kgH₂O) where urine protein was measured by the pyrogallol red method, as in our study⁴. Protein-osmolality ratio, in both healthy population and patients with mild proteinuria of our study did not show a Gaussian distribution and the sample size of children with mild proteinuria was not optimum. Most of the patients with kidney disease were with nephrotic syndrome with glomerular damage, which does not influence the tubular secretion of creatinine as in patients with tubular damage. These factors may have influenced the discrepancy of these values.

In our study, the sensitivity and negative predictive value (NPV) of the protein-osmolality ratio were less than that of the protein-creatinine ratio to evaluate abnormal proteinuria. However, the sensitivity and NPV were closer numerically in detecting nephrotic proteinuria in both protein-osmolality ratio and protein-creatinine ratio (Table 4).

When comparing the ROC curves, urine protein-creatinine ratio seems to be a better measurement to distinguish between normal and abnormal amounts of protein excretion in children (Figure 2). Regarding detection of nephrotic range proteinuria, comparison of ROC curves revealed that there was no difference between both ratios in detecting nephrotic range proteinuria (Figure 3).

Morgenstern et al. reported a stronger predictive value for the urine protein-creatinine ratio (positive predictive value (PPV 94.4%, NPV 97.8%) compared to protein-osmolality (PPV 85%, NPV 93.5%) in detecting abnormal proteinuria in children⁴. These data are in contrast to the findings reported by Kim et al.⁸. According to Kim et al. the NPV of 93.5% for protein-osmolality ratio was higher than that of protein-creatinine ratio (90%) and PPV was 100% for both parameters in detecting abnormal proteinuria.

In the study by Morgenstern et al. the results in the paediatric population differed from the adult population, in that both protein-osmolality and protein-creatinine ratios were equally predictive of abnormal proteinuria in adults⁴. Hooman et al. reported that according to ROC curves there is no difference between these two ratios in detecting either abnormal proteinuria or nephrotic proteinuria⁷.

High urine osmolality brought on by glycosuria could underestimate protein-osmolality ratio. Therefore ideally protein-osmolality ratio in a spot urine sample can be used to assess proteinuria only in patients without glycosuria. In the current study patients with glycosuria were excluded. In children with chronic kidney disease glycosuria is not common. In children, glycosuria should be excluded prior to assessment of protein-osmolality ratio in a random urine sample, which is a limitation of the use of protein-osmolality ratio in assessing proteinuria.

However, in a study done on adult patients with glycosuria, albumin-osmolality ratio in a random urine sample was closely correlated with 24-hour microalbuminuria and was not appreciably affected by glycosuria¹³. This highlights the need for further studies to assess the effect of glycosuria in the assessment of urinary osmolality in children.

In conclusion, both the protein-osmolality ratio and the protein-creatinine ratio in a spot urine sample are clinically valuable predictors of 24-hour urinary protein excretion in normal children as well as in children with proteinuria.

Both the protein-creatinine ratio and the protein-osmolality ratio in a spot urine sample can be used to determine nephrotic proteinuria. However, urine protein-creatinine ratio was more sensitive than urine protein-osmolality ratio in detecting patients with mild proteinuria. Therefore protein-osmolality ratio in a spot urine sample could be used in determining the degree of proteinuria. In particular it could be used in the follow up of patients with proteinuria.

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