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USE OF SINGLE VOIDED URINE SAMPLES TO ESTIMATE QUANTITATIVE PROTEINURIA IN CHILDREN*

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Quantitation of protein excretion in urine is used for diagnostic and prognostic purposes and also to assess the effects of therapy in children. The method in common use is to measure urinary protein in a 24-hour urine sample, which may be time consuming and is often inaccurate. The aim of this study was to determine if the urine protein/creatinine ratio in a single-void urine sample had a high correlation with the quantity of protein in a 24-hour urine specimen. We found that there was an excellent correlation between the protein content of a 24-hour urine excretion and the protein/creatinine ratios in single morning urine samples of 50 patients. We also discovered that a protein/creatinine ratio greater than 4.9 could signify "nephrotic-range" proteinuria, while a ratio less than 2.5 indicated nephritic syndrome or other renal diseases. We concluded that the determination of urinary protein/creatinine concentration ratios in a single morning urine sample under most clinical circumstances, especially in nephrotic syndrome, could replace the measurement of protein excretion in 24-hour urine specimens. *Key words: protein/creatinine ratio, 24-hour urine sample, single voided urine sample, nephrotic-range proteinuria.*

Quantitation of proteinuria in children has diagnostic and prognostic importance, and is used to assess the effects of therapy. The quantity of protein excreted in the urine varies during the day. Proteinuria is related more to changes in the glomerular filtration rate and tubular reabsorption than to the plasma concentration of albumin, volume of urine voided daily and volume of extracellular fluid. In children and adolescents there are several additional factors that may also change the degree of proteinura, namely stress, exercise, temperature and the upright postural position[1,2].

The most common method for quantitating urinary protein excretion makes use of a 24-hour urine sample collection. However, since the amount of protein can fluctuate from day to day, a 24-hour urine sample collection must be carried out on three different days to obtain an accurate measurement.

In practice, it may not only be difficult to obtain 24-hour urine collections from children but also from adults, for this method can be rather time consuming, cumbersome and, on occasion, because of forgetfulness, unreliable.

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In recent years, there have been several studies conducted with the aim of excluding the urine volume factor in the quantitation of proteinuria. Some authors have reported that the protein/creatinine ratio in a single voided sample of urine is a successful method used to assess the degree of proteinuria. They found an excellent correlation between 24-hour urine protein excretion and the protein/creatinine ratio in a single random urine sample[2]. However, the methods used and their clinical assessment have not as yet been completely identified.

The aim of our study was to determine if a correlation exists between protein concentration in a 24-hour urine sample and the protein/creatinine ratio in a single urine sample and the role that the protein/creatinine ratio plays in clinical management.

Material and Methods

A total of 50 subjects comprising outpatients and hospitalized patients who were being followed in the Nephrology Unit of the Department of Pediatrics at the Ege University Faculty of Medicine were enrolled in this study. There were thirty boys and twenty girls whose ages ranged between one to twelve years (mean 4.5 ± 0.6 yrs.). Twenty-six patients had minimal change nephrotic disease (MCND), 16 had either membranoproliferative glomerulonephritis (MPGN), membranous glomerulopathy (MG) or focal glomerulosclerosis (FGS), and eight had acute nephritic syndrome (ANS). Studies were carried out on the patients during the period of active disease or during the remission period following treatment. Sixteen patients with MCND and seven patients in the MPGN, MG, FGS group were in the active phase of the disease, while the others were in remission.

All patients were examined. Their respective medical histories, along with their physical and routine laboratory findings were scrutinized. Each patient was asked to submit a morning urine sample in one container, while in the other container urine specimens voided over a 24-hour period were also to be submitted. Protein and creatinine concentrations were measured in the single voided and 24-hour urine samples. Urine protein was measured using the Prudy method[3]. The creatinine excretion rate was determined by the Jaffe reaction (picric acid)[4]. The measurements were all expressed in milligrams per deciliter (mg/dl). A protein concentration exceeding 0.05 g/kg in the 24-hour collected urine sample was considered to be representative of "nephrotic range" proteinuria, while values less than 0.05 g/kg were termed "abnormal proteinuria".

The Student's *t* test was used for statistical analysis.

Results

The protein concentrations in the 24-hour urine samples exceeded 0.05 g/kg (mean 0.068 g/kg) in twenty-three patients. The mean protein/creatinine ratio in

the single voided urine samples was 8.47. The remaining 27 patients had protein concentrations in 24-hour urine excretion measuring less than 0.05 g/kg (mean 0.017 g/kg). The mean protein/creatinine ratio was 1.66 (Table I).

Table I: Mean 24-Hour Protein Excretion and Protein/Creatinine Ratios in Morning Urine Specimens of Both Groups

Patients	N	Mean 24-h Protein Excretion (g/kg)	Mean protein/creatinine Ratio (single urine)
NEPHROTIC SYNDROME (proteinuria > 0.05 g/kg/day)	23	0.068 ± 0.018	8.47 ± 2.40 (> 4.9)
ABNORMAL PROTEINURIA (proteinuria < 0.05 g/kg/day)	27	0.017 ± 0.009	1.66 ± 0.31 (< 2.5)

When we compared the amount of protein in 24-hour urine excretion with the protein/creatinine ratio in single morning urine samples of all 50 patients, it was found that they were well correlated ($p < 0.01$) (Table II). In addition, the protein/creatinine ratio in the single voided urine specimens of the 23 patients with nephrotic-range proteinuria and the remaining 27 subjects were compared,

Table II: Analysis of 24-Hour Protein Excretion and Protein/Creatinine Ratios in Morning Urine Samples of Patients

	N	Mean
Proteinuria in 24-h collected urine (g/L)	50	1.79 ± 1.57
Protein/creatinine ratio (single urine sample)	50	7.91 ± 6.90 $p < 0.01$

and were found to be significantly different ($p < 0.01$). The protein/creatinine ratios in the single-void urine samples of each group of patients were analyzed, and we found that this ratio was more than 4.9 in all patients who were diagnosed as having nephrotic syndrome according to clinical and laboratory findings. On the other hand, the ratio of the patients who did not have nephrotic proteinuria was less than 2.5 (Fig. 1).

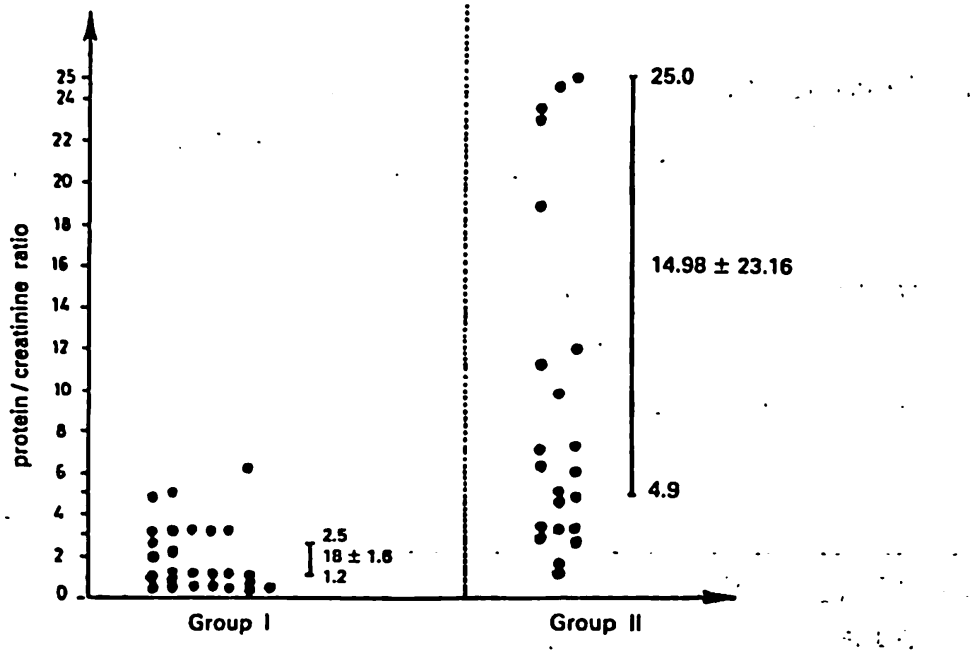

Fig. 1: Analysis of protein/creatinine ratios in morning urine specimens of patients with nephrotic syndrome (Group II) and the other group with abnormal proteinuria (Group I).

Discussion

In pediatric nephrology, measurements of the protein content of a timed 24-hour urine collection is the definitive method of establishing the presence of nephrotic syndrome, whatever the morphologic structure. It is thought that this method is more reliable. It has been reported that urine protein concentration is usually higher in daytime samples than in early morning urine samples in patients with proteinuria[2].

It has been proposed that the daily protein excreted in the urine must be measured per kilogram. Reports in the literature indicate that different amounts of protein excretion in the urine such as 0.05-0.10 and 0.20 g/kg/day, cause the nephrotic syndrome[5]. The expected nephrotic range proteinuria changes according to the proposed degree of proteinuria, however, most authors have reported that minor degree nephrotic-range proteinuria is a value of 0.05 g/kg/day[5]. In our study, we have also used this minor degree measurement to indicate nephrotic proteinuria.

Of 50 patients, 23 had protein concentrations in 24-hour urine excretion in excess of 0.05 g/kg (mean 0.068 g/kg) (Table I). Clinical and laboratory findings of this group of patients evidenced the nephrotic syndrome pattern. The arithmetical average was not very high because the ages and weights of the patients varied.

Twenty-seven patients who had abnormal proteinuria, had protein concentrations in 24-hour excretion with a mean value of 0.017 g/kg, which was a great deal less than the figure associated with minor degree proteinuria. All these results support the opinion that in 24-hour urine specimens, minor degree nephrotic-range proteinuria occurs when the value is 0.05 g/kg.

In the management of nephrotic syndrome, the degree of proteinuria must be assessed along with other clinical findings. Nephrotic syndrome may persist with all its biological changes even though the edema may have disappeared in the patients. Therefore, the degree of proteinuria is a very significant factor when evaluating these patients. A single voided urine sample that would give reliable information regarding the degree of proteinuria would be of great help to the pediatrician.

Ginsburg[6] discovered that the 24-hour protein excretion rate could be estimated from the observed protein/creatinine ratio by multiplying this ratio by the expected creatinine excretion rate which is calculated by using the formulas derived by Cockroft and Gault. He also reported that there was an excellent correlation between it and the 24-hour protein excretion rate. Many other authors have reported that their results correlated very well with the protein/creatinine ratio in single voided urine samples. This method is now used in their daily practice.

In studies conducted on renal disease by Niinomi[7] in 11 patients and by Watana[8] in 41 patients, values for 24-hour urine protein excretion and the protein/creatinine ratio in a random urine sample demonstrated a high correlation. This method is more accurate in determining proteinuria especially in infants and small children. White et al[9] also expressed their results in terms of protein/creatinine ratios of early morning urine samples, and found an extremely good linear correlation with the protein excretion rate in 24-hour urine excretion. Shaw[10] has reported that the protein/creatinine ratio of random urine samples should be used to supplement dipsticks in screening for proteinuria in cases where misclassification could be serious. Wilkin[11] has measured the 24-hour urinary excretion of albumin and the protein/creatinine ratio in early morning and random samples in 14 diabetic subjects and found a significant correlation for early morning samples, but no correlation with the random samples of outpatients. Most investigators are of the opinion that the results of early morning urine samples are more reliable[6,7]. It has been reported that the protein/creatinine ratio of a single urine specimen represented a highly accurate method of assessing renal function in pregnant and preeclamptic women, and was more practical than 24-hour collection[12].

In our study, we also estimated the protein/creatinine ratio in single-void morning urine samples of all 50 subjects and found that there was a high correlation with the proteinuria in 24-hour urine excretion ($p < 0.01$) (Table II). The protein/creatinine ratios in the single voided urine specimens of the patients with nephrotic-range proteinuria were compared with the other group of patients and it was noted that they were significantly different ($p < 0.01$). According to these results, which comply with reports in the literature, it is clear that the protein/creatinine ratio in single morning urine samples represents an accurate and reliable method of assessing proteinuria.

In order to make use of protein/creatinine ratios in single urine specimens in daily practice, we especially have to know the minimum value of this ratio for nephrotic-range proteinuria. Ginsberg[6] reported that when this ratio was more than 3.5, it signified nephrotic-range proteinuria. In Watana's[8] study, the minimum value of this ratio for nephrotic-range proteinuria was two. In our study, the arithmetical mean of the protein/creatinine ratio was 8.47 in the subjects with nephrotic syndrome and 1.66 in the other group (Table I). The protein/creatinine ratio of both groups of patients were examined, and we observed that nephrotic syndrome was present when this ratio was $\geqq$ 4.9, while this ratio was less than 2.5 in the abnormal proteinuria group. We concluded that the subjects with ratios between 2.5 and 4.9 had to be carefully examined and followed up (Fig. I).

We suggest that the determination of urinary protein/creatinine concentration ratios in single morning urine samples under most clinical circumstances, especially in nephrotic syndrome, can replace the measurement of protein excretion in 24-hour urine samples. But, it would be more reliable if urinary protein/creatinine concentration ratios are examined in several random single voided urine samples during normal daytime activities in the same subject and for a few days.

REFERENCES

1. Norman ME. An office approach to hematuria and proteinuria. *Pediatr Clin North Am* 34: 545, 1987.
2. Risdon P, Shaw AB. Which urine sample for detection of proteinuria ? *Br J Urol* 63: 209, 1989.
3. Kessler G. An automated system of analysis. In Frankel S, Reitman S (eds). *Gradwohl's Clinical Laboratory Methods and Diagnosis* (6th ed) Vol. 1. St. Louis: C.V. Mosby 1963, pp. 301-313.
4. Frankel S. Chemical examination. In Frankel S, Reitman S (eds). *Gradwohl's Clinical Laboratory Methods and Diagnosis* (6th ed) Vol. 2. St. Louis: C.V. Mosby, 1963, pp. 1808-1837.
5. McEnery PT, Strife CF. Nephrotic syndrome in childhood. Management and treatment in patients with minimal change disease, mesangial proliferation or focal glomerulosclerosis. *Pediatr Clin North Am* 29: 875, 1982.
6. Ginsberg JM, Chang BS, Matarese RA, Garella S. Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med* 309: 1543, 1983.
7. Niinomi Y, Kawaguchi S, Nonoda T, et al. Quantitation of proteinuria using random urine sample. *Pediatr Nephrol* 1: C51, 1987.
8. Watana D, Laohakunakorn P, Bejraputra O, et al. Use of single voided urine samples to estimate quantitative proteinuria in children. *Pediatr Nephrol* 1: C51, 1987.
9. White RH, Taylor CM, Green A. Measurement of proteinuria [letter]. *Nephron* 55: 437, 1990.
10. Shaw AB, Risdon P, Lewis-Jackson JD. Protein creatinine index and Albustix in assessment of proteinuria. *Br Med J [Clin Res]* 287: 929, 1983.
11. Wilkin T, Mohamed A, Gatling W, Rowe D. Protein creatinine index and Albustix in assessment of proteinuria [letter]. *Br Med J [Clin Res]* 287: 1883, 1983.
12. Chesley LC. Quantitation of proteinuria in pregnancy by the use of single voided urine samples [letter]. *Obstet Gynecol* 71: 142, 1988.