Original Contribution

An Alternative Diagnostic Test rather than 24 hours Urinary Protein to Detect Massive Proteinuria in Nephrotic Syndrome in Mymensingh Medical College Hospital, Bangladesh

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This cross-sectional study was conducted in Department of Paediatrics, Mymensingh Medical College Hospital (MMCH), Bangladesh from February 2016 to December 2016 to detect massive proteinuria by spot urinary protein creatinine ratio as an alternative diagnostic test to 24 hrs urinary total protein in nephrotic syndrome. Fifty one (51) children aged 2 to 12 years admitted with 1st episode of nephrotic syndrome in the pediatric department of MMCH were included in this by purposive sampling technique. All the patients were asked to give a 24 hours urine sample. After this collection the next spot urine samples were collected for protein and creatinine estimation. Among 51 patients 33 were male and 18 were female. The mean age was 5.5+2.3 years. The entire patient had normal renal function. The mean 24 hours urinary protein level was 3.8±1.7 gm/m^2/24 hours, the mean spot urinary protein-creatinine ratio was 5.4±2.5. Mean serum albumin was 1.8±0.6 gm/dl and the mean serum cholesterol was 357.6±74.7 mg/dl. The spot urinary protein creatinine ratio was increased with the increase in the amount of 24 hours urinary total protein and a strong positive Pearson correlation (r=0.805) was found. In all the cases of nephrotic syndrome spot urinary protein creatinine ratio were found more than 2. Based on this study, it can be concluded that the determination of the spot urinary protein-creatinine ratio can replace the 24 hours urine collection in the quantitation of proteinuria in nephrotic syndrome.

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Key words: Nephrotic syndrome, Spot urinary protein creatinine ratio, 24 hours Urinary total protein

Introduction

Nephrotic syndrome (NS) is a fairly common renal disorder in pediatric population[1]. The annual incidence is 2-3 cases per 100,000 children per year in Western countries[2]. Estimated annual incidence of nephrotic syndrome in Bangladesh is 2-7 per 100,000 children[3]. Nephrotic syndrome is characterized by massive proteinuria and the triad of clinical findings associated with large urinary losses of protein: hypoalbuminemia, edema and hyperlipidemia. Nephrotic range proteinuria is defined as protein excretion of >40 mg/m^2/hour that is >1 gm/m^2/24 hours or a first morning protein creatinine ratio of >2 [2]. The underlying abnormality in Nephrotic syndrome is an increased permeability of the glomerular capillary wall, which leads to massive proteinuria and hypoalbuminemia and oedema[2]. Proteinuria is one of major indicator of progression of renal diseas[1]. Disease activity in nephrotic syndrome is determined by amount of protein excretion. So, assessment of urinary protein excretion is not only diagnostic but also has prognostic value in monitoring of these patients[4].

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As the protein excretion in urine varies with stress, exercise, hydration status, posture and diurnally, hence the gold standard test is quantitative estimation of protein done on urine collected over 24 hours. But urine protein estimation by 24 hours collection is a cumbersome task with many errors including incomplete collections due to missed samples, bacterial growth, incorrect timings and incomplete bladder emptying[5]. Furthermore in our country poverty, inadequate healthcare facility and lack of adequate knowledge among parents are great problem in collecting urine samples in child. It also requires hospital admission and causes inconvenience, especially for repeated follow up[5]. All these problems suggest and need for a more convenient, inexpensive and less time consuming method of urinary protein estimation. Measurement of protein-creatinine ratio in a random urine sample has already being documented in various studies by different authors[1,4,5,6,7,8]. They have recommended its value as a substitute for estimation of 24 hours urinary total protein excretion. This approach is based on the fact that in presence of stable glomerular filtration rate, urinary creatinine-protein excretion has been reported to be reasonably constant in a given individual throughout the day[4]. Thus the results of a measurement of spot protein creatinine ratio in a single voided urine sample can provide information that for clinical purpose is a satisfactory substitute for the determination of protein excretion by 24 hours urine collection[1]. This study was done to detect urinary protein excretion with the use of 24 hours urinary total protein and spot urinary protein creatinine ratio and to correlate this two methods of measurement.

Methods

This cross-sectional study was carried out in the Department of Paediatrics, Mymensingh Medical College Hospital (MMCH) from February 2016 to December 2016. Study population was as the patients of 1st episode of Nephrotic syndrome who has been attended in Pediatric Department of MMCH. Sample was collected with purposive sampling technique. Sample size was calculated by using formula, $n=z^2pq/d^2$. According to formula required sample size 61, but due to time constrain and unavaibility of child with 1st attack of nephrotic syndrome 60 patients were assessed for eligibility to study. Among them 9 patients were excluded due to haematuria and low urine output. Subsequently total 51 patients were studied. Inclusion criteria were patients with 1st episode of NS, age 2-12 years and renal function within normal limit and exclusion criteria was children with secondary NS. Patient's guardians were approached about the study method and those who were willingly wanted to participate were included in the study and written informed consent was obtained. Careful detailed history was taken from guardian and thorough clinical examination was done in all cases. All the patients were asked to give a 24 hours urine sample with collection starting at 8:00 AM on the first day and completing by 8:00 AM the following day. After this collection the next spot urine samples (about 3 ml) were collected for protein (mg/dl) and creatinine (mg/dl) estimation. Urinary protein concentration was determined by using pyrogallol red in colorimetric method. With all aseptic precaution 5 ml blood were taken from all patients and sent to reference laboratory for Serum albumin, Serum cholesterol, Serum creatinine and for HB%, TC, DC, ESR. Serum and urine creatinine concentration was determined by using modified Jaffe method.

This study was approved on 02.02.2016 by the Research Ethical Committee of Mymensingh Medical College, Bangladesh. Data were processed and analyzed by using SPSS soft ware program version 18.0. Pearson's correlation co-efficient was used to investigate the relationship between 24hour urinary total protein and spot urinary protein-creatinine ratio. A p value of less than 0.05 was considered statistically significant.

Results

A total of 51 children admitted in Department of Paediatrics, Mymensingh Medical College Hospital (MMCH) with 1st episode of nephrotic syndrome were included in this study. The age of the patients were ranged from 2 to 12 years with a mean age of 5.5±2.3 years (Table I).

There was male predominance and male female ratio was 1.8:1 (Table II). Most of patients (67.0%) came from rural area.

Puffy face was in all patients, ascites was in 48(94.1%), swelling of genitalia was in 14(27.5%), skin infection in 8(15.7%), abdominal pain in 12(23.5%) and burning micturition was in 8(15.7%) patients (Tab III).

Bed side urine albumin '+++' was found in a total of 35(69.0%) patients and '++++'was found in 16(31.0%) shown in Figure 1.

The mean WBC count was 8987.3±2796.4/cmm, mean Hb was 12.2±1.0 gm/dl, mean ESR was 90.5±21.5 mm in 1st hour, mean serum albumin was 1.8±0.6 gm/dl, mean serum cholesterol was 357.6±74.7 mg/dl, mean serum creatinine was 0.7±0.1 mg/dl, mean 24 hours urinary total protein was 3.8±1.7 gm/m^2/24 hours and mean spot urinary protein creatinine ratio was 5.4±2.5 (Table IV).

Spot urinary protein creatinine ratio was between 2.0-4.0 in 19(37.3%) patients, 4.0-6.0 in 12(23.5%) patients, 6.0-8.0 in 11(21.6%) patients, 8.0-10.0 in 7(13.7%) patients and 10.0-12.0 in 2(3.9%) patients (Table V).

Twenty four hours urinary total protein (UTP) was between 1.0-3.0 gm/m^2/day in 20(39.2%) patients, 3.0-5.0 gm/m^2/day in 17(33.3%) patients, 5.0-7.0 gm/m^2/day in 13(25.5%) patients and the rest 1(2.0%) had >7.0 gm/m^2/day (Table VI).

Among 20 patients with 24 hours urinary total protein between 1.0-3.0 gm/m^2/day, 17(85.0%) had spot urine protein creatinine ratio in between 2.0-4.0. Among 17 patients with 24 hours urinary total protein between 3.0-5.0 gm/m^2/day, 12(70.6%) had spot urine protein-creatinine ratio in between 4.0-8.0. Among 13 patients with 24 hours urinary total protein between 5.0-7.0 gm/m^2/day, 8(61.6%) had spot urine protein creatinine ratio in between 6.0-10.0 (Table VII).

Scatter diagram showed a positive linear correlation between 24 hours urinary total protein with spot urinary protein-creatinine ratio (Figure 2).

Pearson's correlation coefficient test showed strong positive, significant correlation between 24 hours urinary total protein with spot urinary protein-creatinine ratio (r=0.865, p=<0.005).

Table I: Distribution of study patients according to age

Age (2-12 years)	Number of patients (n)	Percentage (%)
2-7	38	74.5
7-12	13	25.5
Mean±SD (years)	5.5±2.3	

Table II: Distribution of study patients according to sex

Sex	Number of patients (n)	Percentage (%)
Male	33	64.7
Female	18	35.3

Table III: Clinical characteristics of study patients

Clinical features	Number of patients (n)	Percentage (%)
Puffy face	51	100
Scanty micturition	51	100
Ascites	48	94.1
Swelling of genitalia	14	27.5
Skin infection	08	15.7
Abdominal pain	12	23.5
Burning micturition	08	15.7

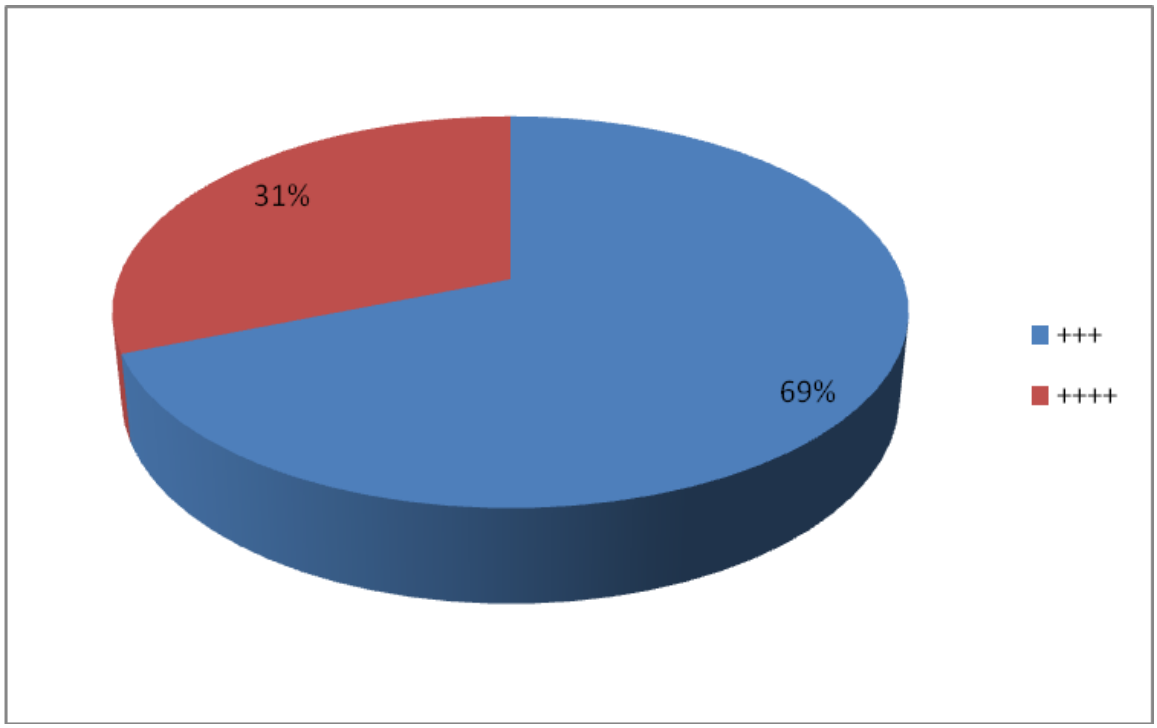

Figure 1: Distribution of study patients by bed side urine albumin examination (n=51)

Table IV: Laboratory parameters of the studied patients

Parameters	Mean±SD
Total WBC count (cells/cmm)	8987.3±2796.4
Hb (gm/dl)	12.2±1.0
ESR (mm in the 1st hour)	90.5±21.5
Serum albumin (gm/dl)	1.8±0.6
Serum cholesterol (mg/dl)	357.6±74.7
Serum creatinine (mg/dl)	0.7±0.1
24hrs urinary total protein (gm/m^2/24 hours)	3.8±1.7
Spot urinary protein creatinine ratio (mg/mg)	5.4±2.5

Table V: Distribution of the study patients by spot urinary protein creatinine ratio (n=51)

Spot urinary protein creatinine ratio (mg/mg)	Frequency (n)	Percentage (%)
2.0-4.0	19	37.3
4.0-6.0	12	23.5
6.0-8.0	11	21.6
8.0-10.0	07	13.7
10.0-12.0	02	03.9

Table VI: Distribution of the study patients by 24 hours urinary total protein (UTP) (n=51)

24 hours urinary total protein (gm/m^2/day)	Frequency (n)	Percent (%)
1.0-3.0	20	39.2
3.0-5.0	17	33.3
5.0-7.0	13	25.5
7.0-8.0	01	02.0

Table VII: Distribution of study patients by 24 hours urinary total protein in relation to spot urinary protein creatinine ratio

24 hours UTP (gm/m^2/day)	Spot urinary protein creatinine ratio (mg/mg)				
	2.0-4.0	4.0-6.0	6.0-8.0	8.0-10.0	10.0-12.0
1.0-3.0	17 (85.0)	3 (15.0)	00 (00.0)	00 (00.0)	00 (00.0)
3.0-5.0	02 (11.8)	5 (29.4)	07 (41.2)	03 (17.6)	00 (00.0)
5.0-7.0	00 (00.0)	4 (30.8)	04 (30.8)	04 (30.8)	01 (07.6)
7.0-8.0	00 (00.0)	0 (00.0)	00 (00.0)	00 (00.0)	1 (100.0)

Data of categorical variable were presented as frequency (percentage)

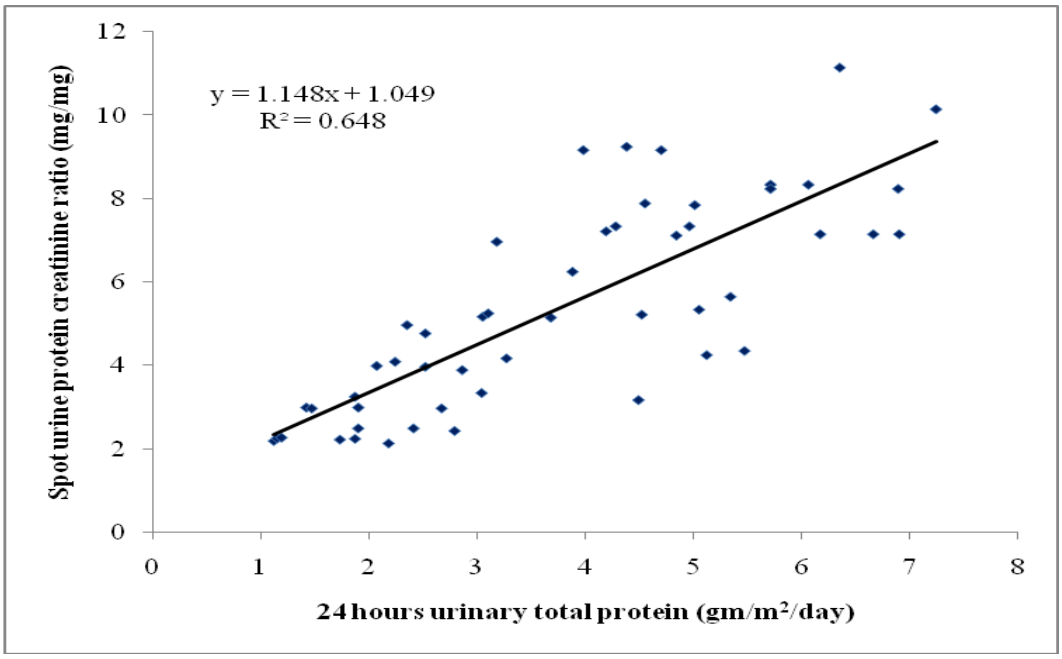

Figure 2: Correlation between 24 hours urinary total protein and spot urine protein creatinine ratio

Discussion

In this study, fifty one Nephrotic Syndrome patients of 2-12 years with first attack were included and the mean age was 5.5±2.3 years. Nash et al.[9] stated that two third of cases of nephrotic syndrome (NS) occurred at the age less than five yrs while Pais P and Avner ED[10] stated that Idopathic NS occurred at the age of 2-6 years. In this study 74.5% patients were below 7 years which was consistent with the observation of Nash et al.[9] and Pais P and Avner ED[10].In this study, most of patients were male 33(64.7%) and female subjects were 18(35.3%). Male female ratio was 1.8:1. Pais P and Avner ED[10] stated that Nephrotic

syndrome is more common in boys then in girls and they observed the male: female ratio as 2:1 which was similar to this study. In this study the mean serum albumin level was 1.8±0.6 gm/dl. Similar measurement was found by Hiraoka et al.[11] and Navale et al.[12] found serum albumin level between 1.5 to 2.4 gm/dl and mean 2.07±0.27 gm/dl which was also close to our observations. Serum cholesterol level was found to be high in all cases Mean serum cholesterol was 357.6±74.7 mg/dl. Similar observations were made by Navale et al.[12]. In this study, the mean 24 hours urinary total protein in NS cases were 3.8±1.7 gm/m^2/24 hours and spot urinary protein creatinine ratio were 5.4±2.5. The range of spot urine protein creatinine ration was found 2.33-5.2 mg/mg by Navale et al.[12] with a mean of 3.28 mg/mg. which was lower than our observation. But mean spot urine protein creatinine ration was found 5.55±2.0 by Iyer et al.[13] which was consistent with our observation. When the relationship between 24 hours urinary total protein with spot urinary protein creatinine ratio is graphically represented the relationship line is reasonably straight and the Pearson's correlation coefficient (r)=0.805, p value <0.05 which is statistically significant. Similar observations were found by Navale et al.[12] (r=0.886), Wahbeh et al.[14] (r=0.83), Lane et al.[15] (r=0.92), Morales et al.[16] (r=0.91) and Gye et al.[17] (r=0.87).

Conclusion

In was concluded that there is a significant strong positive correlation between 24 hours urinary protein excretion and the spot urine protein-creatinine ratio. As quantitation of urinary protein excretion by 24 hours urine collection is time consuming and cumbersome, so instead of this method to diagnose the nephrotic syndrome clinician can use spot urinary protein creatinine ratio as a diagnostic test.

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