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Original Article

Selectivity Index and Urinary Retinol Binding Protein as Predictors of Steroid Responsiveness in Pediatric Nephrotic Syndrome

Ramzi El-Baroudy, Fatina Fadel, Amal Hagras, Somaya El-Gohary*, Salma Mekhemer*, Nohair Abdel-Wahed and Nahla El-Marakby

Departments of Pediatrics and Clinical Pathology, Cairo University, Egypt*

ABSTRACT

Background: Response to treatment of NS in children can be determined by pathologic diagnosis. Several other non-invasive tests have been tried to predict steroid responsiveness.

Objectives: To evaluate the role of noninvasive estimations of selectivity index (SI) and urinary RBP in predicting the response to steroids and the possible pathologic entities, whether minimal change nephrotic syndrome or glomerulonephritis with other histologic patterns and to evaluate the accuracy of calculating 24 hours proteinuria using the spot urinary protein/creatinine ratio, as well as its value in fulfilling the previous purpose.

Methods: In this work leakage of different proteins from the glomerular basement membrane denoted by the selectivity index (SI) using immunoglobulin G and transferrin ratio, and in association with tubular dysfunction denoted by urinary retinol binding protein (RBP), were tested in 40 children with nephrotic syndrome (NS) as well as 10 healthy normal children. The patients were 28 steroid responsive (group I) and 12 steroid resistant (group II) NS.

Results: SI showed 100% specificity and 43% sensitivity in detecting steroid responsiveness where 43% of group I had SI < 0.18 (range 0.05 – 0.43), and all patients of group II had SI ≥ 0.18 (range 0.18 – 0.93). Meanwhile urinary RBP was ≤ 1.20 mg/L in group I and ≥ 2.54 mg/L in group II, with no overlap indicating more predictivity.

Conclusions: These studied parameters may be used to expect response to treatment and indicate early biopsy for pathologic diagnosis and combined immunosuppressive regimens in those suspected to be steroid resistant.

INTRODUCTION

Ninety percent of children with nephrosis have some form of idiopathic nephrotic syndrome, which may have one of three morphologic patterns. Minimal change nephrotic syndrome (MCNS) is found in approximately 85%, diffuse mesangioproliferative glomerulonephritis (MPGN) in 5% and focal segmental glomerulosclerosis (FSGS) in 10%. In the remaining 10% of children with nephrosis, the nephrotic syndrome is largely mediated by some form of secondary glomerulonephritis[1].

Most of the nephrotic patients go into complete remission after steroid therapy (steroid responsive). However a group of patients fail to develop complete remission after a course of daily full dose steroids for 8 weeks (steroid resistant)[2].

It is well known that the amount of protein excreted in urine has a diagnostic and prognostic value for cases with nephrotic syndrome. Accurate quantitative measurement of proteinuria depending upon 24 hours urine collection, particularly in infants and children, is unreliable, incon-

venient and time consuming[3]. Meanwhile urinary protein quantitation by dipstick method is frequently used but is subject to errors and lacks reliability and good standardization[4]. On the other hand calculation of the protein/creatinine ratio in a random urine sample has been used as a rapid and reliable method of assessment[3, 5].

Although renal biopsy is the definitive investigation in kidney disorders, it still is attended by a small percentage of risk to the patient. Furthermore a renal biopsy might miss a foal lesion. Accordingly, non-invasive tests would be helpful to predict the response to steroids and the underlying renal pathology[6, 7].

The urinary markers of steroid responsiveness might be a reflection of glomerular inflammatory reactions which result in leakage of variable amounts of proteins of different molecular weight, measured by urinary protein electrophoresis and different selectivity indices as well as urinary protein/creatinine ratio[5, 8, 9].

The urinary markers may also be a result of tubular defects reflected on the reabsorption of abnormally filtered proteins, or those normally filtered having a molecular weight less than 70000 daltons, as retinol binding protein (RBP), or β2 microglobulin. Meanwhile tubular inflammatory reactions may lead to leakage of cellular constituents as the lysosomal enzyme N-acetyl-beta-D glucosaminidase[7, 10, 11].

AIM OF THE WORK

To evaluate the role of noninvasive estimations of selectivity index (SI) and urinary RBP in predicting the response to steroids and the possible pathologic entities, whether minimal change nephrotic syndrome or glomerulonephritis with other histologic patterns and to evaluate the accuracy of calculating 24 hours proteinuria using the spot urinary protein/creatinine ratio, as well as its value in fulfilling the previous purpose.

SUBJECTS AND METHODS

The study was conducted on 40 nephrotic children during activity (as proven by 24 hour proteinuria) attending the pediatric nephrology clinic or admitted at Cairo University Children Hospital. Their ages ranged between 2-12 years. They were 28 males and 12 females. These children were followed-up for their response to steroid therapy and were classified according to their response into:

- Group I: 28 steroid sensitive NS patients
- Group II: 12 steroid resistant NS patients. All patients of this group were subjected to renal biopsy and proved to be 6 FSGS and 6 MPGN cases.
- Group III: 10 age- and sex-matched healthy children, taken as a control group who were asymptomatic of any acute or chronic illness and without any history of renal disease.

All these children were subjected to the following:

- Full history taking and thorough clinical examination.
- Routine laboratory investigations which included:
  - Full urine analysis.
  - 24 hour proteinuria.
  - Total serum proteins and serum albumin.
  - Serum electrolytes (sodium and

potassium).

- Serum cholesterol.
- Serum complement (C_3 and C_4).
- Blood urea nitrogen (BUN) and serum creatinine.
- Specific investigations:

Urinary protein/creatinine ratio: Protein in mg/dL was measured using the turbidometric method utilising sulfosalicylic acid[12], and creatinine in mg/dL was measured using the Jaffe reaction[13] in a separate early morning urine spot sample.

The 24 hours urinary proteinuria was then calculated from the ratio according to the formula: 0.63 x Upr / Ucr[5].

Selectivity index (SI):

$$\frac{\text{Transferrin (serum)} \times \text{IgG (urine)}}{\text{Transferrin (urine)} \times \text{IgG (serum)}}$$

The above parameters were measured using specific radial immunodiffusion plates (RID).

Urinary retinol binding protein: using RID plates.

RESULTS

Table 1: Statistical comparison of the routine laboratory investigations between the steroid sensitive and steroid resistant cases

	Steroid Sensitive No. = 28	Steroid Resistant No. = 12
Serum protein (g %)	4.71 ± 0.05	4.8 ± 0.69
P	NS	
Serum albumin (g %)	1.63 ± 0.49	18 ± 0.6
P	NS	
Complement C_3 (mg %)	95.61 ± 13.31	95.75 ± 23.09
P	NS	
Complement C_4 (mg %)	27.04 ± 6.2	24.5 ± 11.49
P	NS	
Serum cholesterol (mg %)	408.29 ± 82.18	379.0 ± 92.77
P	NS	
BUN (mg %)	25.32 ± 5.18	24.0 ± 4.79
P	NS	
Serum creatinine (mg %)	0.63 ± 0.14	0.59 ± 0.14
P	NS	

Table 2: **Statistical comparison of the spot urinary protein/creatinine ratio, measured and calculated 24 hour proteinuria between the steroid sensitive and steroid resistant cases**

	Steroid Sensitive No. = 28	Steroid Resistant No. = 12
Spot urine protein (mg %)	1464.29 ± 1027.12	1591.67 ± 1572.76
P	NS	
Spot urine creatinine (mg %)	163.17 ± 28.77	140.89 ± 32.04
P	< 0.05	
Urine protein/creatinine	9.55 ± 9.11	10.28 ± 6.47
P	NS	
24 hr proteinuria ($g/m^2/day$)	6.04 ± 5.75	6.49 ± 4.09
P	NS	
Calculated proteinuria ($g/m^2/day$)	6.02 ± 5.74	6.48 ± 4.08
P	NS	
P # routine estimation	NS	NS

Table 3: *Statistical comparison of the SI and the urinary RBP between* the steroid sensitive and the steroid resistant cases

		Steroid Sensitive No. = 28	Steroid Resistant No. = 12
Transferrin/IgG S.I.	M	0.21	0.38
	SD	± 0.14	± 0.27
	range	0.05 – 0.43	0.18 – 0.93
	selected critical value	< 0.18	> 0.18
	fulfilled %	43 %	100 %
	P	< 0.01	
Urinary RBP (mg/L)	M	0.85	3.98
	SD	± 0.02	± 0.69
	Values	≤ 1.2	≥ 2.54
	P	< 0.05	

Table 4: Statistical correlation between both SI and urinary RBP versus the studied variables

Studied variable	Correlation value (significant > 0.3)	
	SI	Urinary RBP
BUN	- 0.32	0.13
Serum creatinine	- 0.21	0.12
Serum cholesterol	0.22	0.18
Urine albumin	0.42	0.02
Spot urine protein	0.89	0.14
Spot urine creatinine	0.27	0.37
24 hrs proteinuria ($g/m^2/day$)	0.84	0.03
Urine protein/creatinine	0.84	0.03
SI	-	0.45

Table 5: Frequency of the renal pathologic diagnosis among the studied groups

	Steroid Sensitive No. = 28 Biopsy in 18/28	Steroid Resistant No. = 12 Biopsy in 12/12
Minimal change	18 (100%)	0
Focal glomerulosclerosis	0	6 (50%)
Mesangioproliferative GN	0	6 (50%)

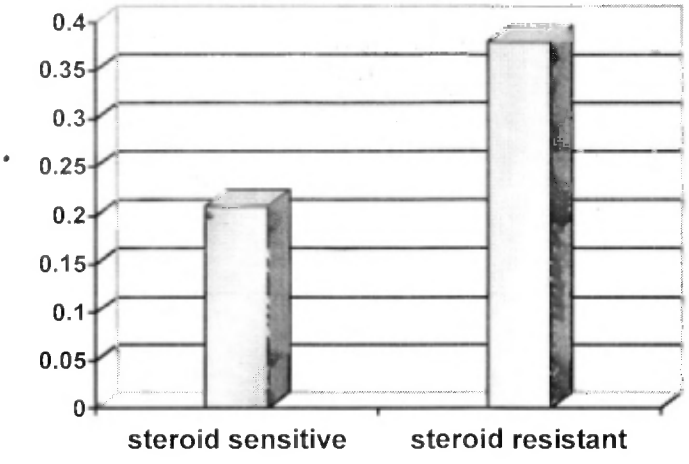

Fig. 1: Comparison of SI in studied groups

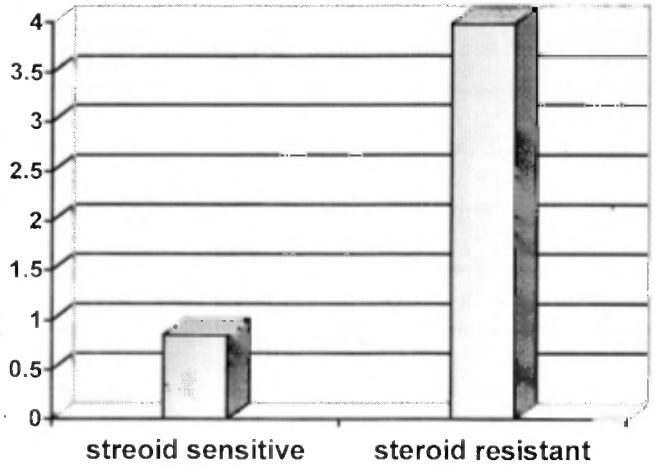

Fig. 2: Comparison of urinary RBP in studied groups

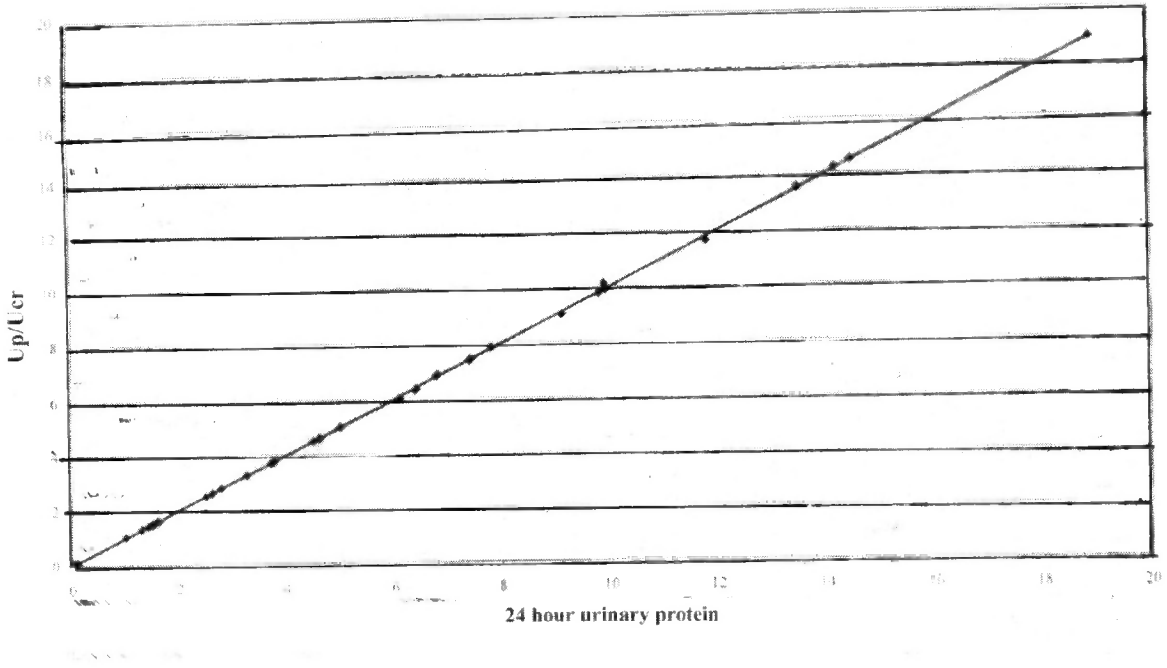

Fig. 3: Relation between 24 hour urinary protein and Up/Ucr

DISCUSSION

The severity of pathologic involvement of the kidney in NS is reflected on the glomerular basement membrane as well as the tubular epithelium. With more severe lesions the glomerular basement membrane becomes more permeable allowing the leakage of proteins of higher molecular weight, which encounter greater resistance to tubular reabsorption. The pattern of urinary proteins was previously assessed by several methods including urinary protein electrophoresis as well as the ratios of proteins of higher molecular weight as IgM, IgG, in comparison to those with relatively smaller molecular weight as albumin and transferrin[14, 15].

Children who develop what would later prove to be steroid resistant NS would be given a trial of steroid treatment on first presentation. There is still some controversy about the duration after which the patient is considered to be steroid resistant. This ranges between 4 and 8 weeks in different protocols[6]. This implies the use of prolonged inefficient courses of steroids, which would affect the morbidity of the patient and the prognosis of the disease, hence the importance of early diagnosis of steroid resistant cases.

Steroid resistant NS on first presentation may be suspected from the age of the patient, presence of hypertension, hematuria and/or hypocomplementemia[16, 17, 18].

Our results showed that the SI in steroid responsive cases (Gp I) ranged between 0.04 – 0.43, while it was 0.18 – 0.93 in steroid resistant cases. SI < 0.18 was

encountered in 13/28 of group I indicating 46.4% sensitivity of this cut off point to detect steroid responsiveness, with 100% specificity. Meanwhile, 15/28 cases of group I had SI > 0.18 indicating 44.4% specificity of this cut off point in suggesting steroid resistance. SI of > 0.45 is indicative of steroid resistance with 100% specificity but only 25% sensitivity, being encountered in only 3/12 cases of group II. It is to be noticed that 50% of the cases of group II had FSGS and 50% had mesangioproliferative glomerulonephritis, while all the biopsied cases of group I were MCNS.

These results are comparable to those of Ramjee et al. in 1997[6], who encountered a SI ranging between 0.02 – 0.38 in 24 steroid sensitive cases, and ranging between 0.189 – 0.937 in 20 steroid resistant FSGS cases. Yet they considered a cut off value of < 0.1 and > 0.1 to indicate steroid sensitivity and resistance respectively.

A recent re-evaluation of the SI considered the value ≤ 0.1 to be highly selective, ≥ 0.11 to be moderately selective and ≥ 0.21 to be non-selective[9].

Meanwhile our results of the urinary low molecular weight RBP showed a significantly higher value in the steroid resistant cases (3.98 ± 0.69 mg/L) than in the steroid sensitive cases (0.85 ± 0.02 mg/L) $p < 0.05$, denoting impairment of tubular functions with more severe forms of glomerulonephritis. Our data clearly denote steroid sensitivity for values ≤ 1.2 mg/L or steroid resistance for values ≥ 2.54 mg/L, with no overlap even beyond double the highest value in the steroid sensitive cases, suggesting 100% sensitivity and specificity.

Sesso et al. in 1992[19] encountered nearly similar findings with more elevation of the urinary RBP in their steroid resistant than in their steroid sensitive cases. Their data showed 100% sensitivity but only 70% specificity, where they had steroid sensitive MCNS cases with elevated urinary RBP who probably had a reversible tubular insult.

Although there was no statistically significant difference between the values of daily proteinuria between the two groups, there was a positive correlation between the SI and the amount of proteinuria ($r = 0.84$). This signifies that the quantity of higher molecular weight proteins is affected by the amount of 24 hours proteinuria regardless of their percentage in the leaking proteins. The positive correlation between the proteinuria and the SI is a reflection of the glomerular permeability. Meanwhile the low molecular weight urinary RBP showed no correlation with the degree of proteinuria, but significantly positively correlated with the SI ($r = 0.45$). This is in concordance with Tomlinson et al.[20] who in 1997 demonstrated that urinary RBP is least affected by the degree of proteinuria. This signifies that both SI and RBP denote the severity of the glomerulonephritis regardless of the amount of proteinuria.

Our results also showed no statistical difference between the calculated daily proteinuria (estimated using the spot urine protein/creatinine ratio) and the measured proteinuria in urine collected over 24 hours. This is in agreement with the findings of Chahar et al. in 1993 and Shield et al. in 1995[3,5]. This finding is of utmost importance in the pediatric population, where the collection of the 24 hours urine is

mostly inconvenient and inaccurate.

The protein/creatinine ratio in our control group was in the range of 0.1 – 0.15, while it was between 1.6 – 30 in the relapsing nephrotic group, and a cut off value ≥ 1.6 would signify nephrotic range proteinuria. While Chahar et al. in 1993[3] and Shield in 1995[5] reported a ratio of more than 3 to signify nephrotic range proteinuria, Mir et al. in 1992[21] found a ratio greater than 4.9 in their nephrotic cases, and considered lesser ratios to indicate nephritis or other renal diseases.

It can be concluded that the analysis of the spot urine sample may fulfill several goals in NS. It can estimate the amount of protein leaking using the protein/creatinine ratio to diagnose the syndrome and follow up the response to treatment. Also analyzing any of the different selectivity indices, as well as measuring the urinary low molecular weight proteins as RBP, can predict the severity of glomerulonephritis and response to steroid treatment as a sole immuno-suppressive agent. Urinary RBP is more conclusive and predictive than SI in this respect.

It is recommended to apply these noninvasive tests to all newly diagnosed cases to avoid unnecessary steroid courses and delay of induction of remission in steroid resistant cases. Delaying proper immunosuppressive protocols would affect the morbidity and response to treatment. Initial pathologic diagnosis may be required as guided by these screening tests.

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