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Original Research Article

Urine protein electrophoresis as a prognostic factor in childhood nephrotic syndrome relapse – An observational study in govt. medical college, Mulankunnathukavu, Thrissur

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ABSTRACT

Background: Childhood nephrotic syndrome (NS) is a chronic illness characterized by relapses and remissions. In many there will be disability, caused by the disease and from the treatment in the form of immunosuppression or end stage renal disease. Progression to renal failure will require anticipation for dialysis and/or renal transplant. A noninvasive biomarker such as urine protein profile that accurately predicts responsiveness to steroid therapy would be valuable in predicting the course of nephrotic syndrome. Our study tries to correlate between the selectivity of proteinuria and the outcome of relapses and thus prognosticate the course of the disease. Few similar studies have been done in India.

Materials and Methods: Our study is a cross-sectional hospital based study, comprised of a population of 43 children with nephrotic syndrome relapse, between 1 and 14 years of age. Blood and urine samples were collected, to look for selective or nonselective proteinuria using the Agarose gel electrophoresis (Age) method. The treatment for relapse was started with steroid and, outcome was then related to the selectivity of proteinuria.

Results: Majority of our patients had non selective proteinuria (NSP) 60.5%, the rest had selective proteinuria (SP) 39.5%.

There was a significant relation between nonselective proteinuria and- 1: Hypertension (p =0.049); 2: Low GFR p<0.001(p =0.0005); 3: Steroid dependent nephrotic syndrome. (p =0.0199).

Non selective proteinuria was more in children, above the age of 6 years (p=0.001).

The nonselective proteinuria group took more time for remission pointing to the lesser degree of steroid sensitivity.

Conclusion: Non selective proteinuria can be used as a biomarker to predict the course in childhood nephrotic syndrome. The higher prevalence of non-selective proteinuria could be due to severe forms of nephrotic syndrome in our study. Selective proteinuria had a better outcome.

1. Background

Nephrotic syndrome is an important chronic disease in children. The characteristic features of nephrotic syndrome are heavy proteinuria (40 mg/m2/hr., in children), hypoalbuminemia (<2.5 g/dL), edema, and hyperlipidemia.[1,2] Childhood nephrotic syndrome is characterized by relapses and remissions which can extend throughout childhood. Incidence in India and in Asia 9-10/100,000.[3]

In majority there is disability which is caused by the disease and from the treatment in the form of

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immunosuppression or end stage renal disease. Progression to renal failure will require anticipation for dialysis and/or renal transplant. For this histopathology findings on renal biopsy have been relied on, but the response to steroid therapy remains the most important feature.[4–6] A noninvasive biomarker such as a urine protein profile that accurately predicts responsiveness to steroid therapy would be valuable in predicting the course of nephrotic syndrome.

Our study aims at detecting proteinuria which may be selective or nonselective, with the help of a noninvasive, cost effective and reliable method the agarose gel electrophoresis. It then correlates the selectivity of proteinuria and the outcome of relapses and thus prognosticates the course of the disease. In diseases like focal and segmental glomerulosclerosis, IgA nephropathy, lupus nephritis and diabetic nephropathy, the degree of interstitial mononuclear cell infiltrates correlate with the rate of renal functional decline.[7] The presence of chronic tubulointerstitial lesions in these diseases are correlated with declining renal function.[8]The findings common to all these diseases is non selective proteinuria. In IgA nephropathy, the non-selective proteinuria was correlated with tubulointerstitial lesions and a higher incidence of renal failure.[9] A good correlation between the short-term steroid response and the selectivity of differential protein clearances has been shown for European adults,[10] European children,[11] and Nigerian children.[12] Studies in Indian children by Chandra, R. K et al, confirmed this correlation. Good response to steroids is rarer in Indian population than in Europe, but more common than those children with nephrotic syndrome in Nigeria.[13] Other pediatric studies have shown predominant FSGS(focal segmental glomerulosclerosis), IgA nephropathy, MCD (minimal change disease),lupus nephritis, and HSP (henoch schonlein purpura) nephritis as being major causes of pediatric renal diseases.[14,15] Since we could not find much studies in Indian population, we selected this study to see, if there is a correlation between the selectivity of proteinuria and the outcome of relapses and thus to prognosticate the course of the disease.

2. Materials and Methods

A detailed clinical history, physical examination, laboratory investigations and events of nephrotic syndrome in 43 steroid responsive patients between the age of 1 and 14 years, were recorded and relapse documented. Those with first episode of nephrotic syndrome and those who were not treated as per standard regimen during the previous episodes, and those with steroid resistant nephrotic syndrome were excluded from this study. The patients were enrolled between August 2009 and July 2010, a one year period, in the paediatric department Govt. Medical College, Mulankunnathukavu, Thrissur, which is a tertiary care teaching hospital in middle Kerala receiving patients generally from the lower socioeconomic strata. Data was collected and recorded on a structured proforma. Blood and urine samples were collected, to look for selective or non-selective proteinuria, using the Agarose gel electrophoresis (AGE) method. Simultaneously blood and urine sample to document the present episode of relapse were also collected. Haemoglobin, total leucocyte, platelet count, ESR, urea, creatinine, total protein, serum albumin, cholesterol, and those not screened previously for HIV, HBsAg. Urine was also collected for routine analysis, culture sensitivity, 24 hr. protein, protein creatinine ratio. USG abdomen/chest, X-ray chest was done if indicated. Urine for albuminuria by sulfosalicilic acid test, which is a qualitative test was done. Then standard treatment for relapse was started with steroid: 2mg/kg per day prednisolone in divided doses till remission, followed by alternate day dosing as a single morning dose of 1.5 mg/kg and tapering over 4 weeks. The patient's outcome was evaluated.

For the purpose of this study, outcome was defined as:

1. Good outcome - remission within 14 days of starting treatment.
2. Poor outcome - remission after 14 days of starting treatment.
3. The outcome was then related to the selectivity of proteinuria
4. Frequent relapsers/Steroid dependent patients were taken as those having a complicated course.

2.1. Procedure for serum electrophoresis

The agarose gel electrophoresis (Age) was done, using a purified neutral fraction of agar called agarose as a medium. Serum is dissolved in warmed agar and applied to a precut well, kept in an electrophoretic chamber for 30 to 90 minutes, the buffer used was barbital with a pH of 8.6. Usually five bands are seen (albumin, α1globulin, α2globulin, βglobulin, γ globulin).

In selective proteinuria (SP), albumin and γ- bands are decreased in conjunction with an increased α2 band.

In non-selective proteinuria (NSP) -Albumin and all the other bands are decreased.

2.2. Procedure for urine electrophoresis

5 ml of urine is placed in a concentrator, the inner surface of which is a membrane of selective permeability backed by absorbent pads. Water is absorbed and proteins left behind and concentrated. The concentrate is kept in electrophoresis chamber with a buffer and run for one hour. Five bands are formed albumin, α1globulin, α2 globulin, β globulin, γ globulin according to the order of their weight.

Inference: Selective proteinuria — prominent albumin bands with no other bands.

Nonselective proteinuria — prominent albumin bands along with any other band.

2.3. Statistical analysis

The data obtained was entered using SPSS version .It was analysed using appropriate statistical technique (Chi-square test).

3. Results

Our study population comprised children of age ranging from 2.5 years to 13 years, mean age being 7.60 years. 69.8% of patients were below the age of 10 years.

Majority of our patients had non selective proteinuria (NSP) 26 (60.5%). Selective proteinuria (SP) was found in 17/43 (39.5%) It was observed that, nonselective proteinuria was more in older children. Chi square corrected (Yates)-0.001

Atypical presentation with hypertension was more in non-selective group. 18.6% of patients had hypertension.9.3% had diastolic hypertension while 9.3% had systolic as well as diastolic hypertension. In the patients with nonselective proteinuria, cholesterol levels were higher than in those with selective proteinuria.

Table 1: Protein electrophoresis & GFR

GFR	Protein Electrophoresis		Total
	SP	NSP	
Normal	11	18	29
Low	6	8	14
Total	17	26	43

Chi square - corrected (Yates) 0.0005

GFR was low in 14 patients, 42% in selective proteinuria group and 58%in non-selective proteinuria.

Table 2: Protein electrophoresis & course

Course	Protein Electrophoresis		Total
	SP	NSP	
Complicated	8	16	24
Uncomplicated	9	10	19
Total	17	26	43

56% children had a complicated course either steroid dependent or frequently relapsing.

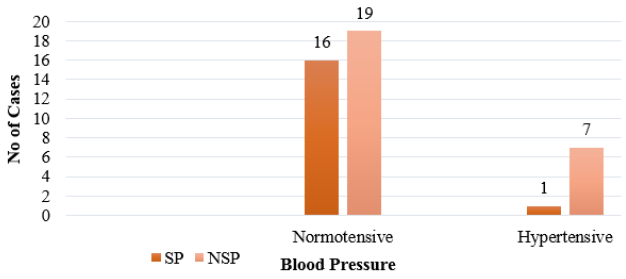

Fig. 1: Blood Pressure & protein electrophoresis.

Hypertension was more in nonselective group 7/8.Only 1 /8 patient in the selective proteinuria group had hypertension.

So atypical presentation with hypertension was more in non-selective group. p=0.049.

This may be because of the possibility of non MCD nephrotic syndrome in the nonselective proteinuria group like FSGS, Membranous nephropathy.

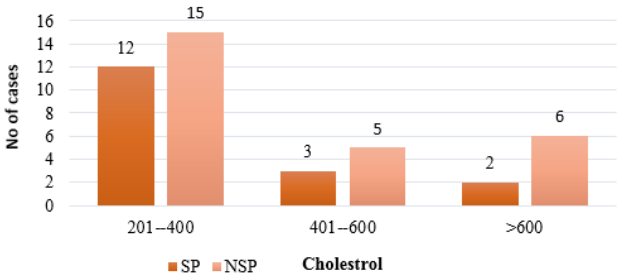

Fig. 2: Serum cholesterol & protein electrophoresis.

Hyperlipidaemia was present in all cases of which, 26(60.5%) were in the nonselective group and 17(39.5%) in the selective proteinuria group. Higher serum cholesterol levels were observed in were in the nonselective group though not statistically significant.

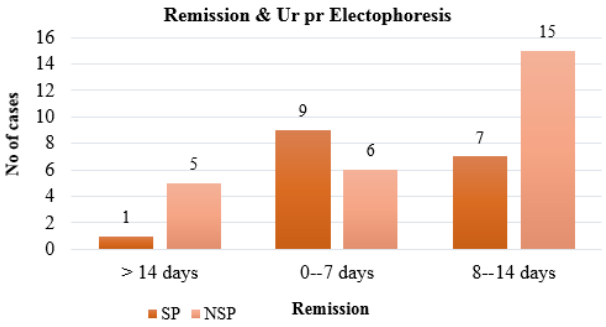

Fig. 3: Remission & protein electrophoresis.

Majority 37/43(86%) of our patients attained remission within 14 days.

15(35%) underwent remission in the first 7 days

22(51%) attained remission between 8th and 14^{th} day.

Those patients who took more time for remission were in the non-selective proteinuria group.

65% (11) of steroid dependent nephrotic syndrome children, had non selective proteinuria and 35% (6) had selective proteinuria.

1. Steroid dependent patients had nonselective proteinuria with albumin, $\alpha 1$, β, γ, $\alpha 2$ bands in urine electrophoresis.

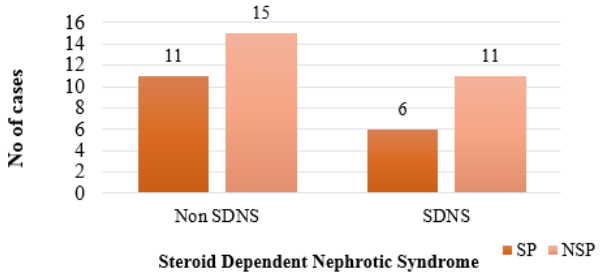

Fig. 4: Steroid dependent nephrotic syndrome (SDNS) & protein electrophoresis. Chi square - corrected (Yates) 0.0199.

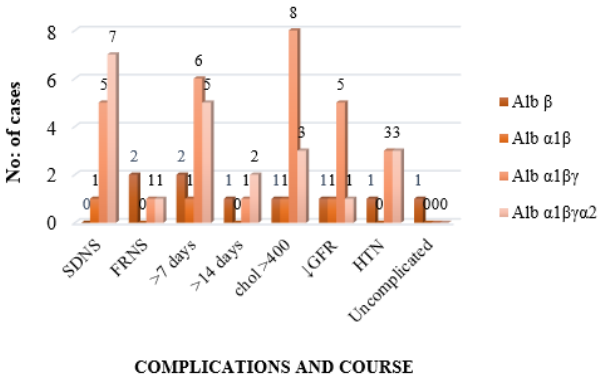

Fig. 5: Complications and Course v/s Urinary Protein Electrophoresis Bands (UPEP)

2. Frequent relapsers showed prominent albumin and β bands.
3. The group with nonselective proteinuria with albumin, $\alpha 1$, β, γ, $\alpha 2$ pattern took more time for remission.
4. Hypercholesterolemia was observed more in those with alb, $\alpha 1$, β, γ bands.
5. Decline in GFR was more common in patients with prominent albumin, $\alpha 1$, β, γ band in urine electrophoresis. Other 3 groups did not differ much.
6. Hypertension was more in those with $\alpha 1$, β, γ and alb, $\alpha 1$, γ, $\alpha 2$ band in urine electrophoresis.
7. Only one patient with only one albumin and β globulin band in urine electrophoresis had an uncomplicated course.

4. Discussion

The study was conducted to evaluate and relate proteinuria with the course of disease in childhood nephrotic syndrome relapse patients. A non-invasive bio marker such as urine protein electrophoretic profile that could predict responsiveness to steroid therapy would be a valuable addition to the diagnostic armamentarium of nephrologists. Renal biopsy has been used to prognosticate long term renal function, but response to steroid therapy is the most important feature in the prognosis of nephrotic syndrome.[4–6] Access to a sensitive and specific non-invasive predictor of steroid responsiveness in nephrotic syndrome would spare a substantial subgroup of patients from receiving high dose, yet ineffective steroid therapy. Difference in the proportion of nephrotic children with poor response and a poorly selective protein excretion varies from one population group to another. This may depend upon the incidence of different causes of the glomerular damage in the different populations.[13] Hypertension is more commonly seen in non-minimal change disease like FSGS and membranous glomerulo nephropathy. An Indian study showed that, Focal segmental glomerulo sclerosis had higher incidence of haematuria (81.63%), hypertension (71.42%) and renal insufficiency (26.53%).[16]

Our study population comprised children of age ranging 2.5 yrs. to 13 yrs.

16.3% were frequent relapses and 39.5% steroid dependant. Studies by Shrivastava RN and Bagga A, showed that those who respond to prednisolone, 40% have frequent relapses and 25 to 35% showed steroid dependence.

18.6 % patients had hypertension. Hypertension is seen more in non-minimal change disease like FSGS and membranous glomerulo nephropathy. An Indian study by AR Reshi, MA Bhat showed that FSGS had higher incidence of hypertension (71.42%) and renal insufficiency (26.53%).[16]

In our study low GFR was seen in 32.6 %. Bohman et al[17] showed a relationship between the degree of foot process effacement and both GFR and filtration fraction suggesting that foot process effacement leads to reduced glomerular filtering area and permeability to water and small solutes.

Majority of our patients had nonselective proteinuria (60.5%), selective proteinuria was 39.5%. Excretion of urinary protein and albumin is probably the best independent predictor of end stage renal disease in non-diabetic proteinuric chronic nephropathies. In selective proteinuria intermediate sized proteins < 100 KD like albumin and transferrin leaks through glomerulus. In nonselective proteinuria a large range of proteins may leak.[18]

Our mean cholesterol level was 415.44 mg/dL. Higher serum cholesterol levels were observed in were in the nonselective group with levels > 600mg/dl. Importance of hyperlipidaemia lies in, contributing to progression of renal damage, aggravating chronic renal failure.[19]

Mean duration of remission after starting standard treatment was 11.9 days. 86% had remission in less than 14 days. A relapse usually takes 10 to 14 days to undergo remission. In this study we took remission in less than 14 days as an indicator of good outcome. Vivarelli et al[20] showed that, in patients who frequently relapsed or

who developed steroid-dependent nephrotic syndrome, the median time to remission was more than 7 days after treatment began. The study concluded that the length of time between steroid treatment onset and remission is an early prognostic indicator for patients with idiopathic nephrotic syndrome relapse.

Non-selective proteinuria was found more in children > 6 yrs. of age. This was statistically significant (p= 0.001). Hypertension was, more in non-selective group. This was also statistically significant (p = 0.049). This may be because of the possibility of non-minimal change nephrotic syndrome in those with non-selective proteinuria, like FSGS.[16]

In our study, GFR was low in non-selective proteinuria (p=0.0005). Bazzi C et al showed that characterization of proteinuria by SDS- PAGE (Sodium dodecyl sulphate – Poly Acrylamide Gel electrophoresis) in progressive glomerulonephritis was of useful predictive value on chronic renal failure.[21]

65% of steroid dependant nephrotic syndrome patients had non selective proteinuria (p=0.0199). As larger protein (e.g. α2-macroglobulin and β-lipoprotein) appear, the proteinuria is less selective, indicating greater damage to the glomerulus (e.g. FSGS membranous nephropathy, proliferative glomerulonephritis).

A study by Adamson recommends the use of the urinary protein electrophoresis (UPEP) as a marker of urinary protein selectivity and to monitor children with high-risk nephrotic syndrome like, those with a frequently relapsing or steroid dependent clinical course, for histological transitions. In this study (54%) children with MCD (minimal change disease) or IGMN (IgM nephropathy) and (94%) of the patients with FSGS (focal segmental glomerulosclerosis) manifested a frequently relapsing or steroid dependent course. A correlation between the selectivity of urinary protein and the clinical course of the disease was made. The mean percent albumin and Gama globulin excretion in the UPEP in patients with MCD and IGMN were 75.5 and 2.9 versus 72.6 and 3.9, respectively. Both patterns were significantly different from that observed in FSGS, where albumin was 62.2%, and gamma globulin 7.1%.[22]

Our study of serum electrophoresis showed decreased albumin with increased α2 band, along with urine electrophoresis in the same patient showing only prominent albumin bands in 17/43 patients, suggestive of selective protein loss.

In the rest of the 26 patients, serum electrophoresis showed decreased albumin with decreased other fractions α1, α2, β, γ globulin suggestive of non-selective proteinuria. In these patients urine electrophoresis showed prominent.

Albumin bands with β, α1, γ globulin in 11 patients.

Albumin with β, α1, γ, α2 globulin in 9 patients.

Albumin bands with β globulin in 4 patients

Albumin bands with α1, β globulin in 2 patients

This study shows that, urine proteomics is a feasible technology to distinguish variants of childhood idiopathic nephrotic syndrome

5. Conclusion

The mean age of the study population was 7.60 years. 55.8% had a complicated course with 16.3% frequent relapsers and 39.5% steroid dependent. 32.6% had low GFR and 18.6% had hypertension. These higher number of complications in comparison to other studies could be due to our inclusion criterion. We had taken only those patients with relapses. Most of the studies had included both new cases and relapses.

Majority of our patients had non selective proteinuria 60.5%. This higher prevalence of non-selective proteinuria could be due to the severe forms of nephrotic syndrome like FSGS and membranous glomerulonephropathy. We did not do the renal biopsy to prove the etiology. Renal biopsy and categorization into different etiological groups may be helpful in the treatment in knowing the sensitivity to steroid, the prognosis and follow up of patients with nephrotic syndrome.

We found that there was significant relation between non selective proteinuria and hypertension (p =0.049), low GFR (p =0.0005), steroid dependent nephrotic syndrome (p =0.0199). Non selective proteinuria was more in older children, above the age of 6 years (p=0.001). The non-selective proteinuria group took more time for remission again pointing to the lesser degree of steroid sensitivity.

Non selective proteinuria can be used as a biomarker to predict the course in childhood nephrotic syndrome. Selective proteinuria has a better outcome.

5.1. What this study adds

Use of UPEP (urine protein electrophoresis) as a marker of urine protein selectivity, and thus make us more alert in those patients with non-selective proteinuria with features of hypertension, low GFR as they may be high risk groups going in for chronic renal failure.

6. Abbreviations

MCD – Minimal change disease, FSGS – Focal segmental glomerulosclerosis, SDNS – Steroid dependent nephrotic syndrome, FRNS – Frequently relapsing nephrotic syndrome, MCNS – Minimal change nephrotic syndrome, MGN – Membranous glomerulonephritis, UPEP – Urine protein electrophoresis, SP – Selective proteinuria, NSP– Non selective proteinuria, AGE– Agarose gel electrophoresis.

7. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

8. Source of Funding

None.

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