

Research article

Electrophoretic pattern of serum and urinary proteins in nephrotic syndromeAmi Mehul Mehta¹, Revathi P. Shenoy², Varashree B. S.², Suneel C. Mundkur³, Monalisa Biswas²²Department of Biochemistry, ³Department of Paediatrics, ¹Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, 576104, Karnataka, India

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Corresponding author: **Revathi P. Shenoy**. Email: revathi.shenoy@manipal.edu**ABSTRACT**

Introduction and Aim: Nephrotic syndrome is a pathological condition in which the filtration of blood by the kidneys is defective. Patients with nephrotic syndrome will show abnormal serum albumin and protein levels in the urine. This study aims at filling the gaps in the knowledge of nephrotic syndrome based on the biochemical studies of samples from paediatric patients with the disease. The purpose of this study was to compare and differentiate the protein electrophoretic pattern of the serum and urine of patients having nephrotic syndrome with the time of diagnosis, time since treatment and management, using polyacrylamide gels.

Materials and Methods: Nine serum and urine samples of known patients, were collected from clinical biochemistry laboratory, sent for investigations and stored at -80°C till we proceeded with the experiments. The total protein concentration was determined using Biuret method. Protein electrophoresis was then carried out using polyacrylamide gel, which sieves proteins in size range of 5-250 kD. The obtained electrophoretogram was compared for urinary and serum proteins patterns of the same patient, at the time of diagnosis, monitoring during treatment or as the disease progresses.

Results: The abnormalities in the lipid profile were noted to complement the electrophoretic results obtained above. On correlation of the urine and serum patterns, we found that the albumin, alpha 2 and beta globulin bands were visible and comparable.

Conclusion: Since some proteins other than albumin are comparable in serum and urine, we would like to extend these preliminary findings with the SDS PAGE pattern of urinary and serum proteins to facilitate the investigation of patients not only at the time of onset of the disease, but also at different levels of management of the disease in larger prospective studies.

Keywords: Nephrotic syndrome; polyacrylamide gel electrophoresis; proteinuria; paediatric.

INTRODUCTION

Nephrotic syndrome, a pathological condition in which the filtration of blood by the kidneys is defective, is a major public health problem. Patients with nephrotic syndrome will show abnormal levels of albumin and protein in serum and urine. The average incidence of nephrotic syndrome among children is 2-16.9 per 100,000 worldwide (1) and among adults is 3 per 100,000 worldwide (2). Childhood nephrotic syndrome has an incidence of 90-100 per million population of India (3). The 5-year (2016-2021) incidence of paediatric nephrotic syndrome in Kasturba Hospital, Manipal was 808.

Due to the insufficiency of relevant statistical data, experimental evidence, and biochemical analysis, uncertainty still exists regarding the diagnosis and management of the disease. Clarity regarding the progression of the disease along with the effectiveness of treatment can throw light on the lines of therapy for future patients.

It is well known that electrophoretic patterns of serum protein in nephrotic syndrome show a marked decrease in total protein, albumin, and gamma-globulin, and a marked increase in α 2-globulin and fl-globulin (4) and serum protein electrophoresis is among the gold standard evidence for the diagnosis of nephrotic syndrome. Research suggests the lack of CD2-associated protein in mice with nephrotic syndrome (5) and that repetitive fragmentation products of albumin and α 1-antitrypsin may be associated with nephrotic syndrome (6). A variety of protein components of serum may be excreted in the urine of these patients including C-reactive protein (7). In 1985, Park *et al.*, (4) studied the electrophoretic pattern in nephrotic syndrome. It was found that in serum protein electrophoresis, nephrotic syndrome showed a marked decrease in total protein, albumin, and gamma-globulin, and marked increase in α 2-globulin and fl-globulin (4). Kalantari *et al.*, (8) in their article highlighted the advantages of studying the protein composition of the urine in nephrotic

syndrome over renal biopsy. According to the author, the non-invasiveness of the technique is a significant benefit as it is convenient yet effective. Other advantages include the ability of the test to be done repeatedly and the stability of the samples containing the proteins. However, the author also mentions that the major disadvantage is an inter individual variability.

On the other hand, Tuazon (9) described electrophoresis as an “Underused but Very Useful Test,” and the article brings to light the procedure, indications, contraindications and graphical representation of the results of serum protein electrophoresis. It describes the various zones as seen in an SPE including Albumin– $\alpha 1$ -Interzone, $\alpha 1$ - $\alpha 2$ interzone and $\alpha 2$ interzone among the other commonly described zones. The interpretation of the results relies on the absolute and relative values of the different bands. The author mentions that in nephrotic syndrome, there is an increase in the $\alpha 2$ -zone (Ceruloplasmin, $\alpha 2$ -macroglobulin, and haptoglobin) due to the impermeability of the glomerular membrane for these large proteins. The author also describes the procedure as inexpensive and widely available which will confer an advantage to the clinical and biochemical aspects of medicine.

The article also mentions the indications and applications of serum protein electrophoresis which are of great importance in the diagnosis of disorders such as nephrotic syndrome. Some of the hints include hypergammaglobulinemia and unexplained proteinuria. Khurana *et al.*, (10) highlight the importance of urine proteomics in the diagnosis of steroid-resistant nephrotic syndrome.

B₂-microglobulin was one of the peaks that were identified in the study. The importance of this finding lies in the fact that it has been determined using an unbiased highly accurate technique. However, one of the main advantages of this method was the ability to

conduct a parallel analysis of many patient samples. Serum and urine electrophoresis at different stages of the disease can aid in the assessment of the levels and nature of the proteins being excreted, which is vital in the prognosis and management of nephrotic syndrome. Polyacrylamide gel electrophoresis, according to Chrambach and Rodbard (11) provides a versatile, gentle, high-resolution method for fractionation and physical-chemical characterization of molecules on the basis of size, conformation, and net charge. This study aims to identify proteins, which could be a biomarker for the evidence for recovery and progression of the disease and compare and differentiate the protein- electrophoretic pattern in serum and urine of patients having nephrotic syndrome using polyacrylamide gels.

MATERIALS AND METHODS

This is an observational, analytical cross-sectional study carried out in the Department of Biochemistry, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, for three months from July to September 2018. Ethical clearance was taken from the Institutional Ethical Committee (IEC) before the commencement of the research project (IEC no 445/2018). Nephrotic syndrome was diagnosed as per the diagnostic criteria by Hull *et al.*, (12).

Samples from newly diagnosed patients as well as those under treatment were included in the study. Demographic and clinical findings were obtained from the medical records. Laboratory findings were extracted from the laboratory information system, residual serum and random urine samples were collected from the clinical biochemistry laboratory and stored in -80°C till analysis. Written informed consent was obtained from all the guardians of the patients before collecting the samples and the clinical data.

Table 1: Demographic and clinical findings among the study participants

Age in years/ Gender	Diagnosis	Urine protein	ESR (mm/hr)	C3 (mg/dl)	C4 (mg/dl)	Total Cholesterol (mg/dl)	First follow-up urine protein
10 / M	1st episode in remission	>300 mg/dl (3+)	39	149	32	353	>300 mg/dl (3+)
6 / M	4th relapse with peritonitis	6g/24hr (3+)	87			446	3+
5 / F	FSGS confirmed by biopsy	3.5 g/24hr	61	105	23	510	3+
6 / M	1st episode	>300 mg/dl (3+)	59	139		386	30 mg/dl (1+)
5 / M	1st relapse	>300 mg/dl	52				>300 mg/dl

		(3+)					(3+)
6 / M	1st episode	6.06g/24hr (3+)	64	149	37	512	2+
2 / M	1st episode	768mg/24 hr, (4+)	96	135	38	402	300 mg/dl (3+)
7 / F	Spontaneous remission after 1 st presentation	Negative	42	188	26	376	Negative

Protein electrophoresis was carried out using polyacrylamide gel, by the method of Laemmli (13) using 10% acrylamide gel, 0.025 M Tris-Glycine buffer, pH 8.4. The protein bands were stained with Coomassie Brilliant Blue R-250. The obtained electrophoretograms were compared.

RESULTS

Eight patients, including 6 males and 2 females of age from 2 to 10 years were included in the study. All serum samples showed albumin between 2-3 % and urinary proteins from 500 mg to 1500 mg /24hrs. Even though the majority of the patients (4/8) were in the first episode of diagnosis, all showed high (3+ />300mg/dl) protein in urine and hypercholesterolemia. On the other hand, both C3 and C4 protein levels in serum were in the normal range. However, increase in ESR in all the cases reveals the increased inflammatory activity in those patients.

The electrophoretic pattern of urine proteins is observed to be quite similar to that of serum proteins,

however, in the serum, the electrophoretic bands moved a very small distance. The serum electrophoretogram showed four bands whereas the urine protein electrophoretogram showed only three bands (Fig. 1 to 3).

The serum pattern obtained for all the samples cumulatively showed major amounts of albumin, alpha-2, and beta globulins whereas gamma globulins concentration was found to be the least. Compared to serum, urinary proteins showed albumin, alpha-2, and beta globulins, conversely alpha-1 globulins and gamma globulins were absent. Standard protein Bovine serum albumin showed multiple bands due to self-oxidation. Due to the limited time and unavailability of the samples, it was only possible to run native or normal PAGE and not the SDS- PAGE which could have shown clarity. It was found that most patients were treated with a course of steroids and antibiotics. This study is only for two months, following up of the patients and repeated sample collection was not done.

Table 2: Distances moved by different serum and urinary proteins in polyacrylamide gel electrophoresis

Samples	Mean± SD (cm)			
	Serum	3.75 ±0.41	2.3 ± 0.29	1.3±0.18
Urine	3.88 ± 0.61	1.85 ±0.68	1.35± 0.07	-

DISCUSSION

Polyacrylamide gel electrophoresis (PAGE) is a widely accepted effective tool to differentiate various proteins according to their molecular weights. Serum protein electrophoresis (agarose gel electrophoresis) is used as one of the diagnostic tests for nephrotic syndrome. However, urine protein electrophoresis is seldom performed to aid the diagnosis and staging of nephrotic syndrome. Our study reports comparable urine and serum electrophoretic patterns in patients

and highlights that low to medium molecular weight proteins like beta globulins and albumin were excreted in urine. This emphasizes that a novel diagnostic battery of urine and serum protein electrophoresis may provide a holistic picture of the stage of nephrotic syndrome, and the prognosis and thus might aid tailoring treatments (steroid-responsive versus steroid-resistant) for favourable outcomes.

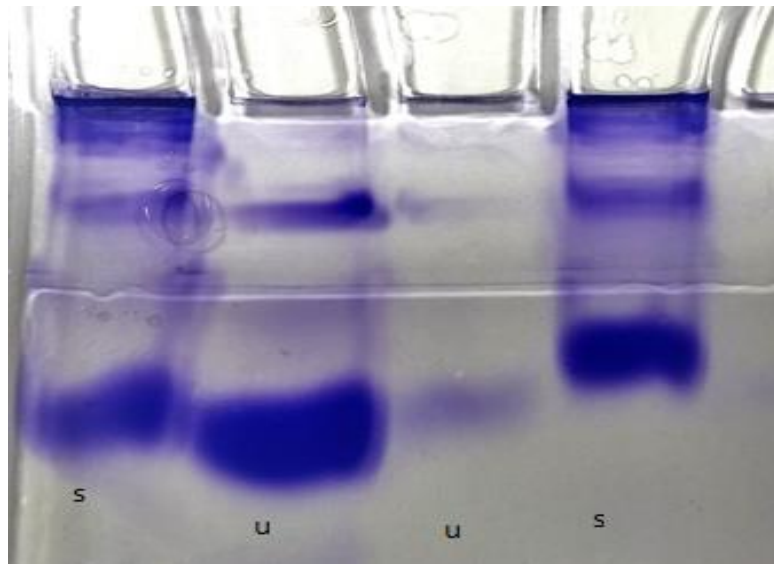


Fig. 1: Electrophoretogram of serum and urinary proteins

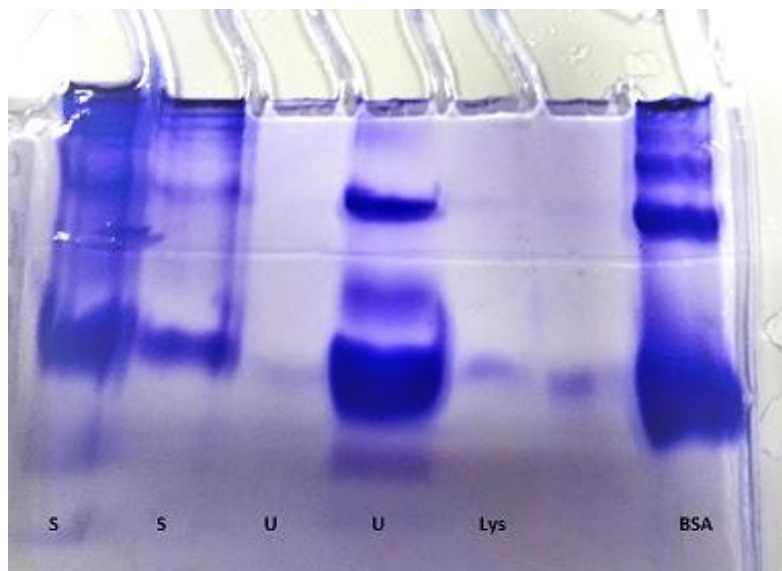


Fig. 2: Electrophoretogram of serum and urinary proteins with standard protein BSA (bovine serum albumin)

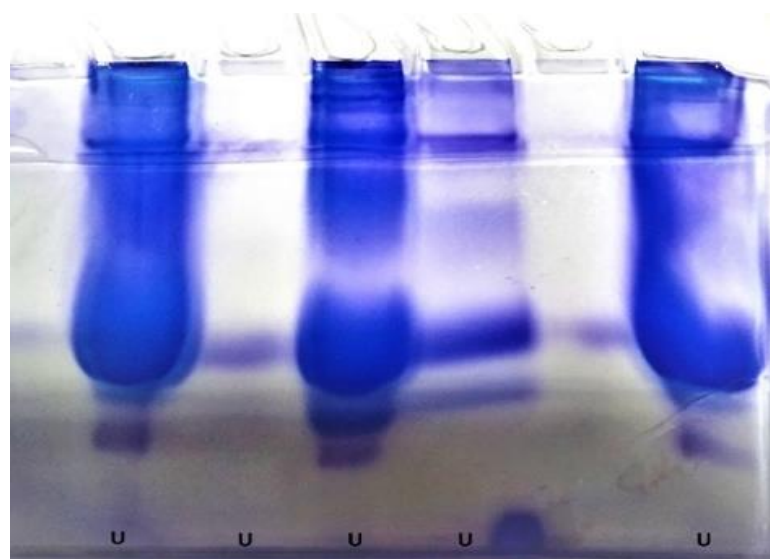


Fig. 3: Electrophoretogram of urinary proteins

Figures 1, 2 and 3 represent the pattern obtained with serum and urine proteins. 'S' represents serum and 'U' represents the urine samples.

Ramjee *et al.*, (14) in their study, observed the presence of albumin, transferrin, haptoglobulins, IgG, lysozyme and β -2 macro globulins in the urine of children with nephrotic syndrome. This study supports our observation that the urinary protein pattern corresponds with the serum samples. The presence of major proteins in the urine is indicative of the severity of the glomerular damage. According to Cil and Perwad (15), it is easy to diagnose nephrotic syndrome in children, but its complex etiology and mechanisms by which the glomerular filtration barrier is disrupted to induce proteinuria are challenging to treat. They also revealed that regardless of histopathological diagnosis a majority of patients respond to immunosuppressive therapy.

In their review article "Pathophysiology of Proteinuria," D'amico and Bazzi(16) discussed the various mechanisms of proteinuria. According to this, excretion of low molecular weight proteins like alpha-1 and beta-2 microglobulins along with IgG and IgM correlates with the severity of the histologic lesions. It was suggested that severity and scarce reversibility could be reliably indicated by elevated urinary excretion of these high molecular weight (HMW) and low molecular weight (LMW) proteins.

It was observed, in our study, that the LMW proteins like beta globulins were excreted with albumin in the urine. This shows a comparatively similar pattern to serum proteins. Since these samples are anonymized and from freshly diagnosed patients, PAGE with urine and serum proteins together can provide a valuable diagnostic marker for the stage of nephrotic syndrome. This is also supported in the study by Ramjee *et al.*, (17). The study involved SDS-PAGE analysis of urine to distinguish steroid-responsive from steroid-resistant patients, which is vital for the management of nephrotic syndrome in children. This suggests that our results also describe the initial presentation of nephrotic syndrome.

Due to the time constraint and the limited number of available samples, the study could not be extended to assess the patterns in treatment response management. Also, the comparison of the concentrations of proteins in urine and serum was not feasible. This warrants further validation before its full potential is appreciated. Our study is being extended to quantification and comparison of both native and SDS PAGE, which would provide a better understanding of the etiology of nephrotic proteinuria.

Thus, our preliminary findings, despite the lack of clarity due to small sample size, motivated us to continue the study with the SDS PAGE pattern of urinary and serum proteins. This would facilitate the investigation of patients not only at the time of onset of the disease, but also at different levels of management of the disease by larger prospective studies. The study will be further continued with a

larger sample size to facilitate identification and quantification of protein patterns in patients at the time of disease onset and multiple time points of management and treatment.

CONCLUSION

In the present study, both urine and serum samples were assessed. On comparison and analysis of these samples, it was revealed the possibility of isolating proteins that seem to be unique to the urine and serum of nephrotic syndrome patients. The study also identified that the patterns of urine and serum are similar except for the band consisting of gamma globulins (which is present only in the serum).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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