Protein Clearances and Selectivity Determinations in Childhood Nephrosis: A Reappraisal

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To critically evaluate the clinical utility of determining specific proteins in patients with extensive proteinuria, we used immunonephelometric methods to measure albumin, transferrin, IgG, and α\textsubscript{2}-macroglobulin in serum and in 24-h urine specimens from 37 children with idiopathic nephrotic syndrome. Renal biopsy demonstrated minimal change disease (I) in 15, focal glomerulosclerosis (II) in 15, and membranoproliferative glomerulonephritis (III) in seven patients. A three-group nonparametric rank test and three-group discriminant function analysis of the protein excretion and clearances of the four proteins we measured revealed significant differences in the excretion of IgG and the clearance of α\textsubscript{2}-macroglobulin among the three groups of patients (p < 0.05). Only patients with III had low serum complement C\textsubscript{3} concentrations. Patients with I or II were best discriminated by differences in the excretion of transferrin and IgG, the clearance of α\textsubscript{2}-macroglobulin, and the selectivity index (the clearance ratio of IgG/transferrin). These data indicate that measurement of specific urinary proteins and selectivity determinations may be helpful in predicting the type of histopathology and the prognosis of nephrotic children who have normal complement concentrations.

\textbf{Additional Keyphrases:} nephrotic syndrome • pediatric chemistry • urinary proteins • protein selectivity • immune nephropathy

The process of macromolecular filtration at the glomerulus has been experimentally shown to depend on the particular anatomy and biochemical composition of the glomerular basement membrane, the transcapillary hydrostatic and oncotic pressures, and the size, configuration, and charge of the macromolecules considered (1–3). In addition to these factors, the ultimate appearance of a protein of plasma origin in the urine may depend greatly on the efficiency of tubular re-absorption of the protein (4).

In clinical studies, most determinants of macromolecular filtration cannot be measured. It is practicable only to control for glomerular filtration rate, dietary intake of salt and acid, and posture of the patient during the urine collection. Nevertheless, despite all the variables involved, urinary protein measurements and selectivity determinations continue to be used to predict the underlying histopathology and prognosis of patients with nephrotic syndrome (5).

The present study was undertaken to determine the clinical utility of measuring protein clearance and selectivity in children with the nephrotic syndrome. We used immunochemical methods that better measure proteins in serum and urine.

\textbf{Materials and Methods}

\textbf{Subjects}

The ages of the patients ranged from 0.5 to 16 years. At the time of the study they were edematous, and the total urinary protein excretion exceeded 40 mg/m\textsuperscript{2} per hour (5). Additional criteria for making the diagnosis of nephrotic syndrome, such as hyperlipidemia and hypoalbuminemia, were fulfilled in all patients. In all instances 24-h urine specimens were collected during hospitalization while the patients received a standard low-salt diet, and activity was limited to walking about the hospital ward. None of the children included for study received corticosteroids, diuretics, or other medications for a minimum of two weeks preceding the urine collection. Urine aliquots were stored at \(-70 \text{oC}\) for as long as four months before being assayed for proteins.

\textbf{Renal Biopsy}

Indications for renal biopsy were (a) nephrotic-range proteinuria after 30 days of prednisone at a daily dose of 2 mg/kg per day (maximum 60 mg/m\textsuperscript{2} per day); (b) persistent hypocomplementemia and nephrotic-range proteinuria; and (c) corticosteroid-responsive but frequently relapsing nephrotic syndrome. The median time from onset of disease to renal biopsy was two months for patients with FGS and MPGN (range two weeks to five months) and 12 months for those with MC (range five months to six years).\textsuperscript{3} In all cases, percutaneous renal biopsies were performed under local anesthesia with ultrasonographic localization of the kidneys. The histological classification criteria used in this study were similar to those described by Habib and Kleinnech (6) for children with idiopathic nephrotic syndrome.

\textbf{Procedures}

All patients had a urinalysis and biochemical evaluation of renal function. Glomerular filtration rate was assessed by endogenous creatinine clearance. Completeness of 24-h urine collections was verified by total creatinine excretion rates. Other routine studies included serum complement (C\textsubscript{3}) profiles, antinuclear antibody determinations, agarose gel electrophoresis of proteins, and quantitation of serum immunoglobulins by standard radial immunodiffusion methods.

The nephelometric assays and performance characteristics of the procedures used to measure urinary and serum albumin (Mr 68,000), transferrin (Mr 80,000), IgG (Mr 160,000), and α\textsubscript{2}-macroglobulin (Mr 820,000) are detailed elsewhere (7). Specific antisera were purchased from Atlantic Antibodies, Westbrook, ME 04092, and their monospecificity was confirmed by immunofixation methods. The higher purity of monospecific antibodies used more recently, as well as minor modifications in technique, have further increased the precision of our assays. The coefficient of variation of the urinary

\textsuperscript{3} Nonstandard abbreviations used: MC, minimal change nephrotic syndrome; FGS, focal glomerulosclerosis; and MPGN, membranoproliferative glomerulonephritis.

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assays ranged from 17.6% for albumin to 4.5% for IgG. Serum protein assays had a coefficient of variation ≤3.4%, as previously reported (7).

The three groups of patients were compared in terms of protein excretion and clearances and selectivity indices, by use of a three-group nonparametric rank test (Kruskal–Wallis H-test) (8) and three-group discriminant function analysis (Mann–Whitney U-test) (9) at the 0.05 level of probability.

**Results**

Renal biopsy disclosed MC disease in 15, FGS in 15, and MPGN in seven children. None of the patients with FGS included in this study had focal global sclerosis; two patients in the MPGN group had dense-deposited disease.

All patients had endogenous creatinine clearances between 100 and 130 mL/min per 1.73 m² of body surface. C₃ complement concentrations were decreased in the seven children with MPGN; none of the patients with MC or FGS had abnormal complement values. Antinuclear antibodies were absent in all patients. Electrophoresis of serum on agarose gel demonstrated similar patterns in all cases, including decreased serum albumin, increased α₂-macroglobulin, and decreased gamma-globulin. Strikingly low serum IgG concentrations were found in most children with MC but also in several with FGS.

Table 1 shows the concentrations of albumin, transferrin, IgG, and α₂-macroglobulin in serum and also the urinary excretion and clearance of these four proteins. The total protein excretion was converted into mg/m² per 24 h, while protein clearance is expressed in standard units of mL/min per 1.73 m². These methods permitted comparison of patients regardless of age and body weight.

Of particular interest in patients with MPGN is the finding of greater excretion of IgG than of transferrin. In patients with FGS, excretion of IgG approached that of transferrin. Nevertheless, transferrin clearance surpassed IgG clearance in each of the three groups. Serum albumin and transferrin concentrations were decreased in all patients with MC, FGS, and MPGN, and the marked decrease in serum IgG concentrations in patients with MC cannot be explained solely on the basis of IgG excretion, which was minimal. Serum α₂-macroglobulin concentrations were in general inversely related to the degree of hypoalbuminemia in MC, FGS, and MPGN.

Multiple-comparison analysis showed no significant difference in the concentrations of any of the four proteins in serum among the three groups of patients (p > 0.05). Table 1 shows the statistical comparison for the urinary protein studies in the three histopathologically differentiated groups of patients. By both statistical methods, the most useful studies discriminating among all three groups were the excretion of IgG and the clearance of α₂-macroglobulin. These two variables differed significantly in each of the three groups. Furthermore, the combination of transferrin and IgG excretion, the clearance of α₂-macroglobulin, and the selectivity index were significantly greater in patients with FGS as compared with those with MC.

In keeping with previous studies comparing the protein selectivity indices in children with MC, FGS, and MPGN (10–12), the selectivity plots of the present patients were computed after linear regression analyses of the mean protein clearances relative to transferrin clearance (Figure 1). Selectivity slopes were identical between patients with MC or FGS (42°) but the angle was smaller (27°) for patients with MPGN.

Multivariate analysis between selectivity indices determined from protein clearances was undertaken to determine their value in distinguishing the three morphological lesions. Despite an apparently significant difference in the selectivity indices between MC and FGS (ratio of IgG to transferrin clearances of 0.215 vs 0.351), five patients in the MC group and nine in the FGS group had nonselective proteinuria (selectivity index ≥0.2). There was no significant difference in the selectivity index in patients with FGS as compared with those with MPGN (0.351 vs 0.378, p < 0.05).

**Discussion**

The assessment of proteinuria and assignment of protein selectivity values have been based on the fact that in a pathological process that leads to proteinuria, the glomerular basement membrane protein barrier is altered, thereby permitting the filtration and excretion of large plasma proteins. It thus follows that the size and relative amounts of plasma proteins excreted in the urine can serve as an indirect measure of the pathological pore size and of the severity of renal involvement. Results obtained by several semiquantitative methods of assessing proteinuria have been used to derive mathematical values for protein "selectivity," which have been
proteins consisting almost entirely of albumin and transferrin. This suggests greater uniformity in the histopathology of these patients. However, despite their low IgG excretion, the children with MC had the lowest serum IgG concentrations of any of the disorders studied, and some investigators believe that this finding may be related to the pathogenesis of MC nephrotic syndrome (16, 17).

A surprising finding in patients with FGS is the relatively high excretion of IgG relative to their serum IgG concentrations. Similar results have been reported in another study (18), but to date a pathogenetic relationship remains to be demonstrated. In that study, a decline in "selectivity" with time was found in patients with FGS. In the present patients with FGS, the α2-macroglobulin excretion was low despite a mean period of follow-up of 3.7 years from the onset of the nephrotic syndrome to the time of the present protein measurements.

Because of the similarities in disease presentation, it is often difficult to distinguish patients with MC from those with FGS. It is under these circumstances, perhaps, that protein studies may be of specific clinical value. This distinction is of practical importance because, unlike MC, FGS is generally nonresponsive to therapy and is associated with a poor prognosis. Indeed, earlier studies on protein selectivities were primarily used to differentiate these two disorders (11). Our results indicate that the most discriminant protein studies for this purpose were the excretion of transferrin and IgG, the clearance of α2-macroglobulin, and, to a lesser extent, the selectivity index, in this case the clearance ratio of IgG to transferrin. The combined predictive value of these studies approaches 90% specificity in making the correct diagnosis. We believe that our use of 24-h (rather than untimed) urine collections and improved methodology is largely responsible for the important differences in the results of the present study; i.e., about 35% of patients with either MC or FGS could not be distinguished by selectivity indices alone.

Children with MPGN were distinguished from those with MC and FGS in having a statistically greater excretion of IgG and clearance of α2-macroglobulin. These protein studies and the low concentrations of C3 complement in serum that frequently are found in children with MPGN permit differentiation of the disorder from other idiopathic forms of the nephrotic syndrome. Yet, even given this characteristic clinical profile, it must be noted that clearances of transferrin, IgG, and α2-macroglobulin in two of the patients with MPGN were indistinguishable from those with MC and FGS. The finding of such significant inter-individual variations in protein data among patients with specific morphological diagnoses limits their clinical usefulness and does not eliminate the need of a renal biopsy in instances in which the diagnosis is in question.

Our data lend support to the notion that many uncontrollable factors influence filtration of proteins in the clinical setting. Although statistical differences do exist between the protein data in these three morphological groups, there is considerable overlap in the excretion and clearance of proteins among patients, even under controlled conditions of urine collections, dietary intake, and physical activity. Nevertheless, our results indicate that these protein studies may provide information on which to base a reasonable initial evaluation and management of a child with nephrotic syndrome. These studies are not intended as a replacement for a renal biopsy when a more definitive diagnosis is required.

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