Clinical Evaluation Of Protein-Sieving Coefficient In Severe Nephrotic Syndrome

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Abstract

Background: The pathogenesis of nephrotic syndrome is thought to be due to a decrease in protein sieving function in the glomerular capillary wall. However, no research has been conducted on the renal sieving coefficient of serum protein in nephrotic patients. We investigated the renal protein sieving coefficient in pediatric patients with severe nephrotic syndrome.

Methods: Thirty pediatric patients (age: 3–20, mean: 9.3, years; 18 male and 12 female) with severe nephrotic syndrome: hypoproteinemia (TP (Total Protein) ≤ 5.0 g/dL), heavy proteinuria (UTP/Cr (Creatinine) >2 g/gCr), and a normal GFR(glomerular filtration rate) (creatinine clearance ≥ 90 mL/min), were studied. The sieving coefficient of the serum total protein (SCTP) was calculated using a simple formula: Total protein clearance/creatinine clearance (%). The correlation with a twenty-four-hour (24hr) urinary protein test corrected by body surface area (24hrUPT/BSA) was analyzed to assess the accuracy of SCTP. For reference, the correlation between 24hrUPT/BSA and UTP/Cr with SCTP was also analyzed, and the differences between these correlation coefficients were statistically examined.

Results: The mean ± standard error of SCTP was 0.13 ± 0.017%. The correlation coefficients of SCTP and U-TP/Cr with 24hrUPT/BSA were 0.90 and 0.78, respectively. SCTP had a significantly stronger correlation with 24hrUPT/BSA than U-TP/Cr ($p < 0.05$).

Conclusion: We found that only 0.13% of sieving coefficient dysfunction causes severe nephrotic syndrome. SCTP has a stronger correlation with 24hrUPT/BSA than with UTP/Cr. If nephrotic syndrome is defined as a protein-sieving dysfunction of the glomerular capillary wall, SCTP can be considered a conceptually correct indicator.

Background

The pathogenesis of nephrotic syndrome is thought to be a protein-sieving dysfunction of the glomerular capillary wall. However, because serum total protein is a diverse set of proteins, the renal sieving coefficient of serum total protein (SCTP) has not been studied. A 24-hour urinary protein test (24hr UPT) is used as a gold standard for evaluating proteinuria as a substitute .[1] The most direct method of assessing the amount of protein leakage from the glomerulus is 24hrUPT. However, performing 24hrUPT on infants and small children is difficult. Because of the difficulty of infant urine storage as well as for infection control purposes, the urinary protein/creatinine ratio of morning spot urine (U-TP/Cr) has long been utilized clinically as an alternative indicator of proteinuria [2]. U-TP/Cr is a measure of the degree of urinary protein. However, U-TP/Cr does not correlate well in severe nephrotic states and/or in renal dysfunction [3], possibly because serum creatinine and total protein levels influence the U-TP/Cr ratio of primary urine under the same sieving coefficient [4]. The SCTP could be calculated using the following simple formula: total protein clearance/creatinine clearance (%) [5]. In this study, we analyzed nephrotic children with various body surface areas (BSA). The absolute glomerular filtration rate (absolute GFR) of

normal subjects is known to correlate well with their BSA [6]. Therefore, standardization is required by dividing 24hrUPT by BSA.

On the basis of the above hypothesis, we tested the relationship between SCTP and U-TP/Cr with 24hrUPT/BSA in children with hypoproteinemia (TP ≤ 5.0 g/dL), heavy proteinuria (UTP/Cr > 2 g/gCr), and normal GFR (creatinine clearance ≥ 90 mL/min), indicating severe nephrotic syndrome.

To determine the differential clearance curve, the "sieving function of the glomerular capillary wall" was originally applied to the fractional clearance of specific molecules of different molecular sizes and charges [7]. It is not considered in routine clinical decisions. Total protein in serum and urine contains a variety of proteins of varying sizes, conformations, and charges, and the sieving coefficient for each is likely to be different. However, there is no conceptual alternative that is a physiologically convenient method for assessing the severity of protein sieving dysfunction in nephrotic patients. Therefore, in this study, we first compared the clinical accuracy of SCTP to 24hrUPT/BSA and then statistically analyzed the relative accuracy with conventional U-TP/Cr in these patients.

Materials And Methods

Study design and subjects

This retrospective observational study was conducted at two Japanese medical centers. The patients were admitted to Nihon University Itabashi Hospital and Tokyo Metropolitan Children's Medical Center between January 2010 and March 2018. This study was approved by the ethics committees of Nihon University School of Medicine Itabashi Hospital (No. RK-180410-04) and Tokyo Metropolitan Children's Hospital (No.H30b-19). All research was performed in accordance with relevant guidlines/regulations. Consent for the study was obtained from the patients and their guardians by opt-out method. This research was performed in accordance with Declaration of Helsinki.

Helsinki declaration.

Fifty-eight serum and urine samples were collected from 30 pediatric patients aged between 3 and 20 years with hypoproteinemia (TP ≤ 5.0 mg/dL), heavy proteinuria (UTP/Cr > 2 g/gCr), and normal GFR (≥ 90 mL/min), (mean: 9.3 years, Male 18, female 12). Twenty of these children had steroid-responsive idiopathic nephrotic syndrome that had either relapsed or was the first presentation. Six of the children had Henoch-Schönlein purpura nephritis, two had IgA nephropathy, and the remaining two had membranous nephropathy and membranoproliferative glomerulonephritis, respectively. Because the influence of tubular protein reabsorption must not be ignored in patients with less heavy proteinuria, samples from patients with UTP/Cr ≤ 2 g/gCr were not included in the analysis.

We collected a 24-hour urine specimen from all the patients to measure their 24hrUPT. To study U-TP/Cr, we collected a small portion of the early morning urine samples separately. Blood tests were only performed when necessary to monitor the patients conditions. SCTP was calculated at that time. The

creatinine levels of all the serum and urine samples were determined using an enzymatic method (LABOSPECT 008α, HITACHI, Tokyo, Japan). BSA was calculated using DuBois' formula [8], with weights and heights measured several weeks before and after blood tests.

The relationship of SCTP and U-TP/Cr with 24hrUPT/BSA was analyzed to evaluate the accuracy of SCTP. As a reference, the correlation between SCTP and UTP/Cr was also analyzed, and the differences between these correlation coefficients were statistically examined.

Statistical analysis

The mean and standard errors (SE) were calculated. Using log-transformed data from 24hrUPT/BSA, UPT/Cr, and SCTP, linear regression analysis was performed, and Pearson's correlation coefficients (R) were determined. A paired t-test was used to compare the three indices using Z-scores. Statistical analyses were performed using JMP® 14 (SAS Institute Inc., Cary, NC, USA).

Results

The mean ± SE of the 24hrUP/BSA, U-TP/Cr, and SCTP (%) were 5.17 ± 0.57 (g/day/ m^2), 10.99 ± 1.33 (g/gCr), and 0.13 ± 0.017 (%), respectively..The R between 24hrUPT/BSA and U-TP/Cr was 0.78 (Figure 1). The R between 24hrUPT/BSA and SCTP was 0.90 (Figure 2). SCTP had a significantly stronger correlation with 24hrUPT/BSA than that of U-TP/Cr ($p < 0.05$).

Discussion

The fractional excretion of serum total protein in a patient with congenital nephrotic syndrome of the Finnish type was reported to be only approximately 1% [9]. The fractional excretion of total protein is calculated in the same SCTP. In this study, we identified that only 0.13% of sieving coefficients caused severe nephrotic syndrome. As a result, minor changes in the protein sieving function of the glomerular capillary wall result in marked hypoproteinemia and edema.

SCTP also has a stronger correlation with 24-hour urine storage than TP/Cr. This suggests that SCTP, rather than UTP/Cr, more accurately reflects the severity of nephrotic states. U-TP/Cr has been utilized as an alternative indicator of 24h-UPT for decades [10]. However, the U-TP/Cr did not correlate well with 24h-UPT in our cases of severe nephrotic syndrome (Fig. 1) when compared to that of the previous report [11].

The physiology of UTP/Cr and SCTP is depicted schematically in Fig. 3. Even if the SCTP remains constant (0.2%), when plasma protein decreases from 5000 mg/dL to 1000 mg/dL, U-TP/Cr would decrease from 20 to 4. Therefore, in cases of severe hypoproteinemia, it was unclear whether a decrease in U-TP/Cr reflects an improvement in nephrotic conditions. However, this phenomenon has not been clinically recognized so far. Thus, SCTP, a novel method to evaluate proteinuria, is a conceptually correct

measure to evaluate the severity of nephrotic syndrome, with the exception of the portion of filtered proteins reabsorbed through the renal proximal tubules [12] and tubular creatinine excretion [13].

Next, we tried to prove the above hypothesis, depicted in Fig. 3, in clinical samples, with a special emphasis on cases with heavy proteinuria and severe hypoproteinemia, because the effects of severe hypoproteinemia on UTP/Cr have not been clinically evaluated. Our findings showed that, in cases with heavy proteinuria and severe hypoproteinemia, SCTP was a statistically better indicator of 24hUPT/BSA than UTP/Cr, as theoretically expected.

The limitations and importance of this study are as follows. We used a limited number of samples from two institutions; therefore, we were not able to investigate the difference of SCTP among groups of nephritis, nephropathy and minimal change disease. Serum creatinine, a factor that might affects SCTP, was not the target of our study. Because we selected patients with normal GFR in order to reduce the number of factors that could affect the results, such as Cr, to make the clinical significance of SCTP easier to understand. However, the clinical significance of the changes of serum creatinine which affects U-TP/Cr has been discussed in the previous case report. Further investigation is needed to clarify the relationship between serum creatinine and U-TP/Cr as well as SCTP.

In a limited number of cases, multiple samples were taken from some of the same patients, but this is not expected to affect the results based on the measurement principle.

The SCTP calculation provides clinicians with a vision of understanding proteinuria through classical renal physiology. Then, most importantly, we were able to show that worsening of the sieving coefficient is related to the severity of nephropathy with high correlation with 24hr UPT. In addition, to perform 24hrUPT in infants and small children is very difficult and the test is thought to be a risk of nosocomial infections. Clinical application of SCTP may facilitate the assessment of urinary protein in infants and small children as a substitution of 24hrUPT and may reduce the risk of nosocomial infection.

Conclusion

If nephrotic syndrome is defined as a protein-sieving dysfunction of the glomerular capillary wall, we hypothesized that SCTP could be considered a conceptually correct indicator. The clinical signficance of SCTP in severe nephrotic patients was then examined. The results proved that SCTP was a reliable predictor of the severity of proteinuria, particularly in the presence of severe hypoproteinemia and severe proteinuria. We also found that only approximately 0.13% of the in the sieving coefficient dysfunction caused severe nephrotic syndrome.

Declarations

Author contribution

S.H., S.S., and S.T. designed the study; S.H., S.S., T.M., R.H., and H.H. collected the samples; S.H., S.S., and S.T. analyzed the data; S.H., S.S., and S.T. made the figures; S.H., S.S., and S.T. drafted the paper; Y.K. and I.M. revised the analysis of the data and the draft; all authors approved the final version of the manuscript.

Disclosures: None

Data availability:
All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Figures

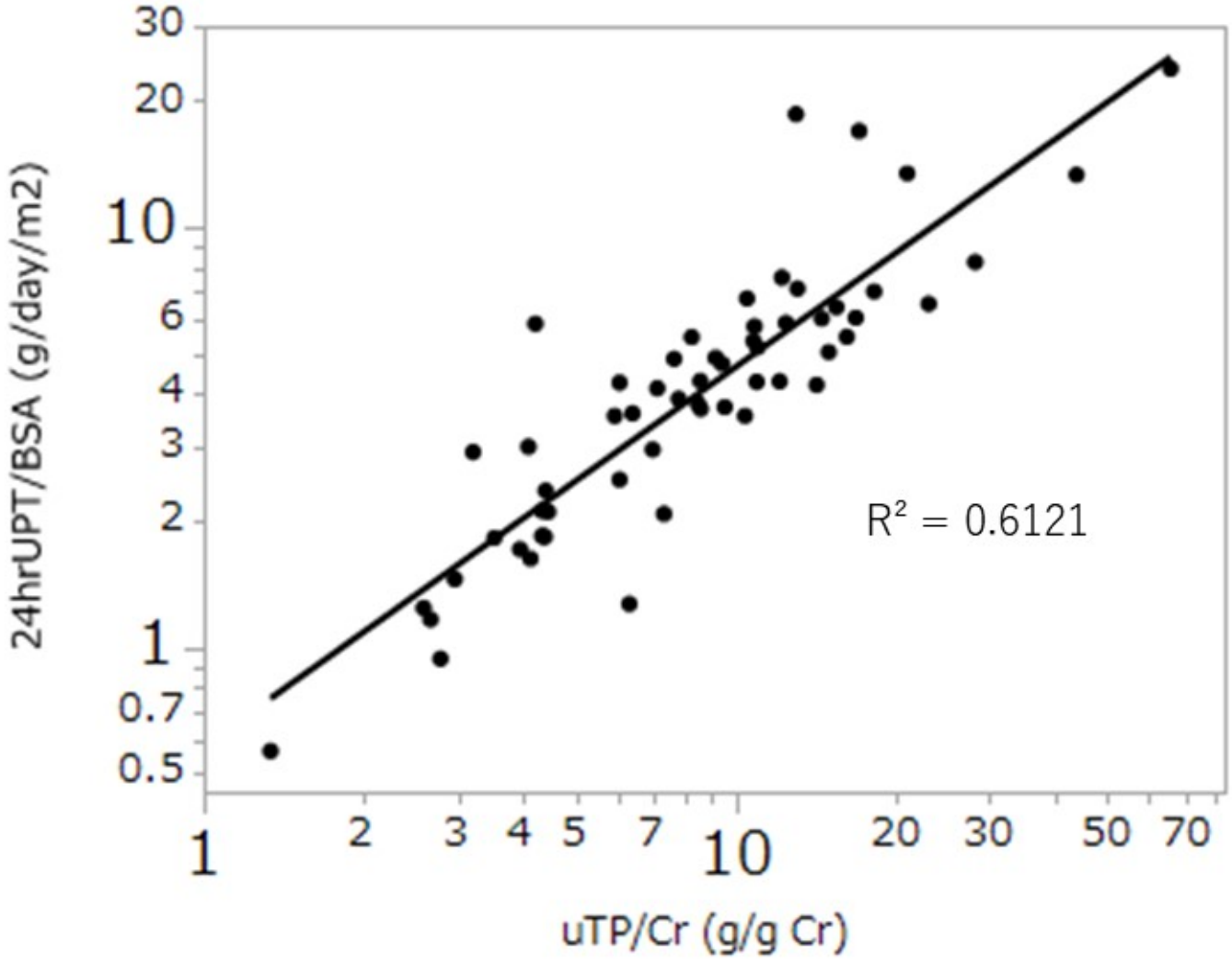

Figure 1

The correlation between the 24hrUPT/BSA and UTP/Cr (n = 58, r = 0.78), measured using a small portion of the early morning urine samples.

24hrUPT: 24-hour urinary protein test.

BSA: body surface area.

UTP/Cr: urinary protein/creatinine ratio.

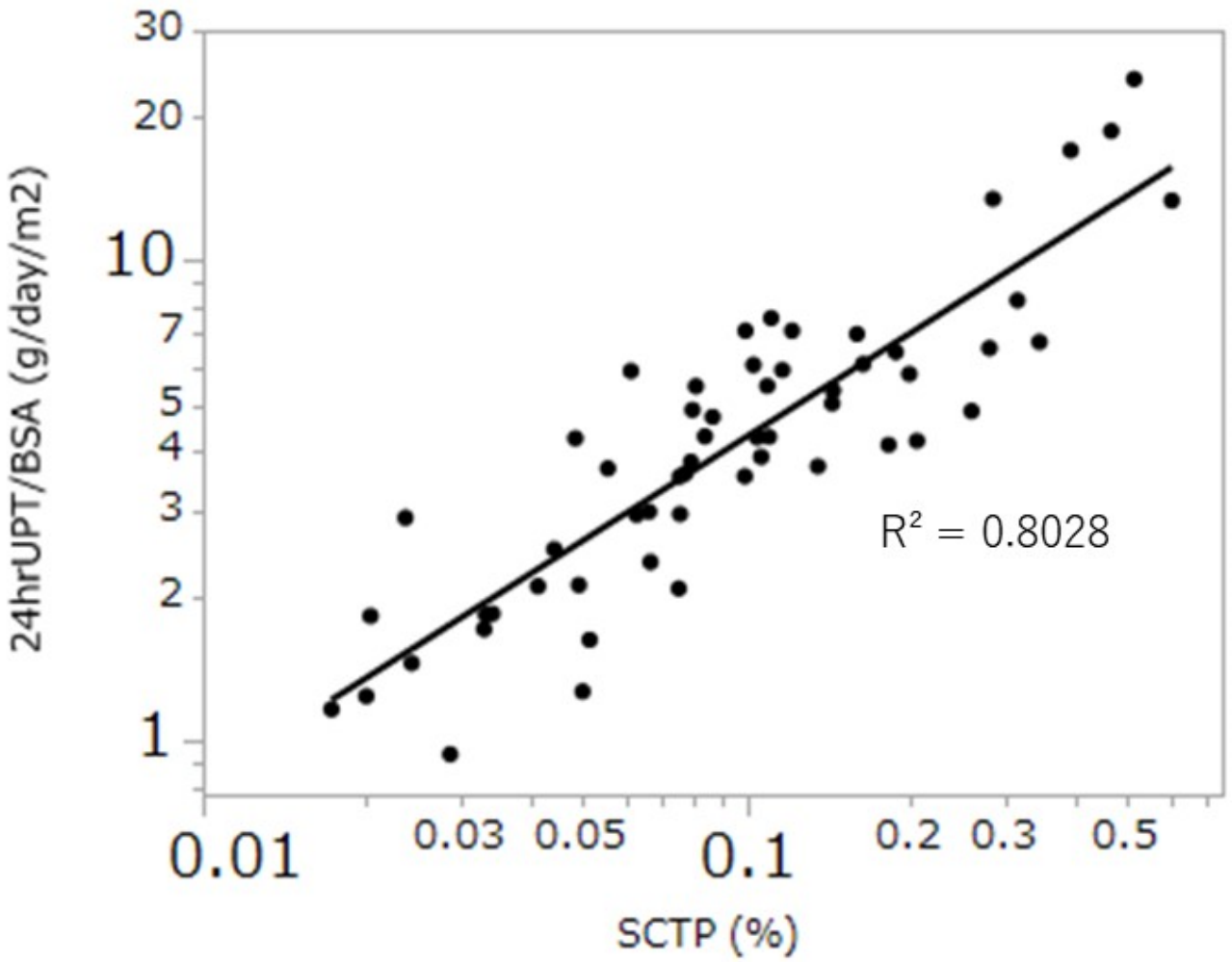

Figure 2

The correlation between 24hrUPT/BSA and SCTP, measured using a small portion of the early-morning urine sample (n = 58, r = 0.90)

24hrUPT: 24-hour urinary protein test.

BSA: body surface area.

SCTP: sieving coefficient of the serum total protein.

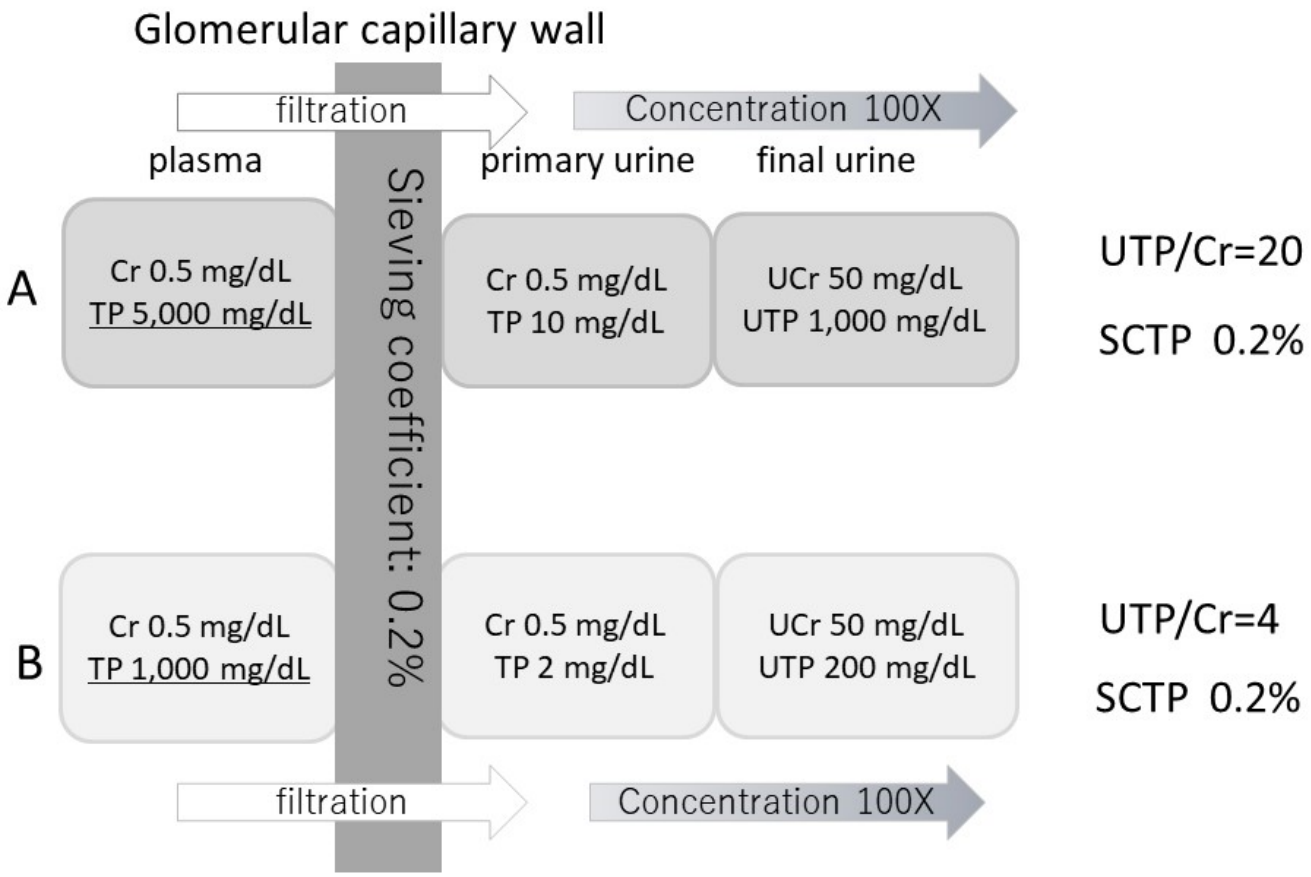

Figure 3

Comparison between UTP/Cr in cases with plasma total protein of 5000 mg/dL (A) and 1000mg/dL (B) with the same sieving coefficient（SCTP: 0.2%).

When plasma protein decreased from 5000 mg/dL to 1000 mg/ dL, U-TP/Cr would decrease from 20 (A) to 4 (B).

UTP/Cr: urinary protein/creatinine ratio.

SCTP: sieving coefficient of the serum total protein

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- RawData.xlsx