

Correlation between glomerular filtration rate and urinary N acetyl-beta-D glucosaminidase in children with persistent proteinuria in chronic glomerular disease

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Purpose: Urinary excretion of N acetyl-beta-D glucosaminidase (NAG) and β_2 -microglobulin (β_2 -M) was increased in the presence of proximal tubular damage. Based on these urinary materials, we investigated the ability of expecting renal function in chronic glomerular diseases. In this study, we evaluated the relationship between glomerular filtration rate (GFR) urinary NAG, and urinary β_2 -M.

Methods: We evaluated 52 children with chronic kidney disease at the Chung-Ang University Hospital between January 2003 and August 2009. We investigated the 24-hour urinalysis and hematologic values in all 52 patients. Serum creatinine, creatinine clearance (Ccr), serum cystatin C, urinary β_2 -M and urinary NAG were measured.

Results: Out of 52 patients, there were 13 children with minimal change in disease, 3 children with focal segmental glomerulosclerosis, 17 children with immunoglobulin A nephropathy, 15 children with Henoch-Schönlein purpura nephritis, 3 children with poststreptococcal glomerulonephritis, and 1 child with thin glomerular basement membrane disease. In these patients, there were significant correlation between the Ccr and urinary NAG ($r=-0.817$; $P<0.01$), and between the GFR (as determined by Schwartz method) and urinary NAG ($r=-0.821$; $P<0.01$). In addition, there was a significant correlation between the GFR (as determined by Bokenkamp method) and urinary NAG ($r=-0.858$; $P<0.01$).

Conclusion: In our study, there was a significant correlation between the GFR and urinary NAG, but there was no correlation between the GFR and urinary β_2 -M, suggesting that the GFR can be predicted by urinary NAG in patients with chronic glomerular disease.

Key words: Proteinuria, Glomerular filtration rate, N acetyl-beta-D glucosaminidase

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Introduction

Proteinuria is defined as a condition where urinary protein level is greater than 150 mg/24-hour in adults, and greater than 4 mg/m²/hr in children. Chronic glomerular nephritis occurs when proteinuria continues for more than a 3 month period¹⁾. Proteinuria exists as several forms; there is glomerular proteinuria that is caused by loss of the function of the glomerular barrier, and tubular proteinuria that is caused by failure of resorption of protein that filtrates in the glomerulus²⁾. The kidney diseases that chief symptom is proteinuria have different pathological findings; in nephrotic syndrome, glomerular injury appears in the early period of nephrotic syndrome, and progressive tubular injury appears in late period, and in tubulointerstitial disease, tubular dysfunction appears in the early period, and secondary glomerular injury appears in the late period³⁾.

The urinary excretion of β_2 -microglobulin (β_2 -M) and N-acetyl- β -D-glucosaminidase (NAG) has been used as a marker of renal tubular cell injury. β_2 -M is a small circulating protein with a molecular weight of 11,800, which is partly filtrated at the glomerulus. After glomerular filtration, β_2 -M is normally 99.9% reabsorbed in proximal tubule and degraded in the lysosome of the renal tubular cell, and finally it is excreted in the urine^{4,5)}. In tubular disorders, this mechanism is impaired, and urinary excretion of β_2 -M is increased. NAG is one of the low molecular weight proteins found in high concentrations in the lysosomes of renal proximal tubules. The increase of urinary excretion of NAG usually implies there is renal tubular injury⁶⁾.

Glomerular filtration rate (GFR) is widely used as the best measurement of kidney function. Serum creatinine is most commonly used to estimate GFR because it can be filtrated through glomerular basement membrane freely, and is not reabsorbed in the proximal tubule^{7,8)}. The creatinine clearance is measured by serum creatinine using the Schwartz method ($K [\text{coefficient}] \times [\text{height}/\text{serum creatinine}]$)⁹⁾. It may also be measured by the Bokenkamp method ($137/\text{cystatin C}-20.4$)¹⁰⁾. The decrease of GFR indicates the glomerular injury, and the increase of excretion of tubular enzyme indicates the tubular injury. As the kidney disease is chronic and the proteinuria continues, the glomerular injury can have an effect on the tubular injury; the increase of the protein that is filtrated through the glomerulus can have an effect on reabsorption of the protein in the renal tubule. We hypothesize that evaluation of the urinary tubular enzyme can be helpful to predict kidney function in chronic glomerular disease.

In this study, we evaluated the association of the GFR and urinary β_2 -M and urinary NAG in children with chronic kidney disease, and we evaluated glomerular function estimated by urinary β_2 -M and urinary NAG measurement in chronic kidney disease.

Materials and methods

1. Study population

Fifty-two children with various chronic glomerular disease, who had continuous proteinuria (≥ 4 mg/m²/hr) for more than 3 months, were examined at the Department of Pediatric Nephrology at Chung-Ang University Hospital, Seoul between January 2003 and August 2009. From the group 28 patients were male and 24 female, the mean age was 8.1 years, and the mean body weight was 30.6 kg.

Of these 52 patients, 13 children were diagnosed with minimal change disease, 3 children diagnosed with focal segmental glomerulosclerosis, 17 children with immunoglobulin A nephropathy, 15 children with Henoch-Schönlein purpura nephritis, 3 children with poststreptococcal glomerulonephritis, and 1 child was diagnosed with thin glomerular basement membrane disease (Table 1).

Nineteen patients were categorized with stage 1 chronic kidney disease, 27 patients were stage 2, 5 patients were stage 3, and 1 patient was stage 4 (Table 2).

2. Materials

We obtained urinary samples in the early morning and investigated the 24-hour urinalysis and the hematologic values in all of 52 patients. Serum creatinine, creatinine clearance, serum cystatin C, urinary β_2 -M and urinary NAG were measured in these patients.

The venous blood samples were kept in a freezer at -20°C. Twenty-four hours urinary samples were kept in a freezer at -20°C, and we added 1.0 NaOH to the urine samples to prevent β_2 -M degradation

Table 1. Distribution of Glomerular Disease Found in a Study Population

Renal diseases	No. (%)
Minimal change disease	13 (25.0)
Focal segmental glomerulosclerosis	3 (5.8)
Henoch-Schönlein purpura nephritis	15 (28.8)
Immunoglobulin A nephropathy	17 (32.7)
Poststreptococcal glomerulonephritis	3 (5.8)
Thin glomerular basement membrane disease	1 (1.9)
Total	52 (100)

Table 2. Distribution of Stages of Chronic Kidney Disease in a Study Population

Stage	GFR (mL/min/1.73 m ²)	No. (%)
1	>90	19 (36.6)
2	60-89	27 (51.9)
3	30-59	5 (9.6)
4	15-29	1 (1.9)
5	<15	0 (0)
Total		52 (100)

GFR, glomerular filtration rate.

in the acidic urine. We measured β_2 -M by radioimmunoassay using Coat A-Conunt Beta-2 Microglobulin IRMA kit (Diagnostic Products Co., Los Angeles, CA, USA). Serum cystatin C was measured by the particle enhanced nephelometric immunoassay (Dade Behring Marburg GmbH, Marburg, Germany).

We investigated the association of the GFR and the urinary β_2 -M and the urinary NAG in patients with proteinuria. GFR was estimated by measuring creatinine clearance using both Schwartz and Bokenkamp methods. We also evaluated the association of the creatinine clearance and the urinary β_2 -M and the urinary NAG at the each stage of chronic kidney disease.

3. Statistical analysis

We used the SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis. Data were expressed as mean values and the variables were evaluated by Pearson's correlation. Differences were considered significant with P value < 0.05.

Results

1. Correlation between GFR and urinary NAG in children with chronic glomerular disease

We evaluated the correlation between the GFR and the urinary NAG by measuring creatinine clearance using both Schwartz and Bokenkamp methods. There was a negative correlation between the creatinine clearance and the 24-hour urinary NAG in all patients with chronic proteinuria ($r = -0.817$; $P < 0.01$) (Fig. 1). As well there was a negative correlation between the 24-hour urinary NAG and the GFR by Schwartz method ($r = -0.821$; $P < 0.01$) (Fig. 2). We found a negative correlation between the 24-hour urinary NAG and the GFR

by Bokenkamp method ($r = -0.858$; $P < 0.01$) (Fig. 3).

2. Correlation between GFR and urinary β_2 -M in children with chronic glomerular disease

The correlation between GFR and urinary β_2 -M using creatinine clearance was evaluated using both Schwartz and Bokenkamp methods. There was no correlation between the creatinine clearance and the 24-hour urinary β_2 -M among the patients with chronic proteinuria ($r = -0.132$; $P = 0.352$) (Fig. 4). There was also no correlation between the 24-hour urinary β_2 -M and the GFR by Schwartz method ($r = -0.123$; $P = 0.386$) (Fig. 5). Additionally, there was no correlation between the 24-hour urinary β_2 -M and the GFR by

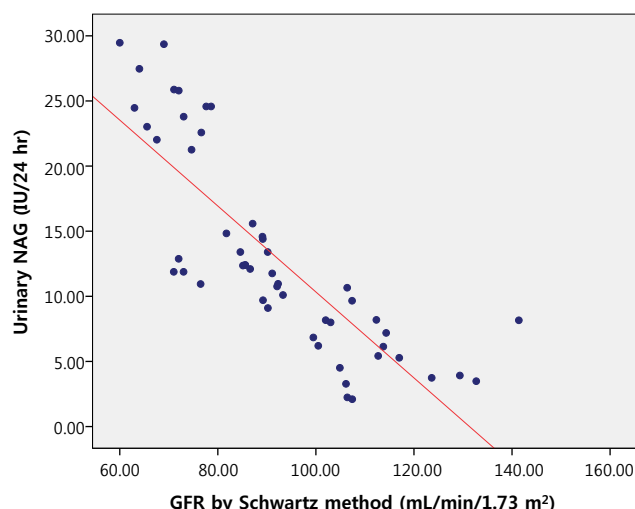


Fig. 2. Correlation between glomerular filtration rate (GFR), as measured by Schwartz method and 24-hour urinary N-acetyl- β -D-glucosaminidase (NAG) in children with chronic kidney disease ($r = -0.821$; $P < 0.01$).

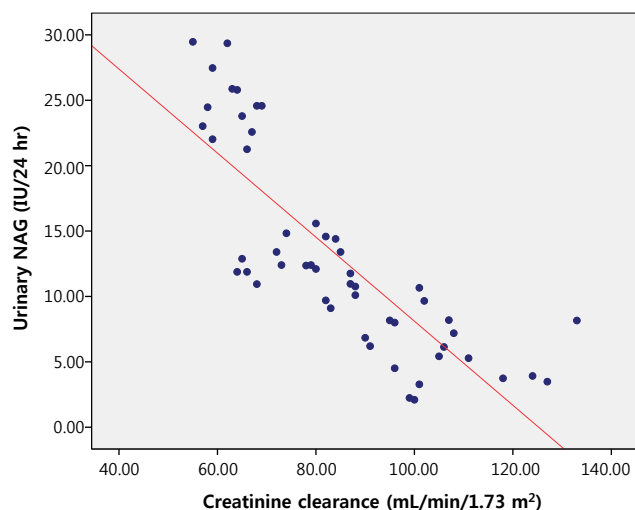


Fig. 1. Correlation between creatinine clearance and 24-hour urinary N-acetyl- β -D-glucosaminidase (NAG) in children with chronic kidney disease ($r = -0.817$; $P < 0.01$).

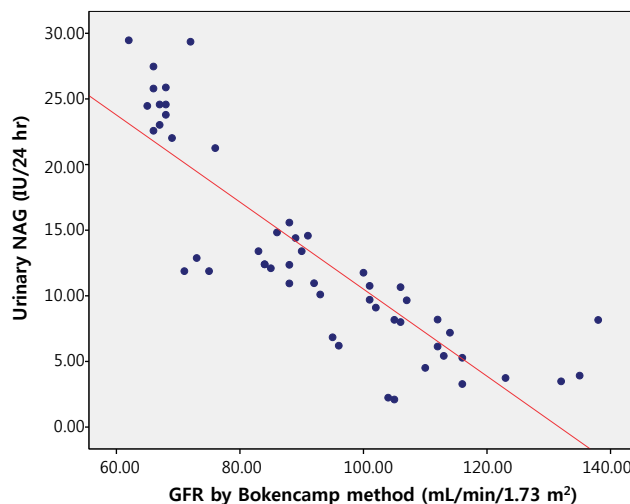


Fig. 3. Correlation between glomerular filtration rate (GFR), as measured by Bokenkamp method and 24-hour urinary N-acetyl- β -D-glucosaminidase (NAG) in children with chronic kidney disease ($r = -0.858$; $P < 0.01$).

Bokencamp method ($r=-0.231$; $P=0.099$) (Fig. 6).

3. Correlation between the creatinine clearance with respect to urinary NAG and urinary β_2 -M in the children with chronic glomerular disease

We evaluated the correlation between the GFR and the urinary NAG as well as with urinary β_2 -M using creatinine clearance in each stage of chronic kidney disease. In stage 1 chronic kidney disease, there was a negative correlation between the creatinine clearance and the 24-hour urinary NAG ($r=-0.503$; $P=0.028$). In stage 2 chronic kidney disease, there was a negative correlation between the creatinine clearance and the 24-hour urinary NAG ($r=-0.651$; $P<0.01$). With regards to stage 3 chronic kidney disease, there was again a negative

correlation between the creatinine clearance and the 24-hour urinary NAG ($r=-0.888$; $P=0.044$) (Fig. 7). But, in stage 1 chronic kidney disease, there was no correlation between the creatinine clearance and the 24-hour urinary β_2 -M ($r=-0.017$; $P=0.945$). In stage 2 chronic kidney disease, there was no correlation between the creatinine clearance and the 24-hour urinary β_2 -M ($r=-0.020$; $P=0.922$), as was also the case in stage 3 chronic kidney disease, where there was no correlation between the creatinine clearance and the 24-hour urinary β_2 -M ($r=-0.632$; $P=0.253$) (Fig. 8).

Discussion

In this study, there was a negative correlation between the creatinine clearance and the 24-hour urinary NAG and no correlation between the creatinine clearance and the 24-hour urinary β_2 -M in all patients with chronic proteinuria. These results are consistent when they are compared using both Schwartz and Bokencamp methods for evaluation. In each stage of chronic kidney disease, there was a negative correlation between the creatinine clearance and the 24-hour urinary NAG and no correlation between the creatinine clearance and the 24-hour urinary β_2 -M.

Among the several methods for evaluating the kidney function, the calculation of GFR is one of the most common methods. GFR is the measure of clearance of inulin, iothexol, Cr-ethylenediaminetetraacetic acid, TC-diethylene triamine pentaacetic acid and I-iothalamate, which are extracorporeal materials, and is also the measure of the clearance of serum creatinine, blood urea nitrogen and β_2 -microglobulin, which are body materials. In the clinical field, serum creatinine is the most commonly used because of

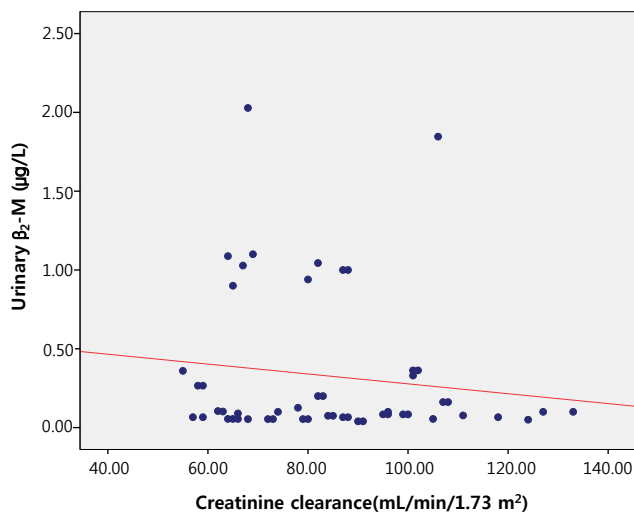


Fig. 4. Correlation between creatinine clearance and 24-hour urinary β_2 -microglobulin (β_2 -M) in the children with chronic kidney disease ($r=-0.132$; $P=0.352$).

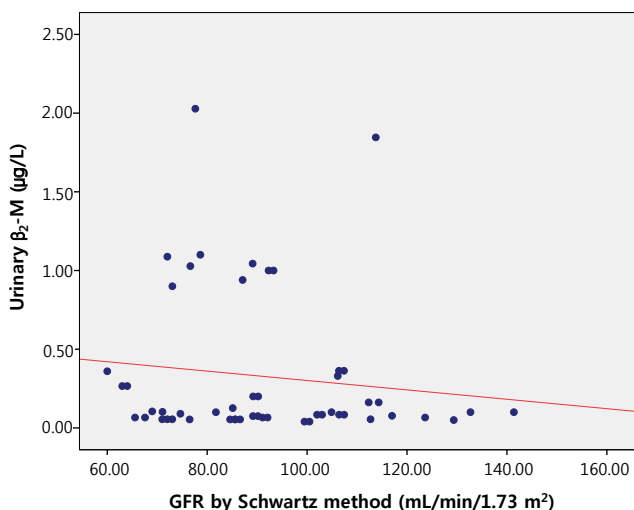


Fig. 5. Correlation between glomerular filtration rate (GFR), as measured by Schwartz method and 24-hour urinary β_2 -microglobulin (β_2 -M) in children with chronic kidney disease ($r=-0.123$; $P=0.386$).

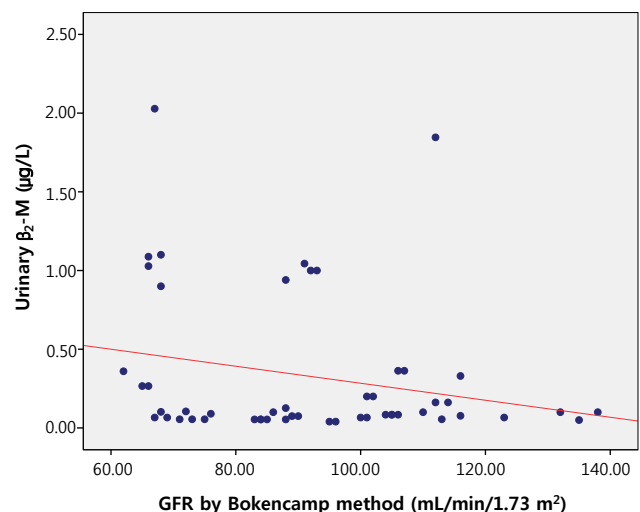


Fig. 6. Correlation between glomerular filtration rate (GFR), as measured by Bokencamp method and 24hr urinary β_2 -microglobulin (β_2 -M) in children with chronic kidney disease ($r=-0.231$; $P=0.099$).

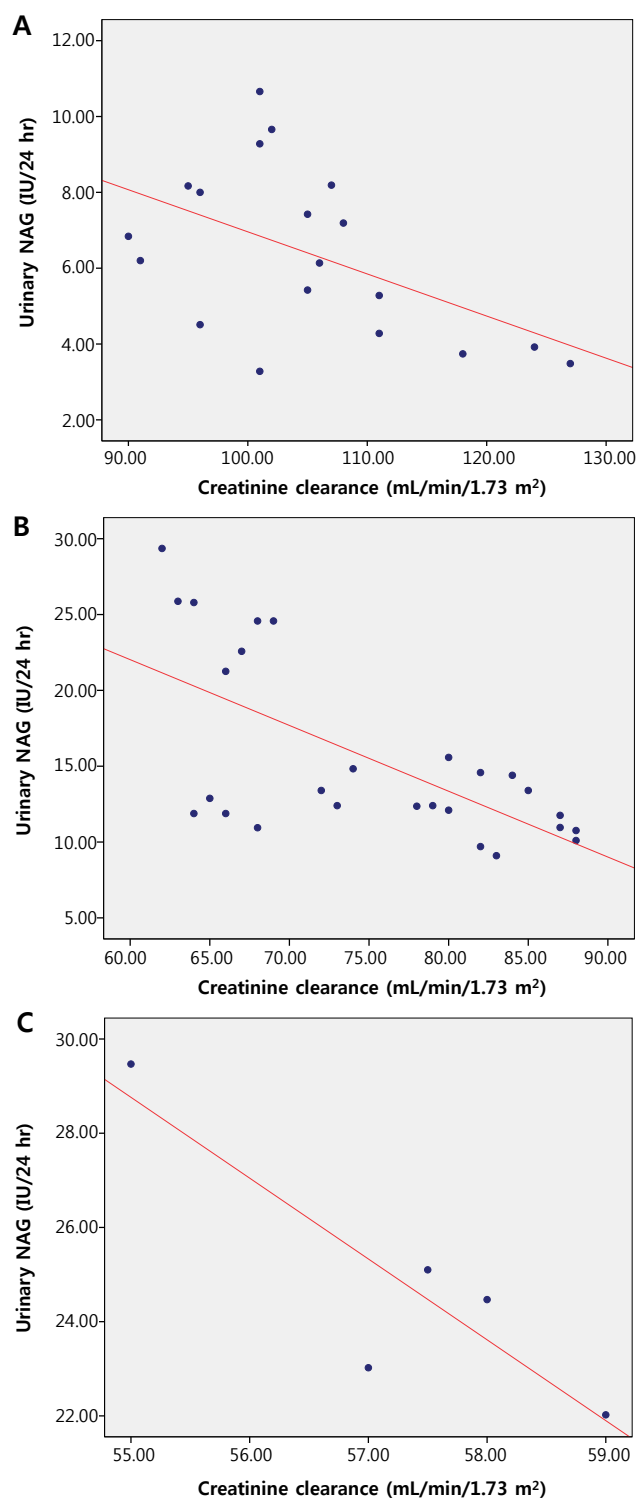


Fig. 7. Correlation between creatinine clearance and 24-hour urinary N-acetyl- β -D-glucosaminidase (NAG) in children with chronic kidney disease. (A) Stage 1 ($r=-0.503$; $P=0.028$), (B) stage 2 ($r=-0.651$; $P<0.01$) and (C) stage 3 chronic kidney disease ($r=-0.888$; $P=0.044$) are shown.

several advantages: it can filtrate through the glomerulus freely and is not reabsorbed in the proximal tubule, and additionally it has cost effectiveness^{7,8}. In this study, the creatinine clearance is represented by serum creatinine measurement, and evaluated by Schwartz method

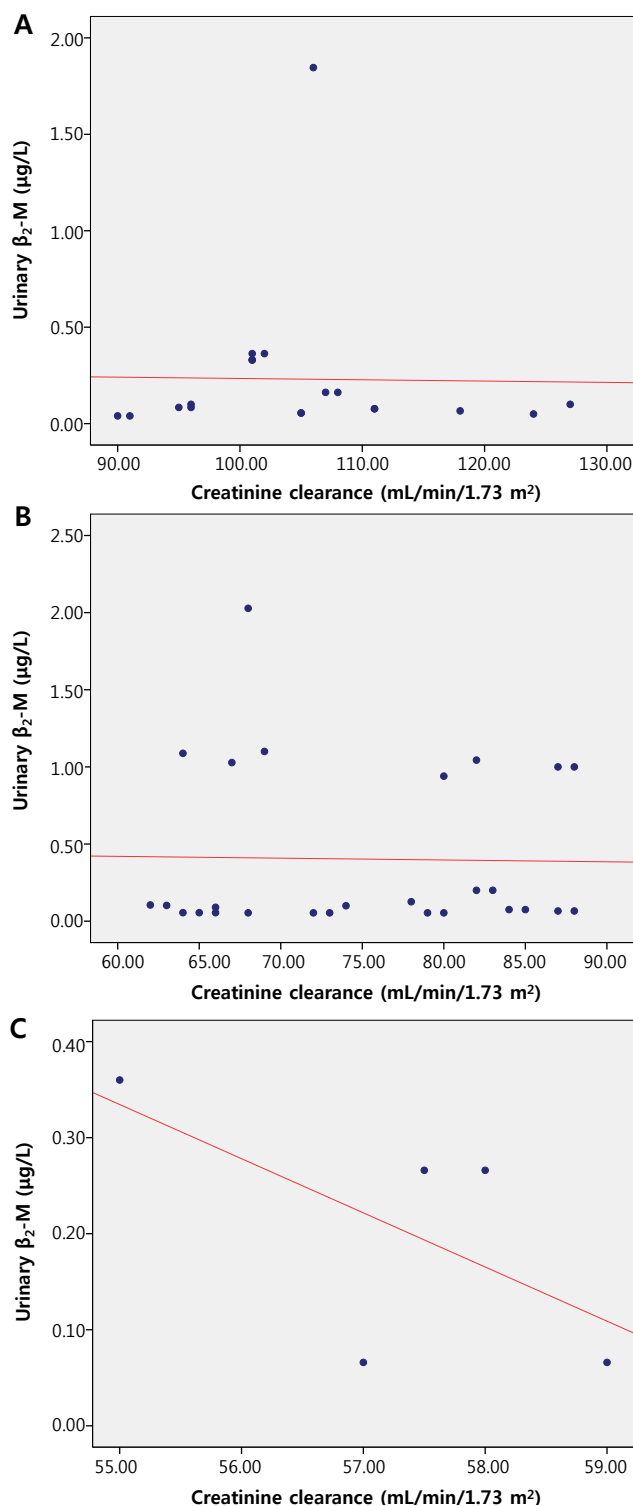


Fig. 8. Correlation between creatinine clearance and 24-hour urinary β_2 -microglobulin (β_2 -M) in children with chronic kidney disease. (A) Stage 1 ($r=-0.017$; $P=0.945$), (B) stage 2 ($r=-0.020$; $P=0.922$) and (C) stage 3 chronic kidney disease ($r=-0.632$; $P=0.253$) are shown.

($K[\text{coefficient}] \times [\text{height/serum creatinine}]^9$). It can also be measured by the Bokenkamp method ($137/\text{cystatin C}-20.4$)¹⁰. Cystatin C is a non-glycosylated protein which consists of 122 amino acids. It is synthesized in nucleated cells, and can filtrate through the glomerular

basement membrane freely and is reabsorbed in the proximal tubule. Thus, the serum concentration of cystatin C is dependent on the GFR^{11,12}.

There are many studies for proteins and enzymes as markers for early detection of renal tubular injury; β_2 -M, retinol-binding protein, alpha1-microglobulin, NAG¹³.

Bazzi et al.¹⁴ had studied urinary NAG as a marker of tubular injury. They measured the urinary NAG of patients with primary glomerulonephritis, and concluded that urinary NAG excretion could be considered as a marker of tubular damage in the early stage of glomerulonephritis, and might be an effective non-invasive test for assessing the damage. NAG is a low molecular weight protein and is found in high concentrations in the lysosomes of renal proximal tubules. The increase of urinary excretion of NAG means that there is renal tubular injury. NAG, especially the isoenzyme A, is in the lysosome of the renal tubular cell, and most urinary NAG comes from the renal tubular cell⁶. The urinary NAG has been used as a marker of renal tubular cell injury, and it increases in glomerular nephritis, diabetes mellitus nephritis, cadmium poisoning, radiographic agent nephropathy, chronic pyelonephritis¹⁵⁻¹⁷.

In our study, we measured the level of urinary NAG in patients with glomerulonephritis, and evaluated the correlation between the urinary NAG and GFR. We found that as the glomerular nephritis was progressive, where GFR is typically lower, the excretion of urinary NAG increased. Our results are similar to Bazzi's study.

According to Piscator¹³, the urinary β_2 -M is a good marker for early detection of proximal tubular damage. β_2 -M is isolated from the urine of patient with Wilson's disease and chronic cadmium poisoning by Berggard and Bearn¹⁸ in 1986. β_2 -M is naturally made in most nucleated cells, especially lymphocytes, and it exists in serum, urine, cerebrospinal fluid, saliva, and ascites^{19,20}. β_2 -M is a small circulating protein with a molecular weight of 11,800, which is partly filtrated at the glomerulus. After glomerular filtration, β_2 -M is normally 99.9% reabsorbed in the proximal tubule and degraded in the lysosome of the renal tubular cell, and finally excreted in the urine^{4,5}. The excretion of urinary β_2 -M does not always increase when the serum β_2 -M increases, and it can increase when the reabsorption decreases due to dysfunction of renal proximal tubule; cadmium poisoning, mercury poisoning, Balkan nephropathy, tubule injury due to antibiotics, acute glomerular nephritis, renal failure, urogenital malformation^{21,22}. In rheumatic arthritis, systemic lupus erythematosus, Crohn's disease, chronic liver disease, leukemia, and lymphoma, the concentration of serum β_2 -M increases. If these disorders do not exist, the concentration of serum β_2 -M is dependent on the GFR, and the excretion of urinary β_2 -M is dependent on the function of the proximal renal tubule^{23,24}.

In our study, we measured the level of the urinary β_2 -M in patients with glomerulonephritis, and evaluated the correlation between the urinary β_2 -M and the GFR. We found that there was no correlation between urinary β_2 -M and GFR, which was a different result in relation to urinary NAG although both urinary NAG and urinary β_2 -M are considered markers of tubular injury.

In Sherman's study, both urinary NAG and urinary β_2 -M increased in patients with progressive glomerulonephritis. Sherman et al.¹⁷ had shown that there was a tubulointerstitial involvement in the renal parenchymal diseases. Our results of study about urinary β_2 -M is different from that of Sherman's study.

Chiaromonte had conducted studies regarding urinary NAG and urinary β_2 -M in patients with renal disease. He found that both urinary NAG and urinary β_2 -M increased further in the progressive renal disease, and he concluded that these were useful and accurate markers of renal injury progression²³. In our study, we evaluated urinary NAG and urinary β_2 -M in each of the three stages of renal disease, which was classified by GFR. Chronic kidney disease is classified into five stages by GFR. Stage 1 is characterized by slightly diminished function with normal or relatively high GFR (>90 mL/min/1.73 m²). In stage 2 there is mild reduction in GFR (60 to 89 mL/min/1.73 m²), stage 3 consists of moderate reduction in GFR (30 to 59 mL/min/1.73 m²), stage 4 has a severe reduction in GFR (5 to 29 mL/min/1.73 m²), and stage 5 is established kidney failure (<15 mL/min/1.73 m²)¹¹. In our study, we found that as the stage was higher, the correlation between urinary NAG and GFR was a more steep negative coefficient of correlation.

Based on our findings, we believe that the urinary NAG can be used to predict the function of the glomerulus in chronic kidney disease. We also suggest further investigation is necessary in larger patient populations, along with follow-up studies. As well, further studies to investigate the differences between urinary NAG and urinary β_2 -M as evaluation markers will be necessary.

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