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Urinary N-Acetyl-beta-D-Glucosaminidase and beta 2-Microglobulin in Children with Various Renal Diseases

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= Abstract =

Purpose : Urinary N-acetyl-beta-D-glucosaminidase(NAG) and beta 2-microglobulin(B2M) is considered to be a marker of tubulointerstitial injury. The aim of this study was to examine the urinary levels of NAG and B2M in children with various renal diseases.

Methods : We studied 21 children(8.9±4.5 years, Male:Female=14:7) and they were divided into three groups: group I(steroid-sensitive nephrotic syndrome-4 patients), group II(various kinds of glomerulonephritis-4 patients), and group III(normal urinalysis or non-glomerular renal diseases-13 patients).

Results : Urinary NAG levels in groups I and II were significantly higher than those in group III(19.4±11.5 and 30.0±30.1 vs. 4.7±3.9, P=0.01), while urinary B2M levels did not differ among the 3 groups, although urinary NAG levels were positively correlated with urinary B2M levels(r=0.49, P=0.03). Urinary NAG and B2M levels were all correlated with proteinuria(r=0.79, $P<0.001$ and r=0.68, respectively, P=0.001) serum albumin(r=-0.72, $P<0.001$ and r=-0.57, respectively, P=0.01) and cholesterol(r=0.58, P=0.006 and r=0.56, respectively, P=0.013) levels.

Conclusions : Urinary excretions of NAG and B2M are increased in children with steroid-sensitive nephrotic syndrome and various kinds of glomerulonephritis, suggesting tubular dysfunction might be present in these diseases. **(J Korean Soc Pediatr Nephrol 2008; 12:143-149)**

Key Words : N-acetyl-beta-D-glucosaminidase, Beta 2-microglobulin, Tubulointerstitial injury, Children

INTRODUCTION

Urinary N-acetyl-beta-D-glucosaminidase (NAG), a lysosomal enzyme of 130 kDa molecular mass, is normally excreted in low amounts as a result of normal exocytosis process[1]. Urinary beta 2-microglobulin(B2M) is a low molecular weight protein that easily pass through the glomerular basement membrane, and is re-absorbed and catabolized at the proximal tubules[2].

It has been reported that their excretions are increased in various conditions associated with renal tubular damage, such as diabetes mellitus, nephrotic syndrome, vesicoureteral reflux, heavy metals poisoning, the use of aminoglycosides, valproate, contrast media or

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other nephrotoxic drugs[3-7]. However, the clinical usefulness of these parameters in children has not been fully elucidated.

In this study, we aimed to evaluate renal tubular function in children with various renal diseases through assessment of the urinary levels of NAG and B2M.

METHODS

We recruited 21 children with various renal diseases(8.9±4.5 years, Male:Female=14:7) from January 2003 to December 2003, and they were divided into three groups: group I(steroid-sensitive nephrotic syndrome-4 patients), group II(various kinds of glomerulonephritis-4 patients), and group III(normal urinalysis or non-glomerular renal diseases-13 patients).

In Group I, three patients were in a nephrotic state(proteinuria>40 mg/m^2/hr and serum albumin<2.5 g/dL) and one in a non-nephrotic state. Group II included focal segmental glomerulosclerosis(FSGS), lupus nephritis, acute poststreptococcal glomerulonephritis(APSGN), and glomerulonephritis due to vasculitis. Group III included normal urinalysis, idiopathic hematuria, hemorrhagic cystitis, lower urinary tract infection, lupus without nephritis, Henoch-Schoenlein purpura without nephritis, nut-cracker syndrome and idiopathic hypercalciuria(Table 1).

Random urine samples were obtained from

Table 1. Clinical characteristics of children with various renal diseases

Group	No.	Age	Sex	Diagnosis	NAG(0-10U/L)	B2M(0-0.25 mg/L)
I	1	3.0	M	SSNS(nephrotic state)	74.0	0.38
I	2	4.8	F	SSNS(nephrotic state)	24.4	-
I	3	4.5	M	SSNS(nephrotic state)	14.5	0.39
I	4	15.2	M	SSNS(non-nephrotic state)	7.2	0.44
II	5	2.3	F	FSGS	33.1	1.08
II	6	13.7	F	Lupus nephritis	24.0	0.55
II	7	6.3	M	APSGN	13.8	0.17
II	8	11.5	M	Glomerulonephritis due to vasculitis	6.8	0.34
III	9	10.5	F	Normal urinalysis	1.6	0.08
III	10	10.2	F	Idiopathic hematuria	2.2	0.29
III	11	2.5	M	Hemorrhagic cystitis	1.8	0.52
III	12	0.2	M	Lower urinary tract infection	6.9	-
III	13	11.1	F	Lupus without nephritis	1.5	0.28
III	14	9.3	M	HSP without nephritis	4.0	0.28
III	15	13.3	M	Nut-cracker syndrome	4.6	0.17
III	16	13.7	F	Nut-cracker syndrome	4.8	0.13
III	17	9.9	M	Nut-cracker syndrome	12.6	0.31
III	18	14.8	M	Nut-cracker syndrome	13.0	0.29
III	19	7.8	M	Idiopathic hypercalciuria	1.2	0.22
III	20	9.8	M	Idiopathic hypercalciuria	3.1	0.34
III	21	12.6	M	Idiopathic hypercalciuria	3.6	0.26

Abbreviations : NAG, *N-acetyl*-beta-D-glucosaminidase; B2M, beta 2-microglobulin; SSNS, Steroid sensitive nephrotic syndrome; FSGS, Focal segmental glomerulosclerosis; APSGN, Acute poststreptococcal glomerulonephritis; HSP, Henoch-Shoenlein purpura

each child. The amount of protein in the urine was assessed as 1+(30 mg/dL), 2+(100 mg/dL), 3+(300 mg/dL) or 4+(1,000 mg/dL)[8], and hematuria was defined as more than 5 erythrocytes per high-power field(in a sediment of approximately 10 mL of freshly voided urine)[9].

Urinary NAG and B2M were analyzed as parameters for renal tubular function. For the measurement of urinary NAG, spectrophotometric assay was performed using chlorophenol red-NAG(CPR-NAG) which is hydrolyzed by NAG with the release of chlorophenol red, which can be measured photometrically at 575 nm on a JCA-BM 12. Urinary B2M was measured by radioimmunoassay(RIA).

Statistical analysis was performed using SPSS version 11.0(SPSS Inc, Chicago, IL). Continuous variables were expressed as mean ± standard deviation. The differences among three groups were analyzed by Kruskal-Wallace test. Correlation between two variables was assessed by Spearmans rank correlation test. $P<0.05$ was considered significant.

RESULTS

Clinical characteristics of the groups are shown in Table 1. Urinary NAG levels in groups I and II were significantly higher than those in group III(19.4±11.5 and 30.0±30.1 vs. 4.7±3.9, $P=0.01$), while urinary B2M levels did not differ among the 3 groups(Table 2).

In group I, NAG levels were increased in three of the four patients and B2M in all 3 patients. In group II, two patients with FSGS and lupus nephritis showed significantly increased levels of NAG and B2M. In one patient with APSGN, NAG was mildly elevated but B2M was within normal range. In one patient with glomerulonephritis due to vasculitis, however, NAG was normal, but B2M was significantly increased. In group III, two of the four patients with nut-cracker syndrome showed increased level of both NAG and B2M.

Urinary NAG levels were positively correlated with urinary B2M levels(NAG=32.6×B2M+0.77, r=0.49, $P=0.03$)(Fig. 1). In 21 patients, urinary NAG levels were correlated with proteinuria (NAG = 7.24 x urinary protein+2.32, r=0.79, $P<0.001$)(Fig. 2), serum al-

Table 2. Laboratory findings among the three groups

	Group I(n=4)	Group II(n=4)	Group III(n=13)
NAG(U/L)	30.0±30.2**	19.4±11.5**	4.7±3.9
B2M(mg/L)	0.4±0.0	0.5±0.4	0.3±0.1
Proteinuria(+)	2.2±0.5*	2.5±0.6*	0.8±0.9
Albumin(g/dL)	1.8±0.9*	2.9±1.1*	4.2±0.4
Cholesterol(mg/dL)	381.7±105.3*	323.3±143.0*	135.2±23.9
CCr(mL/min/1.73 m^2)	94.8±40.3	79.2±36.8	80.1±28.6

*$P<0.01$ in comparison to Group III
**$P<0.05$ in comparison to Group III
Abbreviation : NAG, N-acetyl-beta-D-glucosaminidase; B2M, beta 2-microglobulin, CCr; Creatinine Clearance

bumin(NAG=-9.18×albumin+44.52, r=-0.72, P <0.001)(Fig. 3) and serum cholesterol(r=0.58, P=0.006) levels. Urinary B2M levels were also correlated with proteinuria(r=0.67, P=0.001), serum albumin(r=-0.57, P=0.011) and serum cholesterol(r=0.56, P=0.013). However, urinary NAG or B2M did not correlate with serum creatinine (P>0.05) or uric acid levels(P>0.05).

In various renal diseases, such as HSP without nephritis, lupus without nephritis, idiopathic hematuria, idiopathic hypercalciuria, and cystitis, NAG levels were within normal ranges, but B2M were variably increased.

There was no significant correlation between CCr and NAG or B2M(P>0.05).

DISCUSSION

The present study demonstrates that urinary NAG and B2M are increased in children with SSNS and various kinds of glomerulonephritides, suggesting the presence of renal tubular impairment in these patients.

NAG excretion has been considered as a non-invasive and reliable marker of the tubulo-toxicity of proteinuria in the early stage of membranous nephropathy, FSGS and minimal change disease in adult studies[6,10]. In glomerular diseases, analysis of isoenzymes of NAG demonstrated that the increased urinary excretion of this enzyme is due to an increased release by the renal tubular cells and not to increased filtration across the damaged glo-

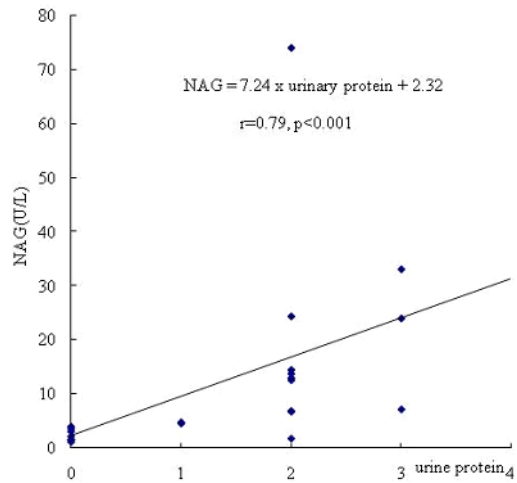

Fig. 2. Relationships between urinary NAG and proteinuria.

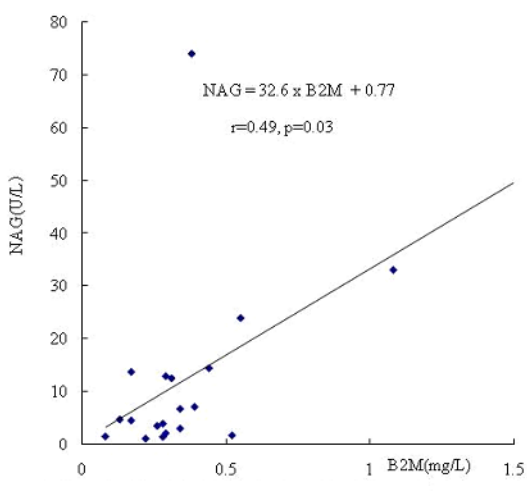

Fig. 1. Relationships between urinary NAG and B2M levels.

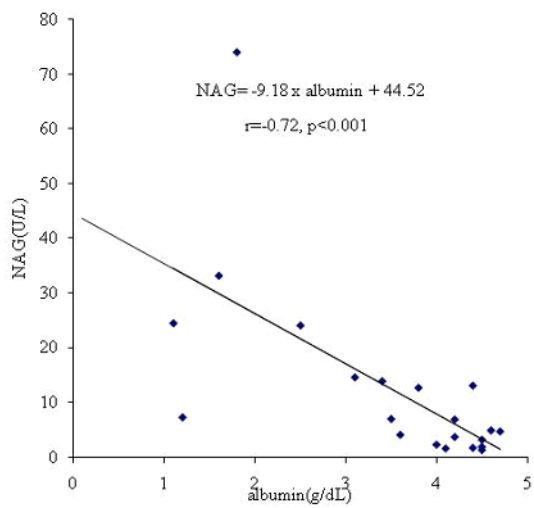

Fig. 3. Relationships between urinary NAG and serum albumin levels.

merular capillary wall[11].

Regarding childhood nephrotic syndrome, Valles et al. reported that urinary NAG and B2M levels were significantly increased in the steroid-resistant nephrotic syndrome(SRNS) group as compared to the SSNS in remission and controls, and there were no differences between the SRNS group and SSNS in relapse[12]. They concluded that proximal tubule cell dysfunction, partially affected by massive albuminuria, might account for the higher values of B2M and NAG excretion in the SRNS patients and urinary B2M and NAG levels are not helpful in identifying histological evidence of structural tubulointerstitial damage in children with SRNS[12]. In our study, there were five patients with nephrotic syndrome (one with FSGS and four with SSNS, and urinary NAG or B2M levels were significantly increased in these patients as compared to those of the control group, which were in accordance with previously reports[4, 12] Furthermore, NAG was significantly decreased in non-nephrotic state compared to nephritic state. However, Tsau et al. had reported the contrasting results that there were no significant changes in urinary NAG levels during heavy proteinuria and after remission, and no correlation was found between urinary protein excretion and NAG in children with nephrotic syndrome[13].

Regarding childhood lupus nephritis, Marks et al. reported that the lupus nephritis group had elevated urinary NAG levels than non-nephritis lupus or normal children[14]. Similarly, our study showed that one patient(No. 6) with lupus nephritis had an elevated urinary NAG level, while the other with non-nephritis lupus showed a normal NAG level. However, Marks et al pointed that tubular dysfunction with elevated urinary NAG levels was present in 2 lupus non-nephritis patients with no evidence of glomerular disease, who eventually developed biopsy-proven lupus nephritis later, suggesting evidence of tubular dysfunction in lupus non-nephritis patients might help to identify lupus nephritis prior to the onset of albuminuria[14]. Therefore, the increase of urinary NAG level can be used as an early index for the damage of tubular epithelium, and may be useful in detecting or monitoring renal disease in lupus patients.

Group III include normal uinalysis, cystitis and Nut-cracker syndrome etc. together because these diseases usually dont have tubular injury.

We had one patient with HSP without nephritis, and urinary NAG level was within normal range. Müller et al. demonstrated that urinary NAG or alpha1-microglobulin levels were highest(>mean +4 SD) in patients with early kidney involvement, intermediately high (>mean +2 SD, <mean +4 SD) in those who developed renal involvement during follow-up, and normal in those with a benign further clinical course. Therefore, urinary NAG levels may be a good prognostic marker for the development of HSP nephritis[15].

In idiopathic hypercalciuria, tubular impairment might occur, especially in patients with urolithiasis or nephrocalcinosis[16]. However, they could not find a direct relationship between the urinary NAG level and the degree of calcium leakage[16], although the relationship between the urinary NAG and the daily urinary calcium excretion was statistically signi-

ficant in an experimental model of hypercalciuria[17]. In our study, all three patients with idiopathic hypercalciuria without nephrocalcinosis showed normal urinary NAG levels, but one of them did increased B2M level.

We also had four patients with nut-cracker syndrome, two of whom(Nos.17 and 18) showed increased levels of NAG and B2M, suggesting left renal venous hypertension might cause the excretion of proteinuria and these urinary enzymes.

It is reported that creatinine correction is rather incorrect because of diurnal variation in NAG level[18].

However, there are some limitations in this study(1) small numbers of patients,(2) a lack of control groups, and(3) a retrospective nature of the study.

Nevertheless, we suggest that the measurement of NAG or B2M may be a useful and non-invasive examination for assessing a proximal tubular damage in pediatric patients with nephrotic syndrome or the early stages of various kinds of glomerulonephritides.

한 글 요 약

다양한 신장질환 환아들에서 요중 N-Acetyl-beta-D-Glucosaminidase와 beta 2-Microglobulin

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목 적 : 요 중 N-acetyl-beta-D-glucosaminidase(NAG) 와 beta 2-microglobulin(B2M) 은 신세뇨관 간질 손상의 표적으로 생각된다. 이 연구의 목적은 다양한 신장 질환 환아 에서 요 중 NAG 와 B2M 수치를 검사해 보는 것이다.

방 법 : 우리는 21명의 환아(8.9±4.5세, 남:녀=14:7)를 조사해서 세 군으로 분류하였다: I군(스테로이드에 반응하는 신증후군 환아-4명), II군(다양한 종류의 사구체 신염 환아-4명), III군(정상뇨 또는 비사구체성 신장 질환 환아-13명).

결 과 : I군과 II군에서의 요 중 NAG 수치는 III군에서보다 유의하게 높았다.(19.4±11.5와 30.0±30.1 vs. 4.7±3.9 , P=0.01) 반면에 요 중 NAG 수치와 B2M 수치가 양의 상관 관계에 있음에도 (r=0.49, P=0.03), 요 중 B2M 수치는 세 군에서 차이가 없었다. 요 중 NAG 와 B2M 수치는 모두 단백뇨, 혈중 알부민, 콜레스테롤과 상관관례를 보였다.

결 론 : NAG 와 B2M 의 배설량은 스테로이드에 반응하는 신증후군 환아와 다양한 종류의 사구체 신염 환아에서 증가되어 있었다. 이는 이런 질환들에서 세뇨관 기능저하를 의미하는 것으로 보인다.

참 고 문 헌

1) Price RG. The role of NAG(N-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin Nephrol 1992;38(Suppl 1): 14-9.
2) Berggard I, Bearn AG. Isolation and properties of a low molecular weight beta-2-globulin occurring in human biological fluids. J Biol Chem 1968;243:4095-103.
3) Mohammadi-Karakani A, Asgharzadeh-Haghighi S, Ghazi-Khansari M, Hosseini R. Determination of urinary enzymes as a marker of early renal damage in diabetic patients. J Clin Lab Anal 2007;21:413-7.
4) Caliskan S, Hacibekiroglu M, Sever L, Ozbay G, Arisoy N. Urinary N-acetyl-beta-D-glucosaminidase and beta 2-microglobulin excretion in primary nephrotic children. Ne-

phron 1996;74:401-4.
5) Miyakita H, Puri P. Urinary levels of N-acetyl-beta-D-glucosaminidase: a simple marker for predicting tubular damage in higher grades of vesicoureteric reflux. Eur Urol 1994;25:135-7.
6) Bazzi C, Petrini C, Rizza V, Arrigo G, Napodano P, Paparella M, et al. Urinary N-acetyl-beta-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. Nephrol Dial Transplant 2002;17:1890-6.
7) Skalova S. The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase(NAG) activity in the detection of renal tubular impairment. Acta Medica(Hradec Kralove) 2005;48:75-80.
8) Loghman-Adham M. Evaluating proteinuria in children. Am Fam Physician 1998;58:1145-52, 58-9.
9) Bergstein J. Conditions particularly associated with hematuria. In: Nelson textbook of pediatrics, 16th ed. philadelphia: Saunders; 2000.
10) Fede C, Conti G, Chimenz R, Ricca M. N-acetyl-beta-D-glucosaminidase and beta2-microglobulin: prognostic markers in idiopathic nephrotic syndrome. J Nephrol 1999;12:51-5.
11) Hultberg B, Ravnskov U. The excretion of N-acetyl-beta-glucosaminidase in glomerulonephritis. Clin Nephrol 1981;15:33-8.
12) Valles P, Peralta M, Carrizo L, Martin L, Principi I, Gonzalez A, et al. Follow-up of steroid-resistant nephrotic syndrome: tubular proteinuria and enzymuria. Pediatr Nephrol 2000;15:252-8.
13) Tsau YK, Chen CH, Yang LF, Sheu JN. Urinary N-acetyl-beta-glucosaminidase in children with idiopathic nephrotic syndrome. Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi 1991;32:286-90.
14) Marks SD, Shah V, Pilkington C, Woo P, Dillon MJ. Renal tubular dysfunction in children with systemic lupus erythematosus. Pediatr Nephrol 2005;20:141-8.
15) Muller D, Greve D, Eggert P. Early tubular proteinuria and the development of nephritis in Henoch-Schonlein purpura. Pediatr Nephrol 2000;15:85-9.
16) Skalova S, Kutilek S. Renal tubular impairment in children with idiopathic hypercalciuria. Acta Medica(Hradec Kralove) 2006;49:109-11.
17) Turkmen M, Kavukcu S, Islekel H, Sarioglu S, Akhunlar H, Gokden N, et al. Urinary N-acetyl-beta-D-glucosaminidase activity in rabbits with experimental hypercalciuria. Pediatr Nephrol 1997;11:481-4.
18) Kui Nyung Yi, Oh Hun Kwon. Urinary beta-N-Acetylglucosaminidase, NAG. In: Clinical pathology file, 3rd ed. Seoul: Eui-Hak Publishing, 2000:411-3.