

## The Clinical Value of Urinary N-Acetyl- $\beta$ -D-Glucosaminidase Levels in Childhood Age Group

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N-acetyl- $\beta$ -D-glucosaminidase is a high molecular-weight lysosomal enzyme found in many tissues of the body. It cannot pass into glomerular ultrafiltrate due to its high molecular weight. However, this enzyme shows high activity in renal proximal tubular cells, and leaks into the tubular fluid as the ultrafiltrate passes through proximal tubules. When proximal tubular cells are injured due to any disease process including glomerular proteinuria, nephrolithiasis, hyperglycemia, interstitial nephritis, transplant rejection or nephrotoxic agents such as antibiotics, antiepileptics, or radiocontrast agents, its urine level increases and thus is used as a reflection of proximal tubular cell necrosis. However, the clinical use of urinary N-acetyl- $\beta$ -D-glucosaminidase determination is limited in childhood because of certain technical problems. In addition, the urinary level of this enzyme changes with the maturational level of proximal tubular cells. Thus, difficulties are involved in assessing normal urine levels of this enzyme for age. On the other hand, successive measurements of urinary N-acetyl- $\beta$ -D-glucosaminidase during the longitudinal follow-up of the patients may enhance its clinical use as an indicator of ongoing tubular injury.

**Key words:** childhood, urine, N-acetyl- $\beta$ -D-glucosaminidase, proximal tubular injury

### Background

Normal urinary protein excretion is about 150 mg/day or less. Urinary protein is heterogenous in type and source. Plasma proteins, renal tissue enzymes, and renal secretions might be the source of urinary proteins. Increased protein excretion is associated with renal pathology, and the qualitative composition of the protein has diagnostic value [1].

N-acetyl- $\beta$ -D-glucosaminidase (NAG) is a high molecular weight lysosomal enzyme. It is not specific to the kidney and found in other tissues as well. However, it cannot pass into glomerular ultrafiltrate due to its high

molecular weight. Thus, urinary NAG is of renal origin. This enzyme shows high activity in renal proximal tubular cells, and leaks into the tubular fluid as the ultrafiltrate passes through proximal tubules. When proximal tubular cells are injured due to disease or nephrotoxic agent, urinary NAG level ( $U_{NAG}$ ) increases. Thus, increased  $U_{NAG}$  reflects proximal tubular cell necrosis [1].

### Factors effecting urinary N-acetyl- $\beta$ -D-glucosaminidase levels (Table 1)

$U_{NAG}$  is measured by means of the colorimetric method. Although urinary excretion of the enzyme alanine aminopeptidase, localized in the brush border of tubular cells, increases as well in the case of proximal tubular necrosis, measurement of  $U_{NAG}$  is preferred for clinical assessment. The activity of alanine aminopep-

tidase is lost when urine is stored frozen. N-acetyl- $\beta$ -D-glucosaminidase, on the other hand, remains stable during these procedures [1], while urinary enzyme levels could be affected by urine flow rate. In order to minimize this effect, the level of enzymuria is expressed as the ratio of enzyme activity to urinary creatinine level.

The difficulty in assessing the normal range of  $U_{NAG}$  for age in childhood limits its clinical use. Longitudinal determination of more than one  $U_{NAG}$  level is more helpful in clinical assessment. For instance, if we administer an antibiotic with nephrotoxic potential to an infant, serial urinary NAG/Cr assessments could reveal the early renal injury related to the drug.  $U_{NAG}$  is affected by age, body weight and height, but not by sex. Urinary NAG/creatinine ratio ( $U_{NAG}/Cr$ ) has been found to decrease during the first 3 years of life [2]. On the other hand,  $U_{NAG}$  reaches its maximum at 14 years of age in boys (Tanner stage 4 of genital development) and at 13 years of age in girls (Tanner stage 3 of genital development) [3].

Lysosomal enzymuria levels change depending on the maturational level of renal tubular epithelium, and it is increased in premature newborns compared to mature newborns [4, 5].  $U_{NAG}$  decreases as the renal tubular cells mature starting from birth. However, it has been reported that  $U_{NAG}$  does not decrease significantly during the newborn period in healthy term newborns [6]. Perinatal asphyxia increases  $U_{NAG}$  levels [4, 7]. On the other hand,  $U_{NAG}$  could be used to detect renal tubular injury during various therapeutic approaches in newborns. For example, it has been reported that vancomycin does not increase  $U_{NAG}$ , and thus can be used for severe infections in very low birth-weight infants [8]. High levels of indirect hyperbilirubinemia was found to increase  $U_{NAG}$  in newborns [9], but the levels lower than 18.4 mg/dl did not affect  $U_{NAG}$  in term newborns [10]. In newborns, urine trehalase/creatinine ratio was found to increase more than the  $U_{NAG}/Cr$  ratio in cases of proximal renal tubular cell injury. However, there was a positive correlation between these parameters [11].

### Urinary NAG levels in childhood diseases

In recent years, the computerized urine protein expert system has been used to evaluate childhood nephropathies in the same manner as in adult patients. Urine levels of creatinine, total protein, albumin,  $\alpha$ -1 microglobulin,  $\alpha$ -2 macroglobulin, and NAG, urinary dipstick levels of granulocyte esterase and hemoglobin, and serum creatinine levels are determined and loaded into a computer program. This program could detect glomerular and tubular proteinuria with 75 and 100% specificity [12].

$U_{NAG}$  has been investigated in detail in pathologies associated with proteinuria. In patients with minimal change disease and focal segmental glomerulosclerosis (FSGS),  $U_{NAG}/Cr$  ratio increases. In addition, in FSGS, urine retinol binding protein/creatinine ratio has been reported to increase [13]. It was reported that  $U_{NAG}$  increased along with proteinuria in glomerular diseases, but this finding could not be regarded as a negative prognostic sign [14]. In glomerular proteinuria,  $U_{NAG}$  could increase independently of the primary renal pathology [15].

$U_{NAG}$  increases before the appearance of severe proteinuria in insulin-dependent diabetes mellitus.  $U_{NAG}$  is related to blood glucose level, and it decreases to normal levels when the blood glucose level is well controlled [16, 17].

**Table 1** Factors affecting urinary N-acetyl-beta-D-glucosaminidase activity

Physiologic factors	- Age - Weight - Height - Urine flow rate
Childhood diseases	- Glomerular proteinuria - Insulin-dependent diabetes mellitus - Short stature (during growth hormone therapy) - $\beta$ -thalassemia major (due to iron overload) - Nephrolithiasis / nephrocalcinosis / hypercalciuria - Upper urinary tract infections / reflux nephropathy - Renal transplantation (acute rejection, cyclosporine toxicity) - Cyanotic congenital heart diseases
Nephrotoxicity	- Antibiotics ( $\beta$ -lactams, aminoglycosides) - D-penicillamine - Cisplatin - Antiepileptics - Radiocontrast agents
Others	- Unilateral nephrectomy, unilateral renal agenesis - Inflammatory bowel disease - Glucose-6-phosphatase deficiency - Increased plasma endothelin-I level

Although some have indicated that  $U_{NAG}$  does not change with recombinant human growth hormone treatment in short children with growth hormone deficiency [18], some studies have reported increased levels of  $U_{NAG}$  [19].

$U_{NAG}/Cr$  has been determined to increase in patients with beta thalassemia major, and was found to parallel urine zinc levels [20]. Some studies have indicated that increased  $U_{NAG}$  in these children is related to iron loading, and with regular deferoxamine treatment  $U_{NAG}$  decreases along with reduced iron load [21].

Increased  $U_{NAG}$  in children with renal calculi has been reported to be related to tubular injury [22]. Likewise,  $U_{NAG}$  has been found to be increased in hypercalciuric children and this was also related to tubular injury [23]. However,  $U_{NAG}$  level was not affected unless the calciuria level was very high [24].  $U_{NAG}$  was also found to increase in nephrocalcinosis [25].  $U_{NAG}$  level also increases during extracorporeal shock wave lithotripsy [26, 27].

Increased  $U_{NAG}$  due to interstitial and tubular injury in upper urinary tract infections (UTI) could help in the diagnosis of pyelonephritis [28]. However, although its level increases in children with febrile UTI,  $U_{NAG}$  is not always correlated to scintigraphic renal parenchymal involvement [29]. On the other hand,  $U_{NAG}$  also increases in reflux nephropathy [30]. In the case of diffuse scarring due to bilateral reflux nephropathy,  $U_{NAG}$  increases as an indicator of tubular dysfunction before glomerular proteinuria ensues. This is related to hyperfiltration in residual nephrons secondary to renal scarring [31]. Although a relation has been found between increased urinary IL-6, IL-8, and  $U_{NAG}$  levels in acute pyelonephritis, there was no relation between  $U_{NAG}$  and the presence of vesicoureteral reflux or renal scintigraphic findings during acute attack and at the end of the first year of follow-up [32]. It has been suggested that late increase in  $U_{NAG}$  levels might be an indicator of reflux nephropathy after ureteral reimplantation for vesicoureteral reflux [33].

$U_{NAG}$  has been used frequently for detection of acute rejection and cyclosporine toxicity after renal transplantation in adults, but was found to be nonrevealing in differentiation of these 2 entities. On the other hand, it was proposed that if  $U_{NAG}/Cr$  decreases during the first month of renal transplantation in children without any complications, the size of the graft could be small for the recipient's body size, and graft survival could be short-

ened [34].

$U_{NAG}$  levels were shown to increase on the first day of radiocontrast injection in adults [35]. Increased  $U_{NAG}$  is an indicator of tubular injury in urography performed with non-ionic iopenthal [36], and is the first finding of tubular dysfunction in cyanotic congenital heart diseases. The detection of microalbuminuria is regarded as the indicator of glomerular dysfunction in these patients [37]. Although radiocontrast material injected during angiographic studies for congenital heart diseases was thought to increase  $U_{NAG}$ , a 5 ml/kg dose of iopromide did not cause a significant increase in  $U_{NAG}$  [38].

### Urinary NAG levels as an indicator of nephrotoxicity

One clinical application of  $U_{NAG}$  measurements is the follow-up of the nephrotoxicity that could develop during the treatment of some diseases. It is known that  $U_{NAG}$  increases when  $\beta$ -lactams and aminoglycosides are used together. Likewise, renal injury increases when ceftazidime is used with an aminoglycoside reflected by increased  $U_{NAG}$ . When ticarcilline/clavulonate is used instead of ceftazidime, renal toxicity and  $U_{NAG}$  decrease [39].  $U_{NAG}$  levels, reflecting proximal renal tubular cell dysfunction, increase in cystic fibrosis patients undergoing inhalational gentamycin treatment [40], in patients with Wilson's disease during the first 2 years of d-penicillamine treatment [41], and in patients using cisplatin for oncologic diseases [42].  $U_{NAG}$  also increases in case of lead nephrotoxicity [43]. One report described that half of the children studied who were treated with sodium valproate showed increases in  $U_{NAG}$  [44], and serum valproate level has been seen to positively correlate with  $U_{NAG}$  level [45]. In addition,  $U_{NAG}$  has been reported to increase due to the changes in serotonin metabolism in epileptic children treated with carbamazepine [46]. Ethosuximide and phenytoin also lead to an increase in  $U_{NAG}$  [47].

### Other abnormalities associated with elevated urinary NAG levels

$U_{NAG}$  was found to be increased in children with unilateral nephrectomy or unilateral renal agenesis [48].

It is known that renal involvement is among the extraintestinal manifestations of chronic inflammatory bowel diseases.  $U_{NAG}$  has been reported to increase in

relation to the primary disease process independent of the medications used in these children [49].

Proximal renal tubular dysfunction and increased  $U_{NAG}$  have been reported in cases of glucose-6-phosphatase deficiency [50].

Plasma endothelin-1 level increases in children with severe biliary cirrhosis, and its level is positively correlated to serum bile acid and  $U_{NAG}$ . Thus, it could be concluded that renal tubular cell injury might develop in association with increased plasma endothelin-1 levels [51].

Since very high levels of NAG isoenzyme B is found in seminal fluid, detection of high urine levels of this isoenzyme in postpubertal boys does not suggest a renal pathology [52].

Although glomerular dysfunction can be caused by nonsteroidal antiinflammatory drugs used for treatment of children with juvenile chronic arthritis,  $U_{NAG}$  level does not change significantly in these children [53].

## Comment

A wide range of normal values in relation to age and technical difficulties in determination of enzyme level limit the practical application of  $U_{NAG}$  measurement in assessing renal tubular injury in the childhood age group. However, serial measurements of  $U_{NAG}$  could increase its clinical value during longitudinal patient follow-up.

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