THE DIAGNOSTIC ROLE OF URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG) ACTIVITY IN THE DETECTION OF RENAL TUBULAR IMPAIRMENT

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Summary: The kidney function can be assessed by a number of methods. The urinary excretion of enzymes, in particular N-acetyl-β-D-glucosaminidase (NAG), is considered a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal tubular function under various conditions. The determination of urinary NAG provides a very sensitive and reliable indicator of renal damage, such as injury or dysfunction due to diabetes mellitus, nephrotic syndrome, inflammation, vesicoureteral reflux, urinary tract infection, hypercalciuria, urolithiasis, nephrocalcinosis, perinatal asphyxia, hypoxia, hypertension, heavy metals poisoning, treatment with aminoglycosides, valproate, or other nephrotoxic drugs. This paper gives an overview of the current use of urinary NAG in the detection of renal injury.

Key words: Urinary N-acetyl-β-D-glucosaminidase; Renal tubular function

Introduction

The kidney plays a major role in maintaining constant volume and composition of the extracellular fluid. In this aspect, the kidney performs three basic functions: glomerular filtration, tubular reabsorption, and tubular secretion. The kidney function can be evaluated by a number of methods, including the assessment of urinary enzymes. Enzyme activity is normally low in urine and may increase when renal tubular cells are injured (15). Urinary enzymes, especially N-acetyl-β-D-glucosaminidase (NAG), alaninopeptidase (AAP), alkaline phosphatase (ALP) are very sensitive indicators of kidney parenchymal damage when compared to functional measurements, such as glomerular filtration rate (GFR) by creatinine or inulin clearance. The relatively low sensitivity of GFR can be attributed to the great functional reserve of the kidney due to its ability to compensate for the damage (15). The assessment of urinary enzymes is considered a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal disorders. The urinary activity of NAG is one of the most frequently evaluated urinary enzymes as it is a very sensitive marker of renal tubular impairment (17,58,59,80). Furthermore, the increased urinary activity of NAG precedes changes in the serum creatinine or endogenous creatinine clearance; and the urinary NAG activity has been reported to correlate with the activity of the disease (15,17,44,58,59,80). This article aims to give an overview of the diagnostic role of urinary NAG.

The characteristics of N-acetyl-β-D-glucosaminidase

NAG is a lysosomal enzyme which is abundantly present in cells of the proximal kidney tubule. The NAG has a relative high molecular weight of 130 000 to 140 000 daltons which does not permit its filtration through the glomerular basal membrane. Therefore, its urinary excretion is relatively constant with minimal diurnal changes. NAG is stable against changes in pH and temperature. The NAG consists of several isoenzymes. The two principal isoenzymes which are present in the kidney and liver, respectively, are the acidic form A and basic form B, together with small amount of intermediate forms I1 and I2. In the serum, NAG is represented predominantly by the A form which is also the only NAG form in the cerebrospinal and synovial fluid. Serum of the pregnant women contains P form of NAG which is similar to the I2 form. C form of NAG is present in the nervous tissue. The urine of healthy human subjects contains small amount of NAG, with the A isoenzyme:B isoenzyme ratio of 4:1 to 10:1, while the intermediary forms are not detectable. In diseases affecting the glomerular membrane, the A isoenzyme is usually detectable in the urine (17,58,59,80).
Methods of assaying NAG catalytic activity in urine

For the evaluation of NAG, spot urine, collected after the first morning void, should be used. As mentioned before, NAG is stable against changes in pH and temperature and its endogenous inhibitors in the urine specimens, such as urea and ascorbic acid, can be easily eliminated by appropriate sample dilution in the reaction mixture and by keeping the rest of the experimental conditions (pH, substrate concentration and incubation time) close to their optima (17,57). Currently, there are several methods of assaying the urinary NAG catalytic activity. The fluorimetric assay based on the fluorescent 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide substrate was introduced in the late sixties and has been followed by more user-friendly colorimetric and spectrophotometric methods (17,57). The fluorometric method is sensitive enough to determine very low enzyme activities in urinary specimen diluted 20–50–fold to eliminate the influence of endogenous low molecular weight effectors (17). Moreover, each laboratory had to establish its own normal reference intervals as a consequence of the activity arisen in the interlaboratory standardization of the procedure (17). However, fluorimetry still remains a useful method in most routine laboratories, as it is cheap, simple and relatively user-friendly.

The spectrophotometric method is based on highly soluble and stable 4-nitrophenyl-N-acetyl-β-D-glucosaminide as substrate. However, the sensitivity of the assay could only be kept at an acceptable level by the addition of large aliquots of the urine samples to the reaction mixtures (17).

Highly sophisticated and powerful colorimetric procedures are based on the use of 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl-β-D-glucosaminide and m-cresolsulphonphthaleinyl-N-acetyl-β-D-glucosaminide as substrate, respectively. In both these cases the colour of the urine does not disturb the assays (17).

The urinary NAG values should be expressed as a ratio to urinary creatinine concentration, as this relationship shows less variability than the urinary enzyme excretions related to volume or time (17). When evaluating the urinary NAG activity in various disease states, most authors used control groups for comparison. However, especially in children, it seems more appropriate to obtain and use age-dependent urinary NAG reference values from sufficiently large healthy population for the proper evaluation of kidney function (16,17,57,69). Due to the fact that the urinary NAG/creatinine ratio tends do decrease with age in children as a result of a concomitant rise in the urinary creatinine concentration (17,57), and as there is a great interindividu- al variability of those values in children, as reflected by the standard deviation (17,69), the use of proper pediatric reference values is quite reasonable (16,17,52,57,69).

The most representative articles on urinary NAG reference ranges consisted of values obtained from 528 healthy schoolchildren (69), 123 healthy children aged 1–14 years (16), and 262 healthy children (141 boys and 121 girls) aged 0 month through 18 years (68), respectively, where strong age-dependency has been always observed.

Urinary NAG activity in various disease states

The determination of urinary NAG is a non-invasive test and provides a very sensitive and reliable indicator of renal damage, such as injury or dysfunction due to diabetes mellitus, nephrotic syndrome, inflammation, urinary tract infection, hypercalciuria, urolithiasis, nephrocalcinosis, perinatal asphyxia, heavy metals poisoning, treatment with aminoglycosides, valproate or other nephrotoxic drugs, vesicoureteral reflux, hypoxia, hypertension. Urinary NAG is used as a routine marker of renal tubular impairment in the above mentioned disease states. As of November 20, 2004, there were 1165 publications on urinary NAG indexed in the Medline/PubMed. Concerning each disease state, the most relevant publications are listed and briefly discussed below.

Developmental kidney abnormalities

Increased urinary NAG was found in 18 pediatric patients with multicystic kidney (56) and in 16 patients aged 1–26 years with unilateral renal agenesis or history of nephrectomy (73).

Vesicoureteral reflex

Evidence of tubular dysfunction is common in children with vesicoureteral reflux (VUR) and renal scarring. The urinary NAG excretion was examined in 84 children with VUR grade III to V, and was elevated in children with grades IV and V, especially in patients with associated renal scarring (48). This was further confirmed in another study involving 40 children with history of VUR without eviden- ce of scarring, 93 children with history of VUR and scarring and 10 children with previous urinary tract infection without VUR (76). However, in another study comparing 90 urinary samples from reflexing patients and 142 samples from nonrefluxers, only VUR grade V had a significant elevation of NAG (88). In adults with reflux nephropathy (n=55) with normal blood pressure, normal renal function and ureteric reimplantation in childhood, the urinary NAG was significantly higher in comparison to the control group and the NAG correlated with plasma renin activity. Such a relationship supports the concept of segmental perfusion and filtration as an important mechanism (24).

In yet another study, 27 pediatric patients (mean age 1.73 ± 1.43years) with at least 2 episodes of urinary tract in- fection in the previous 2 months, received prophylactic re- gimen with cefixime. In the patients with VUR (n=7), high urinary values of NAG were observed in comparison to those children with recurrent urinary tract infection without VUR (n=20), this in spite of cefixime prophylaxis (22).
Obstructive uropathy

High urinary NAG was reported in 40 children aged 3 weeks to 16 years with unilateral ureteropelvic obstruction (n=30) or primary obstructive megaureter (n=10) in comparison to controls (13).

Urinary tract infection (UTI)

Increased urinary NAG was found in children aged 1 to 8 years with upper urinary tract infection (n=96) compared to control group (n=72) (10), and in 24 febrile infants with urinary tract infection, regardless of the level of infection (30). Urinary NAG may be therefore an informative indicator of UTI (30). In children with fever of non-renal origin (n=68) and those with pyelonephritis (n=25), there was an increase in urinary NAG with only moderately significant differences between the two groups. This may indicate that proximal tubular dysfunction may additionally be due to fever-associated function processes (92).

Nephrotic syndrome

Urinary NAG is higher in primary nephrotic children, and especially in those in the relapse phase than in those in remission. A positive correlation between proteinuria and urinary NAG was apparent (11,19,23,77). Furthermore, in a study involving 14 children with cortico-sensitive nephrotic syndrome, 5 with cortico-resistant nephrotic syndrome and 30 healthy controls, the urinary excretion of NAG was higher in nephrotic children, especially in those with cortico-resistant nephrotic syndrome (23). There were correlations between urinary NAG and serum cholesterol and negative correlations between urinary NAG and serum total proteins and albumin (23). In 23 pediatric patients with steroid-sensitive and 21 with steroid-resistant nephrotic syndrome the urinary NAG was correlated with albumin excretion (77). Similar results were reported by Valles et al. (83), with higher values of urinary NAG in steroid-resistant nephrotic syndrome. These results suggest tubular impairment in nephrotic syndrome, especially in cortico-resistant patients.

Nephrotoxic drugs

Increased urinary NAG was observed due to application of various drugs. Both paediatric and adult patients treated with antibiotics, such as aminoglycosides, applied either parenterally or locally, had high urinary NAG (36,53,54,60,85,86). The application of aminoglycosides thus leads to transient tubular dysfunction. Urinary NAG is therefore frequently used to monitor the nephrotoxic effects of gentamicin (53,60), tobramycin (54) and their various dosing regimen (54,60).

In patients treated with anticonvulsants, in particular valproate, high urinary NAG was repeatedly confirmed (5,18,84). Treatment of both pediatric and adult patients with antineoplastic drugs, such as methotrexate or cisplatin, is also accompanied by tubular dysfunction, as reflected by increased urinary NAG (9,25,29,47,55,75,90). Mild-to-moderate subclinical glomerular and tubular damage can be identified in many childhood cancer survivors, most probably as a result of drug toxicity (90). However, most patients experience some spontaneous recovery from acute nephrotoxicity. Out of 115 childhood cancer survivors, pathologically elevated urinary NAG was noted in 38% of leukemia/lymphoma, 54% of solid tumor and 20% of Wilms tumor survivors (9).

Increased urinary NAG was observed in patients with low-flow and high-flow sevoflurane anesthesia (26). However, no synergistic effect of low-flow sevoflurane and amikacin was noticed in surgical patients (27).

Urinary NAG is known to be elevated in patients with rheumatoid arthritis treated with methotrexate, and has been used to monitor and compare nephrotoxicity due methotrexate or infliximab. The introduction of infliximab during methotrexate therapy demonstrated no early or delayed nephrotoxicity of the drug in patients with rheumatoid arthritis (87). In patients with amyloid deposits, the NAG activity exceeded twice the upper normal limit (87).

In renal transplant recipients a substantial dependence of the activity of urinary NAG on cyclosporine doses and period after transplantation was observed (43).

Heavy metals poisoning

There is an extensive amount of evidence concerning tubular dysfunction as reflected by the increase in urinary NAG in heavy metals poisoning, or exposure to mercury (42,72) lead (14,21) or cadmium (64,78,82) due to environmental pollution. For example, as a result of an extreme pollution in the region of Central Asia, the renal tubular function of children around the Aral Sea is profoundly impaired, as indicated by increases in urinary NAG and urinary beta-2-microglobulin (31). Furthermore, high concentrations of cadmium in placenta, amniotic fluid and milk was revealed in pregnant smokers, together with increase in NAG activity in urine, amniotic fluid and milk (46). Cigarette smoking has a nephrotoxic effect and also is synergistic to lead nephrotoxicity on urinary excretion of NAG (21).

Kidney transplants

Low urinary excretion of NAG is helpful in the diagnosis of kidney transplant rejection. As the amount of excreted NAG depends on the graft mass and the amount of urinary creatinine depends on the recipient body mass, a low NAG excretion (related to urinary creatinine) could be a surrogate marker of an unfavorable low graft to body weight ratio, which in turn might be associated with a reduced graft survival (37).

Hypercalciuria, urolithiasis, nephrocalcinosis

In patients with hypercalciuria and/or urolithiasis, the urinary NAG was evaluated (8,33,67,71,74). The excretion of urinary NAG has been reported as either increased (8,67,71,74), or normal (33), in children with idiopathic hypercalciuria.
ciuria. Recently, urinary NAG was reported as significantly higher in children with urolithiasis and nephrocalcinosis, but not in children with isolated idiopathic hypercalciuria alone, and did not correlate with the urinary excretion of oxalate or calcium (67). Therefore, the increased urinary NAG in patients with idiopathic hypercalciuria might be a result of local tubular damage due to cell-crystal interactions rather than a manifestation of impaired tubular re-absorption (67).

**Hypertension**

The urinary NAG excretion was reported as increased in patients with hypertension (2, 61, 65, 66, 81), and significant correlations were found between NAG excretion and systolic and diastolic blood pressures (2). Changes in urinary NAG may evidence early hypertensive disease. High urinary NAG was observed in patients with untreated essential hypertension (61). Tubular injury (as reflected by high urinary NAG) is present in the early stages of hypertensive nephropathy and may precede glomerular damage. Ischemia due to changes in small vessels may not be the only factor responsible for this injury (81). The urinary NAG was increased in women with pre-eclampsia, but there were no correlations between urinary NAG and blood pressure (65, 66). Therefore, high urinary NAG activity in women with pre-eclampsia seems to be a sign of proximal tubular damage (65, 66).

**Cardiac surgery**

Increased urinary NAG were observed during cardiac surgery, suggesting transient perioperative renal dysfunction (3, 40).

**Neonatal disorders**

High levels of urinary NAG have been reported in neonates with perinatal asphyxia (89) and in premature infants where it tended to be higher with the degree of prematurity, and in term and preterm neonates with renal tubular injury (34, 79). The level of inflammatory cytokines in urine was elevated together with NAG (20). The neonatal asphyxia may induce systemic inflammatory response syndrome, which results in postasphyxial renal injury (20).

**Vasculitis (Henoch-Schoenlein purpura)**

Increased U-NAG has been observed in 12 out of 20 children with Henoch-Schoenlein purpura and correlated well with the extent of early and late renal involvement (49). In pediatric patients with Henoch-Schoenlein purpura, the urinary NAG is considered as a possible prognostic marker for the development of nephritis (49). Urinary NAG was higher in 82 children with treated Henoch-Schoenlein purpura and served as a marker of tubular dysfunction in the course of the disease (91).

**Diabetes mellitus**

The urinary excretion of NAG is helpful in the diagnosis of diabetic nephropathy (12, 28, 32, 35, 41, 63), where urinary NAG may be increased in the early stages of diabetes mellitus even before there is any clinical evidence of renal involvement (17). Furthermore, urinary NAG may reflect glycemic control in insulin-dependent diabetic patients (28, 35). Young (age 7.4–25 years) insulin-dependent diabetic patients with microangiopathic complications (n= 50) had an increased rate of urinary NAG excretion (41). The urinary NAG was considered as a predictive marker for the development of microalbuminuria in adolescents with diabetes, as urinary NAG excretion preceded the increase of albumin excretion (35). In yet another study, urinary NAG levels in the children with diabetes were significantly higher than those of controls. In 42 children with type 1 diabetes, there were positive correlations between urinary NAG levels and microalbuminuria, Hb A1c and systolic and diastolic blood pressure values. 59.5% of diabetic children were positive for urinary NAG, while 38.1% of them were positive for microalbuminuria (1). In patients with type 1 diabetes mellitus, proximal tubular dysfunction (manifested by high urinary NAG) may occur independently of glomerular alteration (62). Another study found significant correlations between high urinary NAG values and disease duration (P <0.05), HbA1c (P < 0.05), diastolic blood pressure (P <0.05) and puberty (P <0.05) (50).

In 27 patients with non-insulin-dependent diabetes mellitus (NIDDM), a significant correlation was found between urinary NAG and creatinine clearance. Elevation of urinary NAG may indicate decreased renal function during early stage NIDDM nephropathy (32). Urinary NAG excretion was elevated in patients with type 2 diabetes mellitus compared with healthy individuals and increased as nephropathy progressed. Pentoxifylline administration was effective in reducing proteinuria and urinary NAG excretion in these patients (51).

**Various diseases**

High urinary NAG was found in patients with glycogen storage disease (39) and in children with iron overload in beta-thalassaemia major (45), in patients with Wilson’s disease (70), in children with liver cirrhosis (7), with Lowe’s syndrome (38) and in children with familial Mediterranean fever (4).

**Asymptomatic primary hyper-N-acetyl-beta-D-glucosaminidaseuria**

A rare diagnosis of asymptomatic primary hyper-N-acetyl-beta-D-glucosaminidaseuria was proposed in two patients with high urinary NAG excretion and no renal abnormalities, including normal findings on renal biopsy. This is a probable new clinical entity of renal tubular disorders (6).

**Conclusions**

The assessment of urinary NAG should be considered as a useful marker of renal tubular impairment in various di-
sease states. It is extensively used both in routine practice as well as for research purposes, when it comes to the eva-
ulation of tubular function. Other urinary enzymes (such as alanin aminopeptidase, alkaline phosphatase) are also sen-
sitive indicators of kidney parenchymal damage compared to functional measurements. However, urinary NAG re-
mains the most widely used marker of renal tubular impair-
ment.

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