

Review

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The Role of Urinary N-acetyl-beta-glucosaminidase in Diagnosis of Kidney Diseases

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Introduction

There are about 40 different enzymes with different origins in the urine. They originate from the plasma and blood cells, kidneys, epithelium of the urinary tract, and urinary tract glands. The important locations of these enzymes are the glomerular membrane (like Alanine Amino Peptidase and γ -glutamyl transferase), lysosome (like N-acetyl- β -(D)-glucosaminidase activity), mitochondrion (like Malate dehydrogenase) and cytoplasm (like LDH) [1]. N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme present in the proximal convoluted tubules of the kidneys that may be used as a marker of proximal tubular damage and nephrotoxicity. The NAG molecule has a relatively high weight of approximately 130000-140000 Da which does not permit its filtration through the glomerular basement membrane of the kidney. Finally, NAG is rapidly cleared from the circulation by the liver.

N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme present in the proximal convoluted tubules of the kidneys that may be used as a marker of proximal tubular damage and nephrotoxicity. An abnormal urinary NAG excretion has been reported in different kinds of renal disorders such as acute kidney injury, urinary tract infection, vesicoureteral reflux; diabetes mellitus; nephrotic syndrome; glomerulonephritis; hypertension; perinatal asphyxia; heavy metals poisoning; drug nephrotoxicity, renal allograft rejection; and heart failure. This paper provides an overview of the diagnostic value of urinary NAG in the nephrourology field.

Keywords: N-acetyl-beta-D-glucosaminidase; Acute Kidney Injury; Kidney Diseases; Pyelonephritis; Hypertension; Vesico-Ureteral Reflux.

Running Title: Urinary NAG in Kidney Diseases

Diurnal urinary NAG values for healthy children were evaluated by Feldmann et al in 1989. They showed a circadian variation in urinary NAG excretion by healthy children. In this study, urinary NAG showed diurnal variation, with morning excretion being the highest. The data of this study provided reference intervals for urinary NAG during childhood. According to their report, urinary NAG value was high in children two and three years old, then decreased and the lowest Level of NAG was observed in children six to eight years old [2]. Totally, urinary NAG originates primarily from the proximal tubule, and increased urinary excretion is a consequence of renal tubular damage. Urinary NAG values should be expressed as a ratio to urinary creatinine, as this relationship shows less variability than the urinary NAG excretions related to the volume or time [3,4]. It is believed that the isoenzyme profile

of urinary NAG varies at different stages of renal damage. Urinary NAG is considered a simple, fast, and noninvasive method for the detection and follow-up of renal tubular function under various conditions. Increased levels of urinary NAG would serve as an indicator of the tubular damage due to ischemia, inflammation, and toxins. Regardless of acute kidney injury, an abnormal urinary NAG excretion is also reported in different kinds of renal disorders such as urinary tract infection, vesicoureteral reflux; diabetes mellitus; nephrotic syndrome; glomerulonephritis; nephrocalcinosis; hypercalciuria; urolithiasis; hypertension; perinatal asphyxia; hypoxia; heavy metals poisoning; treatment with aminoglycosides, valproate, or other nephrotoxic drugs; renal allograft rejection; and heart failure [5-9]. Recently, urinary NAG has been reported as a sensitive predictor of chronic kidney disease in animal models [10]. Urinary NAG has been already investigated in healthy infants and logarithmic normal values of the urinary NAG/creatinine ratio have been measured and reference ranges has been calculated. The researchers have found that urinary NAG excretions are almost constant throughout all groups and the urinary NAG/creatinine ratio decreases gradually until 3 years of age [11]. There are two main forms of NAG enzymes: isoenzyme A (acid form) and isoenzyme B (basic form) [12-14]. The A isoform of NAG (NAG-A) is the dominant form in the normal urine [14,15]. Its excretion is related to cell maturation and cellular exfoliative turnover, so it is signed as functional enzymuria. The B isoform of NAG is closely connected with the basal membrane where it appears. In patients with tubular and interstitial renal lesions, the total activity of urinary NAG, in particular its B form, is elevated (6,7). Therefore, large amounts of NAG are released in the tubular lumen only in cases of cytolytic tubular lesion [16]. This paper provides an overview of the diagnostic value of urinary NAG in the nephrourology field.

Urinary NAG in Acute Kidney Injury

Efforts to detect acute kidney injury (AKI) in the earlier stage have resulted in a number of serum and urinary biomarkers. Among them, urine NAG is a marker for the tubular reabsorption function. According to previous data, urinary NAG levels have been shown to function as a marker of AKI, reflecting the degree of tubular damage. The urinary NAG level may precede the increase of

serum creatinine by 12 hours to 4 days. It has also been established that in AKI patients, urinary NAG independently predicts the mortality or need for dialysis [5,17]. Therefore, urinary NAG appears to have the best performance for risk prediction after AKI [18]. Zhang et al evaluated patients with nephrotic syndrome and primary focal segmental glomerulosclerosis and concluded that complications like AKI were significantly more common in those with higher levels of urinary NAG [19]. In a recent study early measurement of urinary NAG helped predict nephrotoxicity of cisplatin [20]. Han et al concluded that combining urinary NAG, matrix metalloproteinase-9 and kidney injury molecule-1 achieved a perfect score diagnosing AKI [21]. In this regards results of Katagiri study showed that combination of urinary L-type fatty acid-binding protein and NAG predicted acute kidney injury after adult cardiac surgery [22]. Coca demonstrated that urinary NAG could be the best for mortality risk prediction after AKI [23]. The results of these studies show that increased levels of urinary NAG would serve as a good predictor for AKI.

Urinary NAG in Pyelonephritis

Increased urinary excretions of NAG have been shown in pyelonephritis. Hence, urinary NAG has the potential to be considered as an additional marker in differential diagnosis of pyelonephritis from cystitis. Previous studies have documented that the urinary excretion of NAG is significantly greater in patients with pyelonephritis than in patients with lower types of UTI [24,25]. After appropriate antibiotic therapy, a significant reduction of the levels of urinary NAG has been detected in patients suffering from acute bacterial pyelonephritis [26]. In a before and after study on children with pyelonephritis, we evaluated the levels of urinary NAG and found a specific difference between the mean levels of urinary NAG ($P < 0.001$). The sensitivity and specificity of the urinary NAG-creatinine ratio for a diagnosis of pyelonephritis in this study was 73.6% and 77.3%, respectively [27]. Some other published data have reported the levels of urinary NAG in febrile children and showed highly significant differences in the excretion levels of urinary NAG between children with fever of non-renal origin and children with pyelonephritis ($P < 0.0001$) [28]. On the contrary, Jantusch et al measured

the levels of urinary NAG and beta-2-microglobulin (B2M) in pediatric patients with febrile UTI and compared them with the DMSA renal scan results. In this study, increased urinary B2M and NAG were not associated with the severity of renal inflammation or pyelonephritis. They found that increased urinary NAG was associated with UTI in febrile patients. They showed that the sensitivity and specificity of NAG in predicting UTI in febrile patients, regardless of the site of infection, was 88% and 88%, respectively [29]. The urine of the patients with pyelonephritis often has an alkaline pH which is due to urease-producing bacterial species. The effect of the urine pH on urinary NAG isoenzymes has been reviewed and it has been shown that the measurement of NAG isoenzyme B is more reliable than the measurement of total NAG or isoenzyme A in the alkaline urine [30].

Urinary NAG in Urological Problems

Vesicoureteral reflux (VUR) is a common condition in the pediatric age group. Evidence of tubular dysfunction is commonly reported in children with VUR and other urological abnormalities. Williams et al demonstrated that urinary NAG could be applied as a screening test for early detection of vesicoureteral reflux (VUR). They evaluated the urinary NAG to creatinine ratio for each patient and then compared it to their voiding cystoureterography (VCUG) result. They showed that only grade V had a significant elevation in urinary NAG above the nonrefluxers ($P = 0.0001$). Therefore, they concluded that except for grade IV VUR, urinary NAG levels did not reliably detect the presence of VUR [31]. Later, other researchers investigated the urinary NAG excretion in children with moderate to high grade VUR and showed an increased level of enzymuria only in patients with grade IV and V and patients with renal scars [32,33]. After that, some studies revealed a significant excretion of urinary NAG in VUR patients [34-36]. One study reported that in adult patients with reflux nephropathy, the levels of urinary NAG were significantly higher than the control group. The results of this study showed a good correlation between urinary NAG levels and the plasma rennin activity [37]. Raghad J Ali et al reported that the mean NAG/creatinine index was significantly higher in patients with pyelonephritis, vesicoureteral reflux, and hydronephrosis than healthy controls ($p < 0.001$). They also showed the highest level of urinary NAG among patients with different grades of

vesicoureteral reflux [38]. Regardless of VUR, high levels of urinary NAG are observed in unilateral ureteropelvic obstruction and primary obstructive megaureter [39]. In our previous study, we evaluated urinary NAG in pediatric patients with urological abnormalities and compared it with the control group. We demonstrated a high level of urinary NAG in children with urinary tract abnormality and in this regard, patients with hydronephrosis had the highest and the patients with urinary stone had the lowest level of urinary NAG [40]. It seems that urinary NAG can be used as a good predictor of urological abnormalities in children.

Urinary NAG in Diabetic Nephropathy

Published data demonstrate that urinary NAG is an important predictor of diabetic nephropathy [41-45]. Increased urinary NAG excretions may be detected in patients with early stages of diabetes mellitus even before any clinical evidence of the kidney involvement [46]. Ellis et al reported that urinary NAG had a positive correlation with blood sugar ($r = 0.82$; $P < 0.05$) and their data suggested that the urinary NAG to creatinine ratio could be a reflection of the blood sugar control in diabetic patients [47]. Urinary NAG has also been considered as a predictive marker for the development of microalbuminuria in young diabetic patients [43]. In a study conducted on diabetic children, urinary NAG was more sensitive than microalbuminuria for the detection of the renal involvement [48]. It is also established that pentoxifylline administration is able to prevent the renal NAG excretion during experimental diabetes [49]. In another study, a positive correlation was found among fasting blood glucose levels and the glycemic status of the pregnant women and the urinary NAG levels [50].

Urinary NAG in Nephrotoxicity

The effects of different occupational exposures like benzene, toluene, xylene, and inorganic mercury on renal tubular cells and urinary NAG levels have been already investigated [51, 52]. To assess the effects of valproic acid (VPA) on the renal tubular function, epileptic pediatric patients who received VPA for at least 6 months were evaluated. The results of this study revealed significant differences in the mean urinary NAG/creatinine ratio between the patient and control groups ($P < 0.05$). Therefore, epileptic children on VPA therapy may develop proximal renal tubular dysfunction [53-55]. Several studies investigated nephrotoxicity of aminoglycosides and introduced

urinary NAG as an early detector of tubulopathy [56-61]. One study assessed urinary NAG after the administration of tobramycin and gentamicin and a significant increase in NAG levels was seen. The results of these studies revealed highly significant differences between all time points for urinary NAG compared to control groups [62,63]. Urinary NAG has been identified as a fast and practical indicator of clinical and subclinical tubular damage after chemotherapy [64,65], anesthesia [66,67], and administration of rheumatologic drugs [68]. To investigate the effect of extracorporeal shock wave lithotripsy (ESWL) on the kidneys of the canine, Fortes et al showed that high-energy shock waves, when applied to the kidney, led to an increase in the urinary NAG excretion 12 hours later with normalization after 24 hours; however, when reapplied after 24 hours, they did not cause a rise in urinary NAG after 36 and 48 hours. Therefore, the authors concluded shock wave reapplication with a 24-hour interval did not cause any increase in urinary NAG levels [69]. Recently, Arakawa et al investigated the urinary NAG excretion in patients who underwent cisplatin-containing first-line chemotherapy and introduced urinary NAG as a good predictor of severe hyponatremia associated with cisplatin-containing chemotherapy [70].

Urinary NAG in Renal Transplantation

Since allograft rejection during the first year after kidney transplantation leads to persistent allograft dysfunction and reduced long-term graft survival, it is important to define a good early predictor of kidney damage which is less invasive than allograft biopsy. Previously, published data determined the urinary NAG excretion in renal-transplant recipients at different times after transplantation and showed a direct correlation between the extent of urinary NAG excretion and both the time after renal transplantation and prednisolone dosage [71]. Increased levels of urinary NAG in some recipients of kidney transplantation are due to the administration of calcineurine inhibitors [72].

Urinary NAG in Glomerulopathies

The available data suggests that the urinary NAG excretion is higher in pediatric nephrotic patients, especially in those with corticosteroid resistant nephrotic syndrome. There is a positive correlation between urinary NAG and serum lipids and a negative correlation between urinary NAG and serum albumin [73-75]. In a study on patients with idiopathic membranous nephropathy, the

researchers compared the accuracy of urinary beta-2-microglobulin (U β 2m) and NAG in the response to treatment and prediction of renal insufficiency and remission rates. The results of this study identified U β 2m as the strongest independent predictor for the development of renal insufficiency with a sensitivity of 81 and specificity of 90%. For NAG, the sensitivity and specificity was 74 and 81%, respectively. They concluded that, although both U β 2m and U β -NAG could be good predictors for the progression and remission in idiopathic membranous nephropathy, U β 2m was more accurate [76]. Another study showed that urinary NAG levels were good predictors of the functional outcome in primary glomerulonephritis [77].

Urinary NAG in Hypertension

Previous studies have shown a significant correlation between the NAG excretion and systolic and diastolic blood pressure and high levels of urinary NAG have been observed in patients with untreated essential hypertension [77-81]. A study evaluated the urinary excretion of NAG in patients suffering from renovascular hypertension and compared the levels of urinary NAG before and after active intervention including surgery or percutaneous transluminal renal angioplasty (PTRA). Urinary NAG levels were found to be significantly higher in renal artery stenosis (RAS) patients as compared with patients with severe hypertension lacking significant RAS. Both groups of patients had significantly higher urinary NAG values than the control group [82].

Urinary NAG in Vasculitis

Increased levels of urinary NAG have been demonstrated in pediatric patients with Henoch-Schoenlein purpura (HSP) that correlate well with the extent of renal involvement. In children with Henoch-Schoenlein purpura, urinary NAG is considered a possible prognostic marker for tubular dysfunction and development of nephritis [83,84].

The urinary NAG level has been examined in patients with Kawasaki disease and has been reported as a sensitive index of renal involvement during the acute phase of the disease [85-88].

Urinary NAG in Neonates

One study evaluated the urinary NAG excretion in full term and preterm neonates. The results of this study showed that daily urinary NAG excretion increased dramatically during the first four weeks of life. No correlation was observed between the

urinary NAG excretion and the postnatal age. At a postnatal age of 7 days, the NAG to creatinine ratio was significantly higher in the preterm group [89]. High levels of urinary NAG excretion have been shown in neonates with perinatal asphyxia and in term and preterm neonates with renal dysfunction and tubular injury [90-92].

Urinary NAG in Thalassemia

The results of the studies conducted on thalassemic patients have revealed that the excretion of urinary NAG is elevated in patients with thalassemia compared with the control group and is directly correlated with the amount of transfused iron. It seems that renal tubular function is impaired in children with β -thalassemia major and intermedia [93]. The abnormal expression of urinary NAG shows that proximal renal tubular damage may be secondary to oxidative lipid peroxidation mediated by the iron overload [94]. It is suggested that kidney dysfunction in thalassemia increases with age, duration, and level of blood transfusion, and hypercalciuria [95]. In our previous study on β -thalassemic patients, there was a statistically significant relationship between urinary NAG levels and the patient's age, duration of deferoxamine therapy, and the duration of blood transfusion. We concluded that renal disorders were not rare in patients with β -thalassemia major and might increase with age, and prolonged duration of transfusion and deferoxamine therapy [96]. In this manner, the levels of urinary NAG were evaluated in patients with α -thalassemia and proximal tubular abnormalities were detected in α -thalassemia patients, as well [97]. Another study conducted on sickle cell/ β -thalassemia patients reported that the urinary NAG excretion could be considered an accurate and reliable index of the tubular toxicity, and a possible predictor of renal dysfunction in these patients [98]. Contrary to children with β -thalassemia major, α -thalassemia and sickle cell/ β -thalassemia, renal tubular dysfunction has not been determined in children with β -thalassemia minor by measuring urinary NAG [99].

Conclusion

We conclude that urine NAG level is a fast, noninvasive, practical, and valuable method for detecting different kidney diseases. It is established that significantly elevated urinary NAG levels can predict early stages of kidney damage due to important kidney pathologies like

AKI, UTI, vesicoureteral reflux, diabetes mellitus, nephrotic syndrome, glomerulonephritis, hypertension, perinatal asphyxia, nephrotoxicity, and allograft rejection.

Conflict of Interest

The authors have no conflict of interest to declare.

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