Urinary N-Acetyl- β -D-Glucosaminidase (NAG) in Lupus Nephritis and Rheumatoid Arthritis

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Increased activity of urinary N-acetyl- β -D-glucosaminidase (NAG) can be used as an early indicator of damage to the tubular epithelium. Systemic lupus erythematosus (SLE) is a multisystem autoimmune rheumatic disease. Nephritis is known as the most serious complication of SLE and the strongest predictor of poor outcome. In this study urinary NAG excretion was investigated in 24 SLE patients with normal renal function (serum creatinine ≤ 1.2 mg/dL) and the results were compared with those from 26 untreated patients with rheumatoid arthritis (RA) and 27 healthy controls. The SLE patients were divided into two groups according to their urinary total protein levels: group A consisted of 16 patients with ≤ 3.5 g/day proteinuria, and group B consisted of eight patients with nephrotic-range proteinuria (> 3.5 g/day). Serum and urinary creatinine, total urinary protein levels, and urinary NAG excretion were measured in patients with SLE and RA. In addition, serum C3 and C4 levels were determined in the SLE patients. Renal biopsies were performed in all of the SLE patients. Glomerular lesions were classified according to WHO criteria for lupus nephritis (LN) I–V. The Systemic Lupus

Erythematosus Disease Activity Index (SLEDAI) was used to assess disease activity. Urinary NAG excretion was significantly higher in the SLE groups than in the healthy controls ($P < 0.001$). In urinary NAG excretion there was also significant difference between SLE groups and RA patients ($P < 0.001$). However, there was no significant difference in NAG excretion between the RA and control groups ($P = 0.062$). Urinary NAG excretion was significantly higher ($P < 0.05$) in group B compared to group A. There were no differences in SLEDAI scores, ages, and serum creatinine levels between study groups ($P = 0.601$, $P = 0.285$, $P = 0.669$, respectively). Elevated SLEDAI values and hypocomplementemia were detected more often in younger patients ($P < 0.010$, $r = -0.529$ and $P < 0.010$, $r = -0.569$, respectively). There was a strong positive correlation between proteinuria and urinary NAG activity ($P < 0.001$, $r = 0.759$). These results suggest that the determination of urinary NAG activity may be a useful supplement to the routine biochemical analysis performed on the urine in cases of SLE. J. Clin. Lab. Anal. 19:172–176, 2005.

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INTRODUCTION

N-acetyl- β -D-glucosaminidase (NAG) is a urinary enzyme that has been proposed as a valuable marker for renal involvement. It is a lysosomal enzyme of 130 kDa molecular mass, and is normally excreted in low amounts in urine as a consequence of the normal exocytosis process (1).

Systemic lupus erythematosus (SLE) is a rheumatic disease characterized by various clinical manifestations. Lupus nephritis (LN) is an important cause of morbidity and mortality in patients with SLE that results from an acute inflammatory and immunological response to

renal immune complex deposition. The acute response is characterized by activation of circulating leukocytes and renal parenchymal cells, which triggers the production of pro-inflammatory cytokines and growth factors. In all too many cases, this response is followed by a

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chronic response that is characterized by excessive deposition of collagen and other extracellular matrix macromolecules and the development of end-stage renal disease (ESRD) (2).

One of the characteristics of SLE is the large inter- and intraindividual variability of the clinical course. LN is no exception. Some patients with kidney involvement may show rapid progression to renal failure, while others may enter complete and stable remission after adequate therapy (3). Renal disease in SLE occurs in 40–75% of patients, most often within 5 years of disease onset, and is one of the strongest predictors of a poor outcome (4).

Rheumatoid arthritis (RA) is a systemic disease that mainly affects the joints but can also lead to extra-articular organ manifestations. Although renal involvement is not uncommon, impairment of renal function is generally mild, and end-stage renal failure is believed to be rare (5).

The tubulo-interstitial damage due to the increased protein traffic across the tubular cells is presently considered the most important determinant for the progression to chronic renal failure (CRF) in patients with proteinuric glomerular disease (6). Urinary NAG activity is probably an indicator of the increased lysosomal turnover that occurs when increased protein is presented to the tubular cells (7).

In this study we investigated urinary NAG excretion in SLE and RA patients and healthy controls, and compared the results to determine whether the excretion pattern could be useful for assessing early renal involvement, and whether the alteration in its excretion involved the presence of active nephritis.

MATERIALS AND METHODS

Subjects

We studied 31 SLE patients (28 women and three men) who at the time were untreated and had been referred to the Rheumatology Department at Ege University. All fulfilled the 1982 revised criteria of the American Rheumatism Association for the classification of SLE (8). Seven patients with SLE who had high serum creatinine levels (>1.2 mg/dL) were excluded. The remaining 24 SLE patients (22 women and two men), who had normal serum creatinine levels (≤ 1.2 mg/dL), were included in this study. Their median (min-max) age was 31 years (range = 19–64 years). The SLE patients were divided into two groups according to their urinary total protein levels: group A consisted of 16 patients with ≤ 3.5 g/day proteinuria, and group B consisted of eight patients with nephrotic-range proteinuria (> 3.5 g/day). The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was used to assess

TABLE 1. Baseline characteristics of SLE patients. Values are expressed as median (min-max)*

SLE patients	
Total number of patients	24
Male/female ratio	2/22
Age at onset (years)	31 (19–64)
SLEDAI score	18 (10–25)
C3 mg/dL	64 (16–131)
C4 mg/dL	14 (3–37)
ANA + %	24/24 (100%)
Class II	3/24
Class III	8/24
Class IV	13/24

*Normal values: SLEDAI, 0 points; ANA, negative; C3, 90–190 mg/dL; C4, 20–40 mg/dL.

disease activity (9). The urine specimens were collected 2 days before the renal biopsy was performed. Blood and urine samples were always collected on the same day. The renal biopsy specimens were classified according to the World Health Organization (WHO) criteria: minimal changes (class I), mesangial alterations (class II), focal proliferative (class III), diffuse proliferative (class IV), and membranous (class V) glomerulonephritis (10). We also studied 24 patients (21 women and three men) with RA. None of these patients had renal disease or a history of it. Their median (min-max) age was 39 years (range = 20–53 years). They all met at least four of the 1987 revised criteria of the American Rheumatism Association for RA (11). Patients who had received any drug as an antirheumatic treatment were not included in this study. None of the RA patients had clinical or laboratory signs of nephropathy (proteinuria ≤ 0.3 g/24 hr, serum creatinine ≤ 1.2 mg/dL). The study was performed in accordance with the principles of the Declaration of Helsinki. Some baseline characteristics of the SLE patients studied are reported in Table 1.

The control group consisted of 27 healthy volunteers (18 women and nine men). Their median (min-max) age was 28 years (range = 20–55 years).

Assays

In this study we collected 24-hr urine samples to eliminate fluctuations in the effect of urine volume on the enzyme concentration in the urine. Urine samples were collected without a preservative and kept at $+4^{\circ}\text{C}$ during the collection period. After the volume was determined, 10 mL of urine were centrifuged at 3000 g for 5 min, and only the top 5 mL urine samples were used for urine analysis. Urine creatinine and protein measurements were performed immediately. Then 3 mL urine samples were subjected to gel filtration on Sephadex G-50 fine (Pharmacia; Uppsala, Sweden)

(12). After gel filtration was performed for the NAG measurements, the samples were kept at -20°C until they were analyzed.

We determined the urinary total protein levels in the supernate by a microturbidimetric method using a Roche Diagnostics urinary/CSF protein assay kit (catalog number 1877801) in a Hitachi 902 (Tokyo/Japan) automatic analyzer. The within-day and day-to-day coefficients of variation (CVs) were 1.3% and 1.5%, respectively, for urinary protein.

Serum and urinary creatinine levels were measured automatically on a Hitachi 902 instrument by the Jaffe method without deproteinization.

We determined urinary NAG activity using an enzymatic colorimetric method (13), with a 2-mmol/L p-nitrophenol standard (equivalent to the reaction product liberated by 80 U NAG/L). Urinary enzyme levels can be affected by the urine flow rate; therefore, to minimize this effect, we express the level of enzymuria as the ratio of enzyme activity to the urinary creatinine level. Urinary NAG activities are expressed in units per mmol of urinary creatinine (U/mmol creatinine). The within-day and day-to-day CVs were 2% and 3%, respectively, for urinary NAG.

In the SLE patients, we determined the serum levels of C3 and C4 by an immunoturbidimetric method using Boehringer assay kits (catalog number 1875078 and 1875051, respectively) in a Hitachi 704 (Tokyo/Japan) automatic analyzer.

For the statistical analyses, nonparametric tests (Kruskal-Wallis, Mann-Whitney U, and Spearman's correlation) were used. All statistical analyses were done with SPSS 10.0 software. A level of $P < 0.05$ was chosen as significant.

RESULTS

Urinary NAG excretion was higher in groups A and B compared to the RA patients ($P < 0.001$). We also found significantly different NAG activities between the two SLE groups and the healthy controls ($P < 0.001$). However, there was no significant difference in NAG excretion between the RA and control groups ($P = 0.062$). In urinary NAG activities, there was

significant difference between groups A and B ($P < 0.05$) (Table 2 and Fig. 1).

No differences were found in age and serum creatinine levels between these groups ($P = 0.285$ and $P = 0.669$, respectively). The serum C3 and C4 levels and SLEDAI scores showed no statistical differences between groups A and B ($P = 0.081$, $P = 0.627$, and $P = 0.601$, respectively).

The 24-hr excretion values of protein in the patient groups are shown in Fig. 2. The urinary NAG activities and protein excretions in the RA and SLE patients are shown in Fig. 3.

The results of the renal biopsy examinations in the SLE group showed 12.5% class II, 33.4% class III, and 54.1% class IV patients. There was no significant difference in urinary NAG activities between classes II, III, and IV ($P = 0.82$).

In this study we observed a strong positive correlation between proteinuria and urinary NAG activity ($P < 0.001$, $r = 0.759$). However, there was no correlation between the urinary NAG activities and the SLEDAI scores ($P = 0.770$). Elevated SLEDAI values and hypocomplementemia were detected more often in younger

Fig. 1. Urinary NAG activities in study groups.

TABLE 2. Urinary NAG and protein excretions in study groups. Values are expressed as median (min-max)

	Control Group (n = 27)	SLE/Group A (n = 16)	SLE/Group B (n = 8)	RA patients (n = 26)
Urinary NAG (U/mmol creatinine)	0.42 (0.18–0.86)	7.62 (0.86–35.85) ^a	25.59 (4.42–63.67) ^{a,b}	1.27 (0.10–5.22)
Urinary protein (g/day)	–	1.45 (0.20–3.14)	5.65 (3.90–7.70)	0.10 (0.05–0.30)
Serum creatinine (mg/dL)	–	0.70 (0.50–1.10)	0.75 (0.47–1.14)	0.79 (0.50–1.09)

^a $P < 0.001$ vs. controls and RA patients.

^b $P < 0.05$ vs. group A.

Fig. 2. The 24-hr urinary excretion values of protein in patient groups.

Fig. 3. Urinary NAG and protein excretions in patients with RA and SLE.

patients ($P < 0.010$, $r = -0.529$; and $P < 0.010$, $r = -0.569$, respectively).

DISCUSSION

Kidney involvement in SLE is a life-threatening complication that must be detected early. In the literature, the development of ESRD occurs with a frequency ranging from 5% to 26% of patients with SLE nephritis after a mean follow-up of 5 years. Its incidence has remained stable over the last three decades (14).

Measurements of serum creatinine and creatinine clearance are not optimal tests of renal function. Creatinine clearance may overestimate the true glomerular filtration rate (GFR) by up to twofold. More accurate tests of renal function, such as determination of GFR by inulin or diethylenetriaminepentaacetic acid (DTPA), are costly and labor-intensive (15). It is known that since creatinine is produced by muscle cells, the level of serum creatinine is affected by muscle mass and age.

Patients with more serious forms of LN (WHO classes III–V) may present insidiously with asymptomatic hematuria and/or proteinuria. Routine monitoring for urinary abnormalities is required to identify these patients early in the clinical course of the disease (16).

Proteinuria is an important clinical feature that indicates the presence of lupus glomerular disease. In general, nephrotic-range proteinuria reflects diffuse glomerular capillary wall involvement, and is often seen in patients with diffuse proliferative or membranous glomerulonephritis. Relatively low-grade proteinuria (approximately 1–2 g/day) may be associated with active nephritis, or it may reflect chronic irreversible glomerular capillary wall injury (17). According to the renal biopsy findings, the majority of our patients with SLE had proliferative glomerulonephritis, and all of them had proteinuria. Few clinicians would advocate baseline renal biopsy in a newly diagnosed patient with SLE, while most specialists would agree that the appearance of any marker of renal disease, such as hematuria, proteinuria, nephritic syndrome, or renal insufficiency, at any time in the course of SLE is a clear indication for renal biopsy (18).

Noninvasive markers of tubular injury in humans are limited in proteinuric diseases. The central role of tubular damage in determining the progression to CRF in proteinuric glomerular disease explains researchers' interest in finding a reliable urinary marker of the tubular damage that is secondary to protein loss (6). NAG is a lysosomal enzyme that is normally excreted in low amounts in urine as a consequence of the normal exocytosis process in the proximal tubular cells. It is excreted in abnormally high amounts in many situations in which these cells are exposed to various toxic substances (1,19). In a previous study of glomerular disease, an analysis of the 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 glomerular capillary wall (19).

In this study, higher excretion of NAG was found in SLE patients with biopsy-proven LN than in healthy controls. This increased urinary NAG excretion may result from a dysfunction of tubular epithelial cells

induced by increased traffic of proteins, as suggested by Bazzi et al. (6). Evidence is accumulating that an abnormally increased entry of proteins into the urinary space, subsequent to an alteration of the glomerular barrier (especially if it is a long-lasting phenomenon) overwhelms the protein reabsorption capacity of the proximal tubular cells and leads to damage of these reabsorbing cells. This protein toxicity for the epithelial tubular cells can be documented in all types of glomerular disease. The amount of proteinuria is a reliable indicator of the severity of the glomerular permeability abnormality, and is also related to tubulointerstitial damage, as shown in several experimental models and in some cases of human glomerulonephritis, probably because of an intrinsic toxicity of proteinuria on tubular cells (20). Indeed, we observed elevated urinary NAG activities in group B compared to group A. Although Falaschi et al. (21) reported a significant difference in SLEDAI scores between SLE patients with and without nephrotic-range proteinuria, we did not observe such a difference between groups A and B in our study. This discrepancy may be due to those patients with juvenile-onset SLE. We found a strong correlation between increased NAG excretion and levels of proteinuria in patients with normal renal function and nephrotic- or non-nephrotic-range proteinuria. This association has also been reported by several investigators (6,22). However, there was no correlation between urinary NAG and the SLEDAI scores in the SLE patients. In our study we confirmed a strong correlation between decreased C3 levels, elevated SLEDAI scores, and patient's age at onset, as reported by some authors (23,24).

In this study none of the RA patients showed a significant difference in urinary NAG excretion compared to controls, since they were normoproteinuric and were not receiving potentially nephrotoxic drugs.

In conclusion, measurements of urinary NAG activity provide much more information than a simple measurement of 24-hr proteinuria. Increased NAG activity in proteinuria reflects the activity of the disease, and its determination may be a useful supplement to the routine biochemical analysis performed on the urine in cases of SLE.

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