

Urinary *N*-acetyl- β -D-glucosaminidase in rheumatoid arthritis

M. Perwaiz Iqbal,^{1,3} Azra A. Ali,²
M. Anwar Waqar¹ and N. Mehboobali¹

1 Departments of Biochemistry and Medicine,
The Aga Khan University, Karachi 74800, Pakistan

2 Department of Medicine, The Aga Khan University, Karachi 74800, Pakistan

3 Corresponding author

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Abbreviations: NAG, *N*-acetyl β -D-glucosaminidase; RA, rheumatoid arthritis; NSAID, non-steroidal antiinflammatory drug

Abstract

Excretion of urinary *N*-acetyl β -D-glucosaminidase (NAG) and its isoenzyme patterns were studied in two groups of patients with rheumatoid arthritis (RA) and in normal control subjects. Urine samples were collected from 30 seropositive RA patients, 19 seronegative RA patients, and 15 normal healthy subjects. All the patients and normal subjects were assessed to have normal liver and kidney functions. A small portion of the urine sample was dialyzed against 0.01 M phosphate buffer, pH 7.0 and NAG activity was monitored. Mean \pm SD values of urinary NAG in seropositive RA patients, in seronegative RA patients and in normal healthy subjects were found to be 4.20 ± 3.73 U/g creatinine, 2.96 ± 2.11 U/gm creatinine, and 1.71 ± 0.6 U/g creatinine, respectively. The mean urinary NAG value in RA patients was found to be significantly higher ($P < 0.05$) in seropositive RA compared to the mean NAG value in normal healthy subjects and patients with seronegative RA when analyzed by one way ANOVA and Tukey-HSD test. The mean proportion of isoenzyme form B to isoenzyme form A in seropositive RA patients was also found to be significantly different ($P < 0.05$) from the mean proportion of these forms in normal healthy subjects and seronegative RA patients. There also appears to be a correlation between the concentration of urinary NAG and severity of the disease in seropositive RA.

Key Words: *N*-acetyl- β -D-glucosaminidase, rheumatoid arthritis, seropositive, seronegative, isoenzymes

Introduction

N-Acetyl β -D-glucosaminidase (NAG) is a lysosomal enzyme, which is mainly present in proximal convoluted tubules. Its levels in urine have been shown to be elevated in a number of diseases, such as diabetes mellitus (Whiting *et al.*, 1979a; 1979b; Hanseus *et al.*, 1983; Shimajo *et al.*, 1987; Skrha *et al.*, 1987; Severni *et al.*, 1988; Watts *et al.*, 1988), renal stone disease (Javed *et al.*, 1992) and impaired renal function disorders (Ellis *et al.*, 1975). More recently, it has been reported that urinary NAG levels are elevated in adjuvant arthritis (Franch *et al.*, 1990) and there appears to be a positive correlation between urinary NAG excretion and severity of the disease. The present study was undertaken to investigate the excretion of urinary NAG and the pattern of its isoenzymes in different types of rheumatoid arthritis (RA) and compare these values with those in normal healthy subjects. Another objective was to see if there was any correlation between urinary levels of NAG and severity of the disease.

Materials and Methods

Forty-nine adult patients (mean age 44 years) who were diagnosed to have RA as defined by the criteria of the American Rheumatism Association were included in this study. Out of these, 30 (24 females and 6 males) were found to have seropositive RA, while 19 (15 females and 4 males) were having seronegative RA. Moreover, 15 normal healthy subjects in the same age group were taken as "controls".

Severity of the RA was assessed to be mild, moderate or severe depending upon the number of swollen joints and deformities. Those having involvement of 1-2 joints and no deformities were placed in the mild disease subgroup, while those with involvement of 3-10 joints and deformities of either hands or feet were placed in the moderate disease subgroup. When there were more than 10 swollen joints and deformities of both hands and feet, these patients were placed in the severe disease subgroup. Mean duration of RA in each subgroup was also taken into account. All the RA patients were also assessed to have normal kidney and liver functions. Those patients who had received gold as antirheumatic treatment were not included in this study.

Analysis of NAG

Morning urine samples were collected from the two groups of arthritis patients as well as from normal healthy subjects. These were dialyzed against 10 mM phosphate buffer, pH 7.0 for 4 h. NAG activity in the dialyzed samples was

determined by the method of Whiting *et al.* (1979b). This procedure involves incubation of 0.1 ml of urine diluted with 0.4 ml of distilled water and 4.5 ml of 20 mM citrate-phosphate buffer, pH 4.4 containing 0.2 mM methyl-umbelliferyl-glucosaminidine at 37°C. One ml from this is then withdrawn at various time intervals (0 min, 5 min, 10 min, 15 min) and transferred to separate tubes containing 2 ml of 0.5 M glycine-NaOH buffer, pH 10.4, to stop the reaction.

The fluorescence of 4-methyl umbelliferone (MU) released in each reaction was monitored spectrofluorimetrically at excitation and emission wavelengths of 368 and 448 nm, respectively using JASCO FP-770 spectro-fluorimeter. One unit of NAG activity was defined as that which released 1 mmole of 4 MU per minute under assay conditions. In order to compensate for the production of concentrated or diluted urine samples, NAG activities in urine were expressed as U per gm of urinary creatinine. Urinary creatinine was measured by Boehringer Mann-heim Diagnostics Kit based on Jaffe' method.

Separation of NAG isoenzymes

Separation of NAG isoenzymes in urine samples was carried out by using a modification of the procedure described by Severni *et al.* (1988). One ml of dialyzed urine sample was applied to a minicolumn of DEAE-Sephrose (1.5 cm × 3.0 cm) which had been equilibrated with 50 mM phosphate buffer, pH 7.2. The flow rate of the column was adjusted to 15 ml/h. One-ml fractions were collected. First 10 fractions, which contained NAG-B isoenzyme, were collected with 50 mM phosphate buffer, pH 7.2, while NAG-A isoenzyme was eluted using 1 M NaCl. NAG activity in the fractions was determined spectrofluorimetrically as described above.

Statistical analysis

To compare the distribution of mean values of urinary NAG among various groups under study, one way ANOVA and Tukey-HSD test were used. Comparison of the mean values of two subgroups was also carried out by student- t test.

Results

Figure 1 shows the mean ± SD urinary NAG values in the seropositive RA group, seronegative RA group and normal control group. The mean NAG values in these three groups were compared by using one way ANOVA followed by Tukey-HSD test. One way ANOVA indicated that the mean NAG values were significantly different (P-value = 0.027, F statistics = 3.8377). Tukey-HSD test at 5% significance level further revealed that the mean NAG value in the seropositive RA group was significantly different from the mean NAG values in normal control group and seronegative RA group.

Since one of the objectives of this study was to see if there was any correlation between urinary NAG activity and severity of the disease, RA patients in this study were classified into mild, moderate and severe disease subgroups as described in the Materials and Methods section. Urinary NAG values in these three subgroups have been reported in Tables 1 and 2.

Comparison of the distribution of NAG values in these subgroups revealed an apparent correlation between mean value of NAG and severity of the disease in seropositive group of RA patients. This was due to the fact that a comparison of the mean NAG values between severe and mild disease subgroups by student-t test yielded a

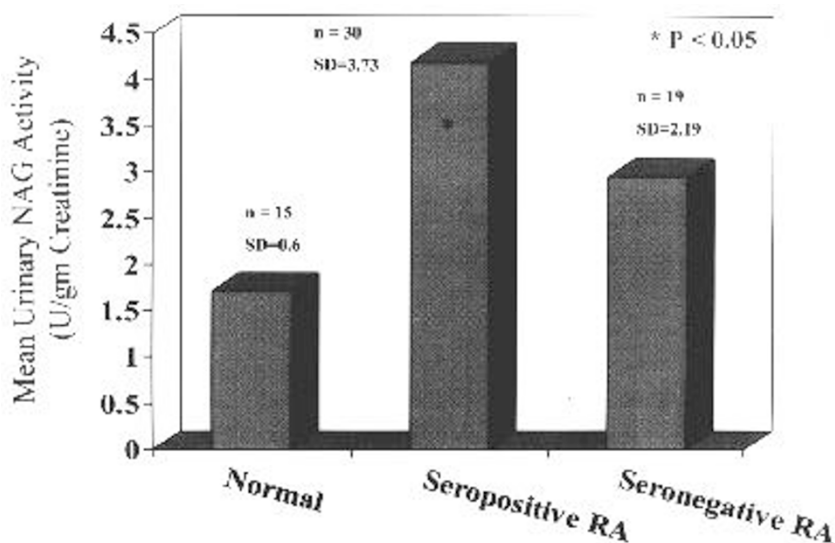


Figure 1. Urinary NAG activity in two groups of rheumatoid arthritis compared to normal control group (n = number of subjects; SD = Standard Deviation). Comparison of the distribution of urinary NAG values in these 3 groups was carried out by using one way ANOVA and Tukey-HSD test.

Table 1. Comparison of the distribution of urinary NAG values in mild, moderate and severe disease subgroups of seropositive RA. One way ANOVA P-value = 0.21. Comparison of the mean NAG values between severe and mild disease subgroups by student-t test, however, reveals a significant difference at P < 0.05.

Disease Condition	Sample size (n)	Mean duration of RA ± SD (years)	Sample Mean NAG value ± SD (U/g creatinine)
Mild	6	4.67 ± 0.47	2.33 ± 1.20
Moderate	12	4.87 ± 3.9	3.82 ± 3.73
Severe	12	7.12 ± 3.45	5.52 ± 4.25

Table 2. Comparison of the distribution of urinary NAG values in mild, moderate and severe disease subgroups of seronegative RA. One way ANOVA P-value = 0.803.

Disease Condition	Sample size (n)	Mean duration of RA ± SD (years)	Sample Mean NAG value ± SD (U/g creatinine)
Mild	13	1.7 ± 1.47	3.16 ± 2.2
Moderate	1	0.33	3.25
Severe	5	5.89 ± 7.03	2.57 ± 2.36

significant difference at P < 0.05 (Table 1). However, when we compared the mean values using more stringent statistical tests, such as one way ANOVA and Tukey-HSD test, no significant difference was observed between the mean NAG values in mild, moderate and severe subgroups of seropositive as well as seronegative groups of RA patients (Tables 1 and 2).

Figure 2 shows a typical chromatogram of NAG isoenzymes in the urine samples of a seropositive RA patient, a seronegative RA patient, and a normal healthy subject

Table 3. Comparison of the mean values of the ratio of isoenzyme B to isoenzyme A in two groups of rheumatoid arthritis and a normal control group. One way ANOVA P-value = 0.017. Comparison of the mean values was carried out by using one way ANOVA and Tukey-HSD test.

Group	Sample size (n)	Mean ± SD isoenzyme B/isoenzyme A
Normal	7	0.407 ± 0.22
Seronegative RA	9	0.795 ± 0.38
Seropositive RA	15	1.284 ± 0.85

eluted from a DEAE-Sepharose column. All the three samples resolved into NAG-B and NAG-A isoenzymes. The mean values of the proportion of NAG-B to NAG-A in the urine samples of three groups of subjects in this study have been shown in Table 3. One way ANOVA showed the mean proportion values of NAG-B/NAG-A to be significantly different among three groups (P-value = 0.017, F statistics = 4.74). Tukey-HSD test further indicated that the mean value of NAG-B / NAG-A obtained in seropositive RA patients was statistically different from the mean values obtained in normal healthy subjects and in seronegative RA patients at 5% significance level. This suggests that the increase in total urinary NAG, at least in seropositive RA patients, is contributed mainly by NAG-B isoenzyme.

Discussion

This study shows that total activity of NAG in urine is significantly elevated in seropositive RA patients compared to normal healthy subjects. Urinary NAG is known to be a sensitive indicator of kidney injury as its levels increase before other renal function tests (especially

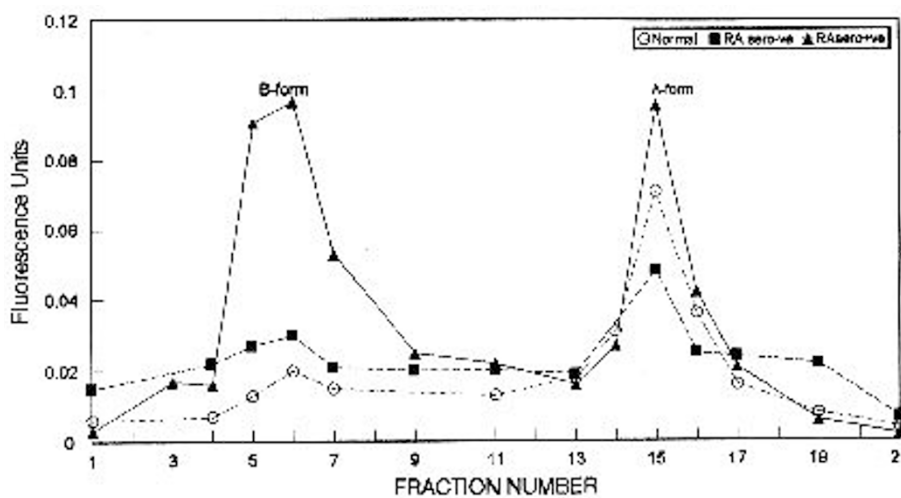


Figure 2. A typical chromatogram of NAG isoenzymes (NAG-A and NAG-B) in urine of a normal healthy subject and two patients suffering rheumatoid arthritis after elution from a DEAE-Sepharose column. Experimental conditions have been provided in the 'Materials and Methods' section.

serum creatinine) become abnormal (Price, 1982). Therefore, increased excretion of NAG may reflect renal damage in RA. This is further supported by the observation that the apparent value of urinary NAG in most cases of severe seropositive RA appeared to be on the higher side compared to the mild cases of this disease. A similar correlation has previously been reported between urinary NAG and degree of nephropathy in diabetic patients (Whiting *et al.*, 1979a; Shimajo *et al.*, 1987; Skrha *et al.*, 1987; Jung *et al.*, 1988; Watts *et al.*, 1988), however, no clear correlation to degree of retinopathy was established in these patients with complications of diabetes mellitus (Agardh *et al.*, 1987, 1991; Watts *et al.*, 1988). This shows that any disease having a direct or indirect effect on kidney tubular function would be expected to be associated with increased levels of NAG in the urine. Franch *et al.*, (1990) have also reported a positive association between urinary NAG excretion and disease severity in adjuvant arthritis.

In an earlier communication we have reported increased levels of serum NAG in different arthropathies including RA (Iqbal *et al.*, 1998). However, it is unlikely that the increased level of NAG in urine is contributed by the increased level of this enzyme in serum as the molecular size of the enzyme is high enough to prevent its passage through the glomerulus. Thus, the origin of urinary NAG appears to be the kidney tubules. Since we measured the values of NAG as units/gm of creatinine, any change occurring in the clearance of creatinine as a result of concomitant use of drugs affecting glomerular filtration rate, such as, non-steroidal anti-inflammatory drugs (NSAIDs) etc. are not likely to interfere with our results.

Isoenzymes of NAG (NAG-B and NAG-A) in urine have been reported to be of value in assessing complications of diabetes mellitus (Severini *et al.*, 1988; Agardh *et al.*, 1991). It has been shown that only low amounts of NAG-B are present in normal urine, however, the activity of this isoenzyme significantly increases in most cases of diabetes mellitus (Severini *et al.*, 1988). Our results also show that the ratio of NAG-B to NAG-A significantly increases in severe cases of seropositive RA.

Renal damage is not uncommon in RA, but the causative role of the disease per se has not been defined as yet (Manganaro *et al.*, 1994). Yoshida *et al.* (1991) have reported two cases of native nephropathy in RA. Our data also suggest that subclinical renal injury without compromising renal function is a possibility in RA especially in severe cases of this disease. It is unclear which factors lead to increased excretion of NAG (especially the isoenzyme form B) in RA patients. The role of (NSAIDs) and certain disease modifying antirheumatic drugs, such as, gold in producing nephropathy cannot be discounted (Yoshida *et al.*, 1991. Kordonouri *et al.*, 1994). Keeping that in view, we have not included in this study those

patients who had received gold therapy. According to the study by Kordonouri *et al.*, (1994) subclinical renal injury due to therapeutic doses of NSAIDs is more likely to affect the glomerular function rather than the tubular function. Hence, NAG, which is an enzyme marker for the tubular function, is not expected to increase in urine due to therapeutic doses of NSAIDs. In a more recent study, Wiland *et al.* (1997) have reported NAG to be a more sensitive indicator of kidney dysfunction in RA than microalbuminuria. They also showed that methotrexate in low dosages does not cause any marked damage to renal proximal tubules.

All these reports lend further support to the notion that subclinical damage to kidney may occur in RA. Determination of serum creatinine which is a routine procedure for assessing kidney function is unlikely to detect those changes as increased concentration of creatinine in serum is not observed until over half of the glomerular function is compromised (Lente and Suite, 1989). Determination of urinary NAG along with excretion of creatinine may, therefore, serve as a more sensitive test for kidney injury in RA and measurement of the levels of its isoenzymes could be of value in providing additional diagnostic information regarding the status of this disease.

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