N-Acetyl- β -D-Glucosaminidase in the Localisation of Urinary Tract Infection in Patients with Spinal Cord Injury

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Summary

This study evaluates the pattern of urinary N-acetyl- β -D-glucosaminidase (NAG) isoenzyme excretion in patients with spinal cord injury (SCI) and its use as a diagnostic tool in localising the site of urinary tract infection (UTI). NAG-B excretion in 27 control SCI patients (mean 207.78 units) was significantly higher than in 10 normal controls (mean 12.6 units) $p < 0.001$. The relative isoenzyme distribution as represented by NAG-B/Total NAG percentage is however similar in both groups, 24.27 and 20.38% respectively. NAG-B excretion in 6 SCI patients with upper UTI was not significantly higher than in 12 SCI patients with lower UTI. NAG-B/Total NAG percentage was significantly different between these two groups (35.3% and 24.98% respectively, $p < 0.05$). There was no significant difference in NAG-B excretion or NAG-B/Total NAG percentage between control SCI patients and those with lower UTI. The results indicate that there is a non-selective increase in urinary NAG excretion in control SCI patients and those with lower UTI. In SCI patients with upper urinary UTI there is a selective increase in NAG-B excretion. The overlap in enzyme values between the different groups suggests that the test may not be clinically useful in localising the site of UTI.

Key words: N-Acetyl- β -D-Glucosaminidase; Urinary tract infection; Spinal cord injury.

Urinary tract infection (UTI) is perhaps the most common cause of morbidity in patients with spinal cord injury (SCI) (Donnelly *et al.*, 1972). The diagnosis and treatment of UTI presents special problems in this group of patients. Clinical localisation of the site of UTI to the upper or lower tract is frequently not possible due to the absence of localising signs and symptoms. Localisation tests are either too invasive (e.g. selective ureteric catheterisation) or not specific enough (e.g. antibody coated bacteria test and Fairley's bladder washout test) (Kuhlemeier *et al.*, 1983; Stamey *et al.*, 1965). It is therefore important to develop suitable localisation tests. In addition to the problem of localisation, standard urine culture

criteria for the diagnosis of UTI are not applicable in these patients due to the frequent occurrence of asymptomatic bacteruria (Gribble *et al.*, 1988).

The use of urinary enzyme excretion in the localisation of UTI has been studied in other patient populations (Vigano *et al.*, 1983; Sandberg *et al.*, 1986; Mengoli *et al.*, 1982; Carrajal *et al.*, 1975; Devaskar and Montgomery, 1978). The most promising of the enzymes tested is N-acetyl- β -D glucosaminidase (NAG) a lysosomal enzyme of 70 000 molecular weight present in A and B isoenzyme forms (Tucker *et al.*, 1980). The normal adult excretes predominantly NAG-A in the urine (Hultberg *et al.*, 1974). However clinical situations where renal tubular injury occur have been associated with elevation of Total NAG (Alderman *et al.*, 1983; Johnston *et al.*, 1983; Kind, 1982) and preferential elevation of NAG-B excretion (Severini *et al.*, 1988; Poon *et al.*, 1979). The proximal renal tubular cells are rich in NAG-B and injury to these cells is thought to result in release of this isoenzyme in the urine.

The aim of our investigation was to study the pattern of NAG isoenzyme excretion in the urine of SCI patients and to evaluate its usefulness as a clinical test in the localisation of UTI.

Subjects and Methods

Forty five SCI patients (44 males and 1 female) were admitted to our Rehabilitation Centre for long term rehabilitation, 34 healthy control subjects were also included in this study. The controls were classified into two groups (I, II) and the patients into three group (III, IV, V). The groups and criteria for inclusion are given below while the clinical profile of the patient groups is given in Table II.

Group I: Normal healthy controls (No. 10)

Criteria: A normal male subject having no dysuria or fever and no clinical evidence of systemic disease with a normal urine microscopy.

Group II: Normal controls with non-urinary tract sources of fever (No. 24)

Criteria: A male subject without evidence of any systemic illness having a fever of less than 1 week duration, $>101.5^{\circ}\text{F}$ on the day of collection of the sample, no symptoms of UTI, a normal urine culture or a normal urine microscopy and a normal serum creatinine.

Group III: SCI patients without UTI (No. 27)

Criteria: A SCI patient having no fever or clinical evidence of UTI.

Group IV: SCI patients with lower UTI (No. 12)

Criteria: A SCI patient having fever $<101.5^{\circ}\text{F}$ on the day of collection of the sample, a significantly positive urine culture ($>100\ 000$ colony forming units), normal serum creatinine and clinical exclusion of an upper UTI and non-urinary

sources of fever. The clinical exclusion of an upper UTI is based on the absence of vomiting, paralytic ileus and loin pain.

Group V: SCI patients with upper UTI (No. 6)

Criteria: A SCI patient having fever $>101.5^{\circ}\text{F}$ on the day of collection of the sample, a significantly positive urine culture, clinically suspected to have an upper UTI based on the presence of features mentioned earlier and one of the following criteria: (1) paralytic ileus; (2) loin pain; (3) elevated serum creatinine (previous serum creatinine values being normal) and (4) a positive blood culture.

Initial urine cultures in Group III were all significantly positive. Hence urine culture was not routinely obtained in the remaining subjects. Urine for enzyme estimation was collected in clean bottles from indwelling Foley's catheters using aseptic precautions or by sterile intermittent catheterisation depending on the voiding method used by the patient.

Urine samples were routinely spun at $400\times g$ for 10 minutes and dialysed against 50 volumes of 0.1M Tris-HCl buffer pH 7.1 for 16 hours at 4°C with two changes of buffer. Dialysed urine samples were assayed either immediately or stored at -20°C and assayed within 7 days for enzyme. NAG was assayed spectrophotometrically at 400 nm using p-nitrophenyl-N-acetyl-glucosaminide as substrate (Basu *et al.*, 1977). The assay system consisted of 100 mmol citrate phosphate buffer pH 4.5, 100 μg bovine serum albumin, 1 mmol substrate and enzyme upto 140 μl in a total volume of 200 μl . Incubation was carried out at 37°C for 60 minutes and the reaction stopped by the addition of 800 μl 0.4M glycine-NaOH pH 10.5.

The differential assay of NAG-B was carried out by heat inactivation of NAG-A at 50°C for 180 minutes of 140 μl enzyme in 100 mmol citrate-phosphate buffer pH 4.5 and 100 μg bovine serum albumin. This was followed by the addition of 1 mmol substrate and incubation and termination of the reaction as given above. Creatinine was estimated by the Jaffe reaction (Brod and Sirota, 1948). Enzyme units are expressed as μ moles p-nitrophenol released/hour/mg creatinine.

Statistical analysis

The NAG-B excretion rate and NAG-B/Total NAG percentage between the individual groups were compared by the Mann-Whitney U test. The NAG-B/Total NAG percentage is used as a measure of the relative isoenzyme distribution. The effect of individual clinical variables on NAG-B enzymuria (Table II) could not be statistically analysed due to the small number of subjects in the individual subgroups.

Results

The mean and range of NAG-B excretion and NAG-B/Total NAG percentage in Groups I to V are given in Table I.

No statistically significant difference in NAG-B excretion was observed between Group I and II indicating that fever alone did not cause NAG-B enzymuria. There were no significant differences in NAG-B excretion between Group III and IV and in NAG-B/Total NAG percentage between Groups I and III, III and IV. The differences in NAG-B excretion between Groups IV and V and NAG-B/Total NAG

Table I

Group	Number of patients	Number of samples	Mean (range) NAG-B excretion Enzyme Units	Mean (range) NAG-B total NAG%
I	10	19	12.57 (0-47.76)	20.38 (0-41.3)
II	24	27	27.20 (0-137.5)	19.63 (0-65.4)
III	27	32	207.78 (0-886.7)	24.27 (0-59.0)
IV	12	12	343.50 (0-1461.5)	24.98 (0-46.1)
V	6	6	1133.18 (119.7-2977.0)	35.30 (30.7-43.1)

percentage between Groups III and V do not reach the level of significance ($p=0.0608$ and $p=0.0531$ respectively).

There were statistically significant differences in NAG-B excretion between Group I and III ($p<0.001$), Group II and III ($p<0.001$), Group III and V ($p<0.01$) and in NAG-B/Total NAG percentage between Group IV and V ($p<0.05$).

The elevation in NAG-B excretion in SCI patients (Group III) as compared to normal controls (Group I) without elevation of NAG-B/Total NAG percentage is indicative of a non-selective increase in NAG excretion. The increase in NAG-B/Total NAG percentage between SCI patients with upper UTI (Group V) and lower UTI (Group IV) and between control SCI patients (Group III) and those with upper UTI (though not significant) suggests that the elevated NAG-B enzymuria in upper UTI is selective.

Discussion

The results demonstrate a non-selective increase in total NAG excretion in control SCI patients (Group III) in comparison to normal controls (Group I). This elevation of NAG excretion maybe due to a chronically inflamed neurogenic bladder, and the enzyme distribution a reflection of isoenzyme composition of the lower urinary tract. Studies in children with neurogenic bladder have yielded similar results (Kunin *et al.*, 1978).

In upper UTI in SCI patients there appears to be a preferential increase in NAG-B enzymuria which maybe indicative of specific renal tubular damage occurring in this situation (Watts *et al.*, 1988). This observation should be interpreted in light of the lack of suitable gold standards to diagnose and localise UTI in SCI patients and the small sample size of SCI patients with upper UTI (Group V) in this study.

The effect of individual clinical variables such as the level and duration of SCI and the method of bladder drainage on NAG-B enzymuria could not be analysed due to the small size of the individual subgroups (Table II). The absence of an increase in NAG-B enzymuria in non-urinary causes of fever (Group II) is contrary to earlier findings (Sandberg *et al.*, 1985). Fever does not independently cause NAG-B enzymuria.

Although there is a difference in NAG-B enzymuria between SCI patients with upper and lower UTI, this does not reach a level of significance. Moreover, the

Table II NAG-B excretion in clinical sub-groups

Clinical sub-groups	Group III			Group IV			Group V		
	No. of patients	No. of samples	Mean NAG-B excretion	No. of patients	No. of samples	Mean NAG-B excretion	No. of patients	No. of samples	Mean NAG-B excretion
Level of spinal cord injury	24 3	29 3	173·19 231·70	11 1	11 1	336·65 too low to be determined	6 —	6 —	1133·18 —
Duration of spinal cord injury	12	16	137·19	4	4	444·55	2	2	641·95
	15	16	220·16	8	8	240·62	4	4	1378·80
Method of urinary drainage	20	25	168·94	8	8	199·63	—	—	—
	7	7	213·46	4	4	526·53	6	6	1133·18

overlap in range of values in the two groups suggests that this test is not clinically useful in localising the site of UTI. This is in agreement with studies of Sandberg *et al.* (1986).

Urinary NAG-B excretion in the assessment of long term decline of renal function has been studied in situations other than SCI (Watts *et al.*, 1988). Early detection of renal failure prior to elevation of serum creatinine may allow preventive measures to be instituted. This remains a possible direction for the future.

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