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CLINICAL STUDY

Urinary N-acetyl-beta-D-glucosaminidase (U-NAG) activity in children with vesicoureteral reflux

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Abstract: *Objectives:* The aim of this study was to measure U-NAG in children with vesicoureteral reflux (VUR) and examine the relationship between selected clinical parameters.
Background: U-NAG/creatinine ratio is a marker of renal tubular impairment and an increase in this ratio have been reported to affect the kidneys in various diseases.
Methods: The U-NAG/creatinine ratio was measured in the spot urine of 22 children (10 boys and 12 girls, mean age 2.83±2.42 years) with VUR. In 8 patients The VUR was unilateral grade I–IV (8 patients), and it was was bilateral, grade I–V in 14 patients. In patients with bilateral reflux and different VUR grade on each side, the highest grade of VUR was taken into consideration.
Results: The U-NAG/Cr levels were significantly higher in VUR patients compared to the reference (p=0.0001). There was no difference in U-NAG/Cr between children with unilateral (n=8) and bilateral (n=14) VUR (p=0.66). There was no difference in U-NAG/Cr between patients with VUR grades I–III and IV–V (p=0.67). The U-NAG/Cr activity was higher in patients with reflux nephropathy (RN; n=9) when compared to reference data (p=0.0001), however there was no difference in comparison to children without RN (p=0.84).
Conclusions: U-NAG/Cr increased in children with VUR grade I–V and there is a very weak relationship with the grade of VUR. U-NAG/Cr is a useful marker of renal tubular impairment, however there is poor relationship with the degree of kidney damage in patients with VUR *(Tab. 1, Ref. 25).* Full Text (Free, PDF) *www.bmj.sk.*
Key words: N-acetyl–D-glucosaminidase, vesicoureteral reflux.

Vesicoureteral reflux (VUR) is defined as an abnormal backflow of urine from the bladder to ureter or kidney (1). International classification of VUR based on the appearance of the urinary tract during voiding cystourethrography (VCUG) distinguishes five grades of VUR, according to its severity (1). VUR is potentially harmful because of the exposure of the kidney to increased hydrodynamic pressure during voiding. Furthermore, the incomplete emptying of the ureter and bladder on voiding predisposes the patient to urinary tract infection and consequent developement of renal scarring, termed as reflux nephropathy (RN) (2). Reflux nephropathy is a condition in which the kidneys are damaged by the backward flow of urine into the kidneys. It is now clear that reflux nephropathy represents two processes, congenital renal dysplasia and acquired chronic pyelonephritis (3). Originally, the term reflux nephropathy was first proposed in 1973 by Bailey to describe the coarse renal scarring of one or both kidneys associated with primary vesicoureteric reflux and urinary tract infection (4). The diagnostic gold standard is microscopic evaluation of biopsy specimens but renal scintigraphy with dimercaptosuccinic acid (DMSA) is widely used in the diagnosis. The pattern of DMSA distribution associated with congenital dysplasia is characterised by global decreased uptake and small renal size, while polar and medial defects with decreased uptake in the upper or lower pole and in medial part of kidney are present in acquired post-infection sequel (5).

Grades I–III VUR tend to resolve faster than grades IV–V, and association between grades IV–V VUR and the presence of renal damage has been observed (2, 6). N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme which is abundantly present in the cells of the proximal tubule and is considered as a very sensitive marker of renal tubular impairment (7–11). Furthermore, increased urinary NAG activity (U-NAG) has been repeatedly reported in patients with VUR grade III to V, suggesting tubular dysfunction (12–15). Our principal aim was to measure urinary NAG activity in patients with VUR grade I through V and relate the obtained results to the grade of VUR and several clinical parameters.

Material and methods

Patients

22 children (10 boys and 12 girls) aged 0.33 to 11.8 years (mean age 2.83±2.42 years) with VUR were enrolled. Informed

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Tab. 1. U-NAG/Cr expressed as Z-scores±SD.

Parameter	Mean	SD	p*
U-NAG/Cr (VUR I–V)	5.63	4.88	0.0001
U-NAG/Cr (VUR I–III)	5.39	4.76	0.0001
U-NAG/Cr (VUR IV–V)	6.35	5.26	0.0001
U-NAG/Cr (RN)	5.69	4.80	0.0001

VUR – vesicoureteral reflux, VUR I–V – vesicoureteral reflux grade I–V, VUR I–V – patients with VUR grade I–V (n=22); VUR I–III – pooled data from patients with VUR I–III (n=9); VUR IV–V – pooled data from patients with VUR IV–V (n=13); RN – patients with reflux nephropathy (n=9); * – compared to reference data

consent was obtained from each parent/guardian (and patient if applicable) prior to any procedures described in this paper. VUR was initially diagnosed on the basis of prenatal ultrasound screening in 14 children. In 8 patients, the VUR was diagnosed postnatally due to pyelonephritis. In all patients, the VUR was confirmed by VCUG. In 8 patients the VUR was unilateral, grade I–IV, and in 14 patients, the VUR was bilateral, grade I–V. In patients with bilateral reflux and different VUR grade on each side, the highest grade of VUR was taken into consideration. Therefore, the diagnostic distribution was as follows: VUR grade I, n=2; VUR grade II, n=3; VUR grade III, n=4; VUR grade IV, n=12; VUR grade V, n=1. Pyelonephritis occurred in 8 patients (36 %) who experienced a total of 20 episodes of pyelonephritis (range 1 to 6 per patient). Renal scintigraphy with 99mTc-dimercaptosuccinic acid (DMSA) was performed in all patients (n=22) in order to reveal renal scarring/RN. In those patients with documented pyelonephritis (n=8), the VCUG was performed at least 6 weeks and DMSA scan 6 months after the initial episode of pyelonephritis, respectively. RN was diagnosed in 9 patients (41 %).

Materials and methods

All patients had their U-NAG and urinary concentrations of creatinine (U-Cr) evaluated. None of the patients suffered from pyelonephritis at the time of the U-NAG and U-Cr evaluation. All patients were free from infection at least 5 months prior to the U-NAG and U-Cr evaluation. U-NAG was evaluated in the spot urine, collected after the first morning void. The blood and spot urine were collected either at the time of the DMSA scan or in a time frame of + 1 month, and within less than 5 months after the VCUG. The influence of endogenous enzyme inhibitors was eliminated by diluting the urine specimens 20-fold. The urinary catalytic activity of NAG was then determined by fluorimetric assay. The U-Cr was estimated by Jaffe's kinetic method on Modular Analyser (Roche Diagnostics GmbH, Mannheim, Germany). The U-Cr values were expressed in mmol/L. The U-NAG values were expressed as the urinary NAG/creatinine (U-NAG/Cr) ratio in nkat/L : mmol/L.

To eliminate the influence of age, the obtained results of U-NAG/Cr were expressed as standard deviation scores (SDS) or Z-scores by the equation SDS = (actual individual value – mean value for age)/standard deviation for age with the use of previously obtained age-related reference data (8). The obtained values were compared to the age-related reference data and correlated with grade of VUR and number of pyelonephritic episodes in the patients' personal history. The presence of RN was also taken into consideration, together with patients' age and VUR grading.

Statistical analysis was performed by t-test. The linear regression analysis was performed to compare the relationship among respective parameters. For all results, a $p<0.05$ was required for statistical significance.

Results

The U-NAG/Cr values were significantly higher in the VUR patients in comparison to the reference data (Tab. 1). There was no difference in U-NAG/Cr between children with unilateral and bilateral VUR ($p=0.66$). As there were low patient numbers with VUR grade I–III and V, we pooled the U-NAG/Cr data for VUR I–III and VUR IV–V, respectively. In both groups the values were still significantly higher in comparison to the reference data (Tab. 1), however there was no significant difference between VUR I–III and VUR IV–V subgroups ($p=0.67$). The U-NAG/Cr activity was high in patients with RN when compared to reference data (Tab. 1), but there was no difference in comparison to children with VUR without RN ($p=0.84$). We found almost no correlation between U-NAG/Cr and grade of VUR ($r=0.38$), which didn't reach statistical significance ($p=0.08$). In addition, significant inverse correlation between patients' age and VUR grade was observed ($r=-0.49$, $p=0.05$). Number of pyelonephritic episodes in patients' personal history was not related to U-NAG/Cr or VUR grade ($r=-0.18$ and 0.21, respectively).

Discussion

The high values of U-NAG/Cr in children with VUR suggest renal tubular impairment and correspond, in part, with previously published data (12–16). However, the U-NAG/Cr values were increased in our patients with VUR, regardless of whether the reflux was unilateral or bilateral. Previous reports indicated high U-NAG in patients with VUR (12–15), especially in children with VUR grade IV and V (12), or grade V only (16), or in patients with VUR and renal scarring (15, 18). It was therefore of particular interest to look for relationship between U-NAG/Cr and the grade of VUR. Our results suggest that high activity of U-NAG/Cr in children with VUR is only poorly related to the degree of renal damage, as there was almost no correlation between U-NAG/Cr and the grade of VUR, with no difference in U-NAG/Cr between VUR grades I–III and IV–V, respectively. Furthermore, there was no difference in U-NAG/Cr between patients with and without RN. This result differs from another observation, where high U-NAG was most prominent in children with renal scarring (15). These results further suggest that U-NAG/Cr is not related to the amount of affected renal tissue, as

strong association between grades IV–V VUR and the presence of renal damage has been repeatedly observed (1, 6, 17–19). However, there is also published evidence of no relationship between severity of VUR, urinary tract infection symptoms and renal scarring (20, 22). This shares some features with our findings of no correlation between number of pyelonephritic episodes in patients' personal history and severity of VUR, weak correlation between VUR grade and U-NAG/Cr, and no difference in U-NAG/Cr in patients with and without RN. Furthermore, it has been already reported that, despite good medical management, even mild and moderate VUR can be associated with renal injury (18, 23), and that VUR is a weak predictor of renal damage in children with urinary tract infection (23). In yet another systematic analysis, the authors questioned the values of identification of VUR after a symptomatic urinary tract infection on subsequent renal parenchymal damage (24). This corresponds to our observation concerning lack of relationship between severity of VUR and number of pyelonephritic episodes in patients' personal history, and weak correlation between severity of VUR and U-NAG/Cr values.

Therefore, the findings of high U-NAG with poor relationship to severity of VUR might reflect the fact that the grade of VUR is not always associated with the degree of renal impairment. Furthermore, we can't rule out that the U-NAG can detect even very mild changes in renal tubular function, which might occur even in low-grade VUR due to low increases of hydrodynamic pressure. We are still searching for the "holy grail", "gold standard" of tubular impairment – a highly sensitive, highly specific marker of functionally significant tubulopathy, which is detectable before deterioration, or before clinical symptoms develop. U-NAG/Cr was considered a candidate for this role, as it is a sensitive marker of tubular impairment. However, this marker is poorly related to the VUR grade and the presence of RN.

The inverse correlation between age and the grade of VUR further supports the well known fact that VUR grade decreases with age (2, 17, 25).

Conclusions

Tubular dysfunction is common in children with VUR. In the absence of a universal "gold standard" of renal tubular impairment, U-NAG/Cr should be considered a useful marker of tubular function, however there is a very weak relationship with the grade of VUR. This might support more recent observations that severity of VUR not always fully corresponds with the degree of kidney damage.

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