Is urinary N-acetyl-beta-D-glucosaminidase a marker of urological abnormality in children?

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Abstract

Background: Hydronephrosis is the most common congenital condition that is detected by prenatal ultrasonography. Moreover, the widespread use of prenatal ultrasonography results in an increased recognition of fetal hydronephrosis. Prenatal hydronephrosis is diagnosed at an incidence of 1:100 to 1:500 by ultrasonographic studies. The presence of hydronephrosis is not synonymous with obstruction. Obstruction signifies impairment of urinary flow, which if left untreated will cause progressive deterioration of renal function. Approximately 10-20% patients with obstruction show progression of hydronephrosis or worsening renal functions.

Methods: The study population consisted of 72 patients who were referred to the Division of Pediatric Nephrology, Mofid Children's Hospital, Tehran, IRAN for evaluation and treatment of pyelonephritis. All patients underwent two-dimensional ultrasonography (2D US) of the urinary tract; immediately afterward. Diagnosis of pyelonephritis has been based on clinical and paraclinical findings and abnormal 99mTc- demercaptosuccinic acid scan (DMSA scan). Glomerular filtration rate was in normal range in all of them. The children were classified in two groups as having normal kidney ultrasonography and abnormal ultrasonography. Fresh random urine samples were obtained on the admission time and at 48th hour of treatment. Urine samples were tested for N-acetyl-beta-D-glucosaminidase (NAG) (ELISA colorimetric, DIAZYME, USA) and creatinine. All of our patients were treated with same medication. We also evaluated our patients with voiding cyctoureterography (VCUG), renal scintigraphy and biochemical studies.

Results: In this study 73.6% of the patients had normal ultrasonography and 26.4% abnormal ultrasonography. In patients with abnormal kidney ultrasonography, condition such as stasis, moderate to severe hydronephrosis, decreased cortical thickening and urinary stone were seen in 47.4%, 26.3%, 5.3% and 21.17%, respectively. The mean for urinary NAG/Creatinine before antibiotic therapy was 36.79 ± 42.24 U/g creatinine in patients with normal ultrasonography, and 46.22 ± 57.53 U/g creatinine in abnormal group. Patients with hydronephrosis had the highest level of urinary NAG (p-value<0.043) and patients with urinary stone, had the lowest level of urinary NAG (p-value<0.004).

Conclusion: The urinary NAG was elevated in children with urinary tract abnormality with or without infection. Hense this protein could be a useful marker in prediction of hydronephrosis and its renal damage in pediatric group, although studies with greater numbers of patients are needed to establish this opinion.

Keywords: Urinary N-acetyl-beta-D-glucosaminidase, children, renal ultrasonography, hydronephrosis, renal stone.

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Introduction

Prenatal hydronephrosis is diagnosed with an incidence of 1:100 to 1:500 by ultrasonographic studies [1]. The presence of hydronephrosis is not synonymous with obstruction. Obstruction signifies impairment of urinary flow, which if left untreated will cause progressive deterioration of renal function. Approximately 10-20% patients with obstruction show progression of hydronephrosis or worsening renal functions [2-4]. Differentiating between obstructive and non-obstructive hydronephrosis requires assessment of renal function, over a period, with repeated radiological and radioisotope imaging. However, the diagnosis of obstruction is difficult to establish in childhood and renal ultrasonography has become the basic imaging modality in pediatric patients. Hydronephrosis may be missed, due to dehydration especially in young infant. Ultrasonographic studies should be performed by those having a great deal of experience in evaluation of kidneys and urinary tract in infants. Conventional two-dimensional (2D) ultrasonography is used as a screening modality for ing of diuretic administration, method of t½ calculation, compliance of renal collecting system and maturity of renal function can influence the results of renography. In a significant proportion of infants, this procedure is equi-vocal and unable to differentiate obstructive from non-obstructive hydronephrosis. The study should be done at places with considerable experience in examining infants and the results must be interpreted with caution. Intravenous pyelography provides excellent anatomic details, but unsatisfactory functional information. A diuretic renogram is superior to intravenous pyelogram due to lower risk of contrast reactions and radiation exposure, and better quantitative assessment of absolute and differential renal function. Imaging along with urinalysis, urine culture and blood tests as well as the urinary markers can be helpful for evaluating hy-

dronephrotic patients [5]. Previous studies have demonstrated that beta 2-microglobulin (beta 2-MG), alpha 1-microglobulin (alpha 1-MG) and NAG were elevated in renal pelvic urine from the hydronephrotic kidney and have showed renal dysfunction accompanying urinary tract obstruction [6]. NAG, a marker of renal proximal tubules, can be excrete in urine due to tubular damage. Urinary NAGs were also elevated in patients with pyelonephritis, renal stones, ureter stones, uretero-cutaneous stomy, nephrocalcinosis, hypercalciuria, multicyctic kidney or a single kidney [7-12]. Ueda et al showed that the urinary NAGs were elevated in all patients with renal stones, ureter stones, uretero-cutaneous stomy or a single kidney. They also reported that electron microscopic observations of the kidney in such patients revealed many primary and secondary lysosomes [8]. Miller et al showed that urinary NAG/Cr values were significantly higher in patients with urolithiasis or nephrocalcinosis or idiopathic hypercalciuria [9]. Then Hoppe et al revealed that urolithiasis or nephrocalcinosis may result in proximal tubular injury, which is rather the consequence of disease activity and of the mechanical influence of calculi, than of the metabolic background. The mechanism of cell damage in these conditions however, seems to be complex. Neither hyperoxaluria nor hypercalciuria alone was sufficient to induce severe tubular damage expressed as an increase in NAG excretion in the patients [11]. The results of these studies indicate that the urinary NAG could be helpful in evaluation and even diagnosis of urological disorders rather than pyelon ephritis. This study was performed to determine the diagnostic value of urinary NAG in hydronephrosis and compare it with other indices traditionally used for this purpose in children.

Method

This Quasi experimental and before and after study conducted from April 2005 to May 2007. The study population consisted of 72 patients

between 1 month and 15 years who were referred to the Division of Pediatric Nephrology, Mofid Children's Hospital, Tehran, IRAN for evaluation and treatment of pyelonephritis. All patients underwent 2D US of the urinary tract; immediately afterward. Diagnosis of pyelo nephritis has been based on clinical [fever, abdominal pain, anorexia and dysuria) and para clinical findings [leukocyturia, positive urine culture, increased ESR (erythrocyte sedimentation rate), positive CRP (C- reactive protein) and abnormal DMSA scintigraphy]. Our Golden standard for diagnosis of urological abnormality was ultrasonography and DMSA scintigraphy for confirmation of pyelonephritis. We excluded all patients with past history of renal or urological disorders, hypertention and voiding dysfunction. All patients were in complete health with no medical or drug history or signs of any renal diseases. Glomerular filtration rate was in normal range for the patients and calculated according to Schwartz' formula.

The sampling method of the study was census. The children were divided into two groups; as having normal kidney ultrasonography and abnormal ultrasonography due to hydronephrosis and urolithiasis. Fresh random urine samples were obtained on the admission time and at 48th hour of treatment. Urine samples were tested for NAG (ELISA colorimetric, DIAZYME, USA) and creatinine (Jaffe reaction, auto analyzer, RA 1000). All of our patients were treated with the same medication (only 75 mg/kg intravenous ceftriaxone). We also evaluated our patients with VCUG, DMSA scan and biochemical studies.

The ethics committee of the Shahid Beheshti University of Medical science have approved this study.

Data were expressed as mean±SD and findings were compared using the Mann-Whitney U test. Pearson's correlation coefficient was used for correlation studies. Statistic test was two-tailed and considered significant when $P \leq 0.05$. The SPSS statistic software program was used for the analyses.

Results

Seventy-two children (25% male and 75% female) with pyelonephritis, with the mean age of 43±39 months were evaluated. The demographic data of the patients is reported in table 1. In this study 73.6% of the patients had normal ultrasonography and 26.4% had abnormal ultrasonography. Stasis, moderate to severe hydronephrosis, decreased cortical thickening and urinary stone were 47.4%, 26.3%, 5.3% and 21.17% respectively, in patients with abnormal kidney ultrasonography.

The mean for urinary NAG/Creatinine before antibiotic therapy was 36.79 ± 42.24 (median=20) U/g creatinine in patients with normal ultrasonography and 46.22 ± 57.53 (median=34.73) U/g creatinine in abnormal group (P=0.528). In post antibiotic phase the mean for urinary NAG/Creatinine was 7.46 ± 8.80 (median=6.52) U/g creatinine in normal group and 21.36 ± 40.76 (median=18.12) U/g creatinine in abnormal group (P<0.002) (Table 2).

In our patients regardless of the impact of pyelonephritis, which proved by DMSA scintigraphy, the urinary NAG significantly correlated with changes in kidney ultrasonography in pretreatment phase. Patients that their ultrasonography showed hydronephrosis had the highest level of urinary NAG (p-value<0.043) and patients with urinary stone in their ultrasonography had the lowest level of urinary NAG (p-value<0.004). Overall, there was a significant difference between the levels of urinary NAG in patients with normal ultrasonography and abnormal ultrasonography (P<0.001).

After treatment of pyelonephritis again the urinary NAG level was correlated with ultrasonographic changes by Kruskal-Wallis test and concluded that patients with decreased cortical thickening due to sever hydronephrosis had the highest level of urinary NAG and patients with renal stone showed the lowest level

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	Std. Deviation	Mean	Maximum	Minimum
Age (Month)	39.23	43.39	144	1.0
Body Weight (kg)	8.30	14.81	46.90	3.50
Systolic Pressure (mmHg)	11.60	95.80	105	75
Diastolic Pressure (mmHg)	5.94	65.33	78	45
WBC (count/mm³)	5.57	13.81	20.30	3.50
ESR (mm/hr)	31.45	48.45	120	8
BUN (mg/dl)	8.10	10.12	27.10	3.0
Creatinine (mg/dl)	1.50	.79	0.98	.20
U NAG/Cr (U/g) (pre-treatment)	46.10	39.01	75.20	15.50
U NAG/Cr (U/g) (post treatment)	24.40	11.90	35.50	7.20
U NAG/Cr (U/g) (Normal US)	8.80	7.46	8.20	2.10
U NAG/Cr (Abnormal US)	40.76	21.36	65.30	10.50

Table 1. Clinical and paraclinical data of study group.

of this protein (p value = 0.09).

No significant association was detected between urinary NAG and the patients' sex, age and weight. In order to evaluate whether there is association between vesicoureteral reflux or DMSA changes and the level of urinary NAG, we used Kruskal-Wallis, Oneway and Post Hoc tests in addition to spearman test. We found no association between the urinary NAG level and the other variants that were described above. And there wasn't any difference in urinary NAG level between patients with vesicoureter-al reflux and without it. We also find no differences in urinary NAG level between patients with scar formation in DMSA scan and normal group.

Discussion

We concluded that patients whose ultra-sonography showed hydronephrosis, at the time of pyelonephritis, had the highest level of urinary NAG and patients who showed urinary stone in their ultrasonography had the lowest level of urinary NAG. However after treatment

Ultra Sonography							Sig.
Stone (n=4)	DCT** (n=1)	HDN* (n=5)	Stasis (n=10)	Normal (n=52)			
24.87	246.00	55.60	28.30	36.79		Pre-tx NAG/Cr (U/gm)	0.528
					Mean		
24.73	246.00	44.12	20.94	20.00	Median		
3.87	246.00	1.77	2.74	.32	Minimum		
46.15	246.00	110.00	92.31	180.00	Maximum		
19.10	-	42.99	27.45	42.24	Std. Deviation		
18.65	158.57	2.56	10.98	7.49		Post-tx NAG/Cr (U/gm)	0.002
					Mean		
14.43	158.57	2.36	7.23	6.52	Median		
13.53	158.57	.92	1.04	.55	Minimum		
28.00	158.57	4.40	33.64	49.04	Maximum		
8.11	.	1.74	11.54	8.80	Std. Deviation		

HDN*: hydronephrosis   DCT**: Decreased cortical thickening

Table 2. Pre and post treatment of U NAG/Cr in patient with normal and abnormal.

of pyelonephritis highest level of the urinary NAG was seen in patients with decreased cortical thickening due to sever hydronephrosis and lowest level of this protein was seen in renal stone group.

This study demonstrated that the urinary NAG was a good indicator of urological abnormalities in patients with and without pyelonephritis. Nonetheless Johnson et al reported that urinary NAG level within 1 SD of the mean in cystitis indicates a low risk of urologic abnormalities [13]. A recent study on infants diagnosed with hydronephrosis showed that some patients presented increased level of urinary NAG and moderate tubular dysfunction during the first year of life [14]. Haung reported that the levels of urinary NAG was more elevated in patients with hydronephrosis than the control group [15].

As far as we are concerned, renal tubular damage that may occur due to hydronephrosis can be the etiologic factor for increased excretion of urinary proteins such as NAG. So increased level of urinary NAG seems to be due to the impact of severe hydronephrosis accompanying with cortical injury. Nevertheless, we have no suggestion for reverse correlation between urinary NAG level and renal stone formation. Therefore it must be explored whether increasing the excretion of some electrolytes or crystals during renal stone formation, can change the composition of urinary NAG.

To the contrary, Yamaguchi reported that hyperoxaluria and crystaluria in rats induced increase urinary NAG excretion [16] and other studies revealed that patients with renal stone had higher urinary NAG level when compared with the control group [17-19]. Although, no significant correlation was seen between the level of urinary NAG and VCUG or DMSA changes, Tomlinson et al showed that elevated NAG levels were found mostly in the 65 of 93 children with bilateral scarring and severe vesicoureteral reflux [20]. Carr et al demonstrated that urinary NAG levels are elevated with high-er grades of reflux and this relatively simple assay may have clinical usefulness in the assessment of tubular dysfunction associated with reflux [21]. It is difficult to make direct comparison between this and other studies, and it is unclear why the results of the studies on correlation between the level of this marker and DMSA or VCUG changes have been varied.

In conclusion, urinary NAG is elevated in children with urinary tract abnormality in presence or absence of infection. This protein could be a useful marker in prediction of the hydronephrosis and associated renal damage in pediatric group, though studies with greater numbers of patients are needed to establish this opinion.

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