Original Article

Evaluation of Human Urinary N-acetyl beta – D-glucosaminidase index in children with urinary tract anomalies

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Abstract:

Background: The kidneys perform glomerular filtration, tubular reabsorption, and tubular secretion and the study of urinary excretion of some enzymes considered as a sensitive test for the detection of early stages of renal disease, particularly N-acetyl-β-D-glucosaminidase (NAG) which is a hydrolytic lysosomal enzyme present in the epithelial cells of the proximal convoluted tubule. Increased urinary NAG due to tubular damage could be used as a marker by a simple non invasive test for prediction of urinary tract problems like pelviureteric junction(PUJ) obstruction ,vesicouretric reflux(VUR) and pyelonephritis.

Objectives: to assess urinary NAG/ urinary creatinine (NAG/ Cr) ratio in children with different urinary tract anomalies and compare it with normal healthy children.

Patients and Methods: A prospective study was conducted from November, 2012 to April, 2013. Urine samples were collected from 51 patients with Urinary tract problems, and 40 healthy children as a control group, their age range (1month-13years). Children were admitted to Pediatric nephrology department, Children Welfare Teaching Hospital / Medical City Complex, Baghdad, Iraq. The Glomerular filtration rate was in normal range in all of them. Urine samples were tested for NAG by (ELISA,Cusabio ,China) while, both Serum creatinine (S-Cr) and urine creatinine (U-Cr) were estimated by Jaffe's kinetic method.

Results: The current results revealed that NAG/creatinine index was significantly higher in patients with vesicoureteral reflux, Pelviureteric junction obstruction and pyelonephritis in comparison with cystitis.

Conclusions: The assessment of urinary NAG could be considered as a useful marker in prediction of the vesicoureteral reflux, hydronephrosis secondary to Pelviureteric junction obstruction .Urinary NAG is elevated in children with pyelonephritis and it can be considered as a further criterion in the diagnosis of upper urinary tract infection.

Keywords: Urinary N-acetyl-beta-D-glucosaminidase, urinary tract infection, vesicouretric reflux,hydronephrosis., Pelviureteric junction obstruction.

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Introduction:

The kidneys play a major role in maintaining constant volume and composition of the extracellular fluid. through three basic functions: Glomerular filtration, tubular reabsorption, and tubular secretion. The kidney function can be evaluated by a number of methods, including the assessment of urinary enzymes. Enzyme activity is normally low in urine and may increase when renal tubular cells are injured(1). Damage to renal tubules can be insufficient to result in a change in a parameter of kidney function such as serum creatinine. In addition, in cases of more extensive tubular injury, there is a lag in time between the injury and an increase in serum creatinine. Sensitive biological markers of renal tubular injury are needed in order to detect early kidney injury(2). Vesicouretric reflux (VUR) is a common urinary tract anomaly seen in children and is potentially harmful because it expose the kidney to the increased hydrodynamic pressure during voiding. furthermore the incomplete emptying of the bladder and ureter on voiding predisposes the patient to urinary tract infection (UTI).This infection carries a relatively high risk of renal scarring (3,4). VUR can be diagnosed reliably by voiding cystogram . This procedure entails placement of a catheter, and may require sedation in toddlers, it also carries a high risk of radiation exposure to gonads (5,6). Pelviureteric junction(PUJ) obstruction is the most common congenital condition that is detected by prenatal ultrasonography at an incidence of 1:100 to 1:500 by ultrasonographic studies. 10-20% of patients show progression of hydronephrosis or worsening renal functions(7, 8)N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme that is present in proximal tubular cells. The NAG has a relative high molecular weight of approximately 130000-140000/daltons,which does not permit its filtration through

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the glomerular basal membrane and it is rapidly cleared from the circulation by the liver. Thus, urinary NAG originates primarily from the proximal tubule, and increased urinary excretion is a consequence of renal tubular cell. Breakdown; therefore, its urinary excretion is relatively constant with minimal diurnal changes. NAG is stable against changes in pH and temperature(9).The urinary NAG values should be expressed as a ratio to urinary creatinine concentration, as this relationship shows less variability than the urinary enzyme excretions related to volume or time (10).

Patients and Methods:

A prospective cohort study was conducted from 1st of November, 2012 to 30th of April, 2013. The study group included 51 patients (35 girls and 16 boys) Their age ranged between (1month-13years),classified as [cystitis (n=10), VUR (n=25), PUJ obstruction (n=16)], who were admitted to the nephrology Pediatric department, Children Welfare Teaching Hospital /Medical City Complex, Baghdad, Iraq. Exclusion criteria included chronic kidney diseases (CKD), Nephrotic syndrome, diabetes mellitus, Jaundice, and use of nephrotoxic drugs or Anti-epileptic agents (Carbamazepine (Tegretol) or Sodium Valproate (Depakin)}. Ethical consideration Permission obtained from the ministry of health to conduct this study and to use the facilities in the hospital. A signed consent from each participant family after discussing with them the purpose of the study and all related matters to the research purpose.

According to imaging studies (ultrasonography and voiding cystourethrography), clinical [fever, abdominal pain, anorexia and dysurea etc) and laboratory data [leukocyturia, positive urine culture, increased ESR (erythrocyte sedimentation rate), positive CRP(C- reactive protein) and all patients had their kidney functions evaluated , Patients were allocated into three groups :-

1-PUJ group : Patients with only distended abdomen ,no other specific symptoms, dilated pelvis (more than seven centimeters is regarded significant)are found on ultrasound and no reflux (normal voiding) cryptography .

2-cystitis group: Patients diagnosed as when patients presented with symptoms of dysurea , frequency ,urgency ,positive urine culture ,no fever ,normal leukocyte count ,normal ultrasound findings ,normal voiding cryptography.

3- VUR group: Patients diagnosed according to findings of evidence of reflux on micturating cryptography (dilated renal pelvicalyseal system and ureters) and this group mainly presented with signs and symptoms of pyelonephritis (fever, abdominal pain or loin pain and anorexia, lab findings were positive for [leukocyturia, positive urine culture, increased ESR, positive CRP,increased echogenicity on ultrasound with positive urine culture ,diagnosed as pyelonephritis (11) and given treatment(ten days course of broad spectrum antibiotic (cefitriaxone)(3rd generation cephalosporin I.V. 100mg /Kg). later MCUG done and discovered as VUR.

While the apparently healthy control group consisted of 40 subjects (23 girls and 17 boys); their age ranged between (1month-13years), they were subdivided into three groups according to their age:-

Control (1) group (n=11) - < than 24 months .

Control (2) group (n=13) - >24-60 months

Control (3) group (n=16) - > 60 months

Glomerular filtration rate (GFR) was in normal range in all of them using: schwartz formula: GFR (ml/min/1.73 m2) = K × Ht ÷ Pcr

(Where K = constant determined by regression analysis provided, Ht = height in cm Pcr = plasma creatinine)(12).

Urine and blood specimens were obtained from both patients and control group. A fresh random urine sample, collected at the first morning void was obtained on the admission time .All urine samples were analyzed by urinary dipstick and centrifuged to remove cellular components.The supernatants were stored at −20°C .These urine samples were tested for N-acetyl-beta-D-glucosaminidase (NAG) (ELISA Kit Catalog No.CSB-E09450h .CUSABIO ,China) Both S-Cr and U-Cr were estimated by Jaffe's kinetic method(Randox Laboratories, England) .(13)The urinary NAG values were expressed as the urinary NAG/creatinine ratio. All of our patients were treated with same medication.The statistical analysis was done by using social sciences software (SPSS version 17) and microsoft excel 2010 data were presented as mean ± SD for continuous data and percentage for categorical variables .Comparison between every two groups were performed by student's t-test, while comparisons among groups were carried out by one-way analysis of variance (ANOVA).For all tests P-value < 0.05 was considered statistically significant and < 0.0001 was considered highly significant. The receiver operating characteristic curve is used to define the diagnostic value (specificity and sensitivity) and best cutoff value of urinary NAG index.

Results:

In this study, 51 patients were evaluated; 35 girls (58.3%) and 16 boys (41.7%). Their age ranged between (1month-13years) with mean age (51.68±40.76 months). While the apparently healthy control group in this study was 40 subjects, 23 girls (57.5%) and 17 boys (42.5%); their age ranged between (1month-13years) with mean age (58.29±42.13 months); There were no statistical differences between cystitis patients and the age matched control group when comparing different laboratory parameters including urinary NAG index as

shown in table (1),while there was a significant statistical difference ,p value < 0.0001 between the VUR group and the age matched group, when comparing different laboratory parameters (S. creatinine, urine creatinine ,U.NAG including mean urinary NAG/creatinine index) as shown in table (2). In PUJ obstruction group ,there was a statically significant difference when comparing the mean urinary NAG/creatinine index levels (398.18±166.77 U/g) , and the age matched control group mean urinary NAG/creatinine index levels (9.1±4.4 U/g) at p value < 0.0001 as shown in tables (3).In order to exclude the effect of infection (pyelonephritis) ,is fifteen patients with VUR and hydronephrosis were selected for follow up after ten days of broad spectrum antibiotic course for pyelonephritis . There was a significant lowering of NAG Index compared to the pretreatment level (p<0.0005),as expressed in the table(4),but it is still very high in comparison with the control group.

Table (1) comparison of the studied parameters levels between control group and cystitis group by t- test

Parameters	Control NO=29 mean±SD	Cystitis NO=10 mean±SD	P value
Age (months)	76.48+34.65	72.0+28.28	0.6886 N.S
BW (kg)	22.26+8.96	21.18+6.29	0.6804 N.S
WBC x10^3/mm^3	8.12+1.04	8.4+1.05	0.3731 N.S
S. urea (mg/dl)	26.41+4.78	27.0+4.62	0.7361 N.S
S. creatinine (mg/dl)	0.7+0.13	0.74+0.12	0.2431
U. creatinine (g/l)	0.48+0.11	0.54+0.1	01707 N.S
U. NAG (U/l)	4.51+1.57	4.64+1.48	0.8125 N.S
U. NAG-Index(U/g)	10.26+5.54	9.1+4.4	0.5107 N.S

N.B. the control whose age ≤ 24 months has been excluded.
BW =body weight, WBC=white blood cell count, U. NAG = Urinary N acetyl Beta - D glucosaminidase expressed in unit per liter (U/l), S =serum, U = urine, N.S = Not significant, SD=Standard deviation.

Table (2); comparison of the studied parameters levels between control group and VUR group by t-test

Parameters	Control NO.=40 mean±SD	VUR NO. =25 mean±SD	P value
Age (months)	58.29±42.13	53.18±48.4	0.6659 N.S
BW (kg)	18.4±10.06	12.7±7.04	0.0041**
WBC x10^3/mm^3	8.39±1.08	10.26±3.15	0.0079**
S. urea (mg/dl)	24.15±6.06	36.88±16.3	0.0008**
S. creatinine (mg /dl)	0.58±0.12	1.04±0.35	< 0.0001**
U. creatinine (g/l)	0.41±0.16	0.24±0.07	< 0.0001**
U. NAG (U/l)	6.45±3.56	160.12±45.75	< 0.0001**
U. NAG-Index(U/g)	25.91±33.5	687.47±176.1	< 0.0001**

BW =body weight, WBC=white blood cell count, U. NAG = Urinary N acetyl Beta – D glucosaminidase expressed in uint per liter (U/l), S =serum, U = urine.

Table (3); comparison of the studied parameters levels between control group and PUJ obstruction group by t-test

Parameters	Control (NO.=40) mean±SD	(NO=16) PUJ obstruction mean±SD	P value
Age (months)	58.29±42.13	31.59±29.48	0.0105*
BW (kg)	18.4±10.06	10.16±4.4	0.0001**
WBC x10^3/mm^3	8.39±1.08	12.13±5.97	0.0247*
S. urea (mg/dl)	24.15±6.06	35.38±15.16	0.0106*
S. creatinine (mg/dl)	0.58±0.12	1.01±0.62	0.0154*
U. creatinine (g/l)	0.41±0.16	0.3±0.15	0.0123*
U. NAG (U/l)	6.45±3.56	103.12±33.26	< 0.0001**
U. NAG-Index(U/g)	25.91±33.5	398.18±166.77	< 0.0001**

BW =body weight, WBC=white blood cell count, U. NAG = Urinary N acetyl Beta – D glucosaminidase expressed in uint per liter (U/l), S =serum, U = urine.

Table (4); comparison of the studied parameters levels between pre and Post treatment VUR with Pyelonephritis groups by paired t-test

Parameters	Pre treatment mean±SD	Post treatment mean±SD	P value
BW (kg)	18.3±7.55	17.5±6.98	0.3739 N.S
WBC x10^3/mm^3	11.8±1.01	9.4±0.52	0.0175 *
ESR (mm/hr)	57.0±36.14	22.8±6.72	0.1191 N.S
S. urea (mg/dl)	48.0±21.2	26.8±6.61	0.0397 *
S. creatinine (mg/dl)	1.32±0.45	0.92±0.22	0.0647 N.S
U. creatinine (g/l)	0.22±0.04	0.34±0.04	0.0296 *
U. NAG (U/l)	187.2±59.31	160.2±18.79	0.2339 N.S
U. NAG-Index(U/g)	826.7±148.18	485.47±103.84	0.0005 **

BW =body weight, WBC=white blood cell count, U. NAG = Urinary N acetyl Beta – D glucosaminidase expressed in uint per liter (U/l), S =serum, U = urine.

Discussion:

A biomarker has been defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [14]. Cells of the renal epithelium synthesize and excrete many enzymes to the urine. Determination of enzyme activities in urine is a sensitive and non-invasive method for the evaluation of renal tubular function.(15) .So far, however, there has been little discussion about the role of N-acetyl-beta-D-glucosaminidase (NAG) in children with Urinary tract anomalies ,The use of biomarkers as a noninvasive tool in the diagnosis and management of various diseases is increasingly reported in the literature,also the use of these biomarkers is especially important in the pediatric age group as the use of invasive investigation is a matter of concern in children. It is difficult to convince parents of the need for radiological investigation of their child after a UTI, therefore, there is a need for noninvasive and sensitive test (16).Lee et al,and Shokeir AA. described the role of various biomarkers in pediatric urological anomalies like vesicoureteral reflux (VUR) and pelvi-uretric junction obstruction (PUJO) .They found that The urinary NAG was elevated in children with urinary tract abnormality (17&18).The urinary NAG/creatinine values were not gender dependent in the current study and this is in agreement with other studies conducted by (Mohkam MKarimi A etal,and Skalova.S et al.) (19,20)), In this study, the measurement of NAG index of 51 patients admitted for diagnosis and treatment of urological problems ,the U-NAG/ Cr index levels were significantly higher in VUR patients ,PUJ obstruction and Pyelonephritis compared to the control group at ($p = 0.0001$), as shown in the tables(2,3,4),while cystitis group had normal NAG index level, this is due the evidence of tubular dysfunction is common in children with upper urinary tract problems and not in cystitis (21) .So increased level of urinary NAG seems to be due to the impact of severe hydronephrosis accompanied with cortical injury(20). Haung also reported that the level of urinary NAG was more elevated in patients with hydronephrosis than the control group (21) and in the Iranian study the urinary NAG was elevated in children with pyelonephritis and it can be considered as a further criterion in the diagnosis of upper urinary tract infection(19).

Conclusions:

The assessment of urinary NAG could be considered as a useful marker in prediction of the VUR, hydronephrosis and renal tubular impairment in various disease states. Urinary NAG is elevated in children with pyelonephritis and it can be considered as a further criterion in the diagnosis of upper urinary tract infection.

Author contributions:

Study conception and design: Firyal H Al-Obaidi, Nariman F Ahmed

Acquisition of data: RaghadJ Ali

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