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ORIGINAL ARTICLE

Renal tubular markers as screening tools for severe vesicoureteral reflux

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Abstract

Severe (grades IV and V) vesicoureteral reflux (VUR) is a risk factor for acute pyelonephritis, renal scars, and renal failure. This study evaluates albumin and *N*-acetylglucosaminidase (NAG) urinary excretion, and renal concentrating ability as screening tools to select patients for voiding cystourethrogram (VCUG). Children (111 M, 52 F) aged 10.97 ± 21.17 months (mean + SD), diagnosed with UTI, and who had undergone renal ultrasound and a VCUG, underwent a desmopressin test and had albumin/creatinine and NAG/creatinine urinary excretion measured. Urine osmolality was significantly lower in 27 children with severe VUR (375.3 ± 171.8 mOsm/kg; mean + SD) compared to 100 patients with normal VCUG (611.5 ± 175.8 mOsm/kg), $p < 0.001$, and to 36 patients with VUR grades I to III (636.2 ± 180.2 mOsm/kg), $p < 0.001$. NAG/creatinine ratio was significantly elevated in 20 children with severe VUR (26.4 (28.3) U/g); median and interquartile range compared to 67 children with normal VCUG (10.8 (17.9) U/g), $p = 0.003$, and to 20 patients with VUR grades I to III (7.6 (21.1) U/g), $p = 0.009$.

Conclusions: Urinary osmolality is significantly decreased and urinary excretion of NAG is significantly increased in patients with severe VUR. These tests could select patients for VCUG to assess for severe VUR.

What is Known:

- *Severe vesicoureteral reflux (SVUR) may contribute to renal damage. Severe vesicoureteral reflux is diagnosed by voiding cystourethrogram and represents about 10% of all patients with VUR. Currently, there are no reliable tests used prior to VCUG to help on the decision of obtaining a VCUG to diagnose SVUR.*

What is New:

- *This study shows that renal tubular markers (concentrating ability and N-acetylglucosaminidase (NAG) excretion) are useful tests prior to voiding cystourethrogram to screen for severe vesicoureteral reflux.*
- *This study suggests the use of renal concentrating ability and urinary N-acetylglucosaminidase (NAG) excretion to screen for severe vesicoureteral reflux before requesting a voiding cystourethrogram.*

Keywords *N*-Acetylglucosaminidase · Urinary concentrating capacity · Urine albumin · Vesicoureteral reflux · Voiding cystourethrogram

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Abbreviations

DMSA	Dimercaptosuccinic acid
NAG	*N*-Acetylglucosaminidase
NPP	Negative predictive value
PPV	Positive predictive value
UTI	Urinary tract infection
VUR	Vesicoureteral reflux
VCUG	Voiding cystourethrogram

Introduction

Severe VUR (grades IV and V) is seen in about 10% of patients with VUR diagnosed after UTI and it is considered a predisposing factor for acute pyelonephritis. Severe VUR is diagnosed on a VCUG, an uncomfortable procedure. Because VUR grades I to III neither predispose to UTI nor are they associated with renal failure [6, 23], there is the need for tests that could help to differentiate severe from lower levels of VUR or no VUR and, therefore, may help in selecting patients to undergo VCUG to assess for severe VUR.

Previously, the suggested predictors prior to obtaining a VCUG were male gender, family history of urinary pathology, the levels of C-reactive protein or procalcitonin, and the presence of dilatation of the urinary tract on ultrasound examination. However, these predictors do not differentiate between severe and lower grades of VUR as well as their sensitivity and specificity are rather low [16, 21].

Preliminary data have suggested that VUR interferes with tubular function resulting in a decreased urinary concentrating ability in those patients [15, 28–30]. However, the studies did not differentiate among the types of VUR and they did not evaluate the role of renal scars on the observed decreased urinary osmolality. The purpose of the study was to evaluate urinary albumin and *N*-acetylglucosaminidase (NAG) excretion and urinary concentrating ability, known tubular markers, as predictors of severe VUR.

Patients and methods

Patients

Inclusion criteria This study included 163 children and adolescents (111 M, 52 F) up to 18 years of age who had a UTI between January 2010 and December 2015 and who underwent a renal ultrasound and a standard VCUG as well as the following renal tubular markers, urine osmolality, albumin/creatinine, and NAG/creatinine. Those < 3 months of age with pyelonephritis and children > 3 months who lived far from our hospital or who were dehydrated, hypotensive, or unable to tolerate oral feeds/antibiotics were hospitalized during their UTI.

VUR was classified into five groups according to the International Reflux Study Committee classification [13]. When the VUR was bilateral, the higher grade value was taken. On renal ultrasound, the term moderate to severe hydronephrosis was used to denote patients with an anteroposterior diameter of the renal pelvis ≥ 2 cm and the term mild hydronephrosis for those cases with the diameter of the renal pelvis being between 0.5 and 2 cm. Dimercaptosuccinic acid (DMSA) scintigraphy was performed on 54 patients.

Exclusion criteria Patients 18 years or older and those who had been diagnosed with renal multicystic renal dysplasia, ureteropelvic or vesicoureteral stenosis, double complete collecting system, or posterior urethral valves were excluded.

Renal tubular markers

Urine samples for tubular markers were obtained 1 or 2 months after an acute cystitis and at least 4 months after a suspected acute pyelonephritis [3, 14, 33]. Urine samples were collected after informed consent was obtained.

Desmopressin test to assess urine osmolality Ten micrograms of intranasal desmopressin was administered in the morning to infants with less than 1 year of age. Three following urine samples were then collected. If only two samples were available, the test was discontinued 8 h later. Bottle-fed infants were restricted to half the bottle content from the first bottle in the morning until 18:00 h to reduce the risk of water intoxication [2, 11, 20]. In children over 1 year of age, renal concentrating capacity was determined after administration of 20 μg of intranasal desmopressin or 0.12 mg (120 μg) of oral desmopressin lyophilized that dissolves immediately in the mouth. After emptying the bladder, three urine samples were collected at 90-min intervals. In this case, it was recommended not to abuse liquids. The highest osmolality value obtained was taken as the test result.

Urinary creatinine was determined by a kinetic colorimetric test based on the Jaffe method (Creatinine Jaffé Gen.2, Roche). Albumin was measured using a nephelometric technique (Array) and NAG activity was determined using an enzymatic colorimetric assay based on the hydrolysis of 3-cresolsulfonephthalein-*N*-acetyl-β-D-glucosaminidase (Roche). Urinary osmolality was measured by freezing point depression in an Osmo Station OM-6050 Osmometer (Menarini Diagnostics).

The measurements taken closest to the date of the VCUG were used. In the case of acute pyelonephritis, urine was collected at least 4 months after infection.

Statistical methods

The Kolmogorov–Smirnov test was used to analyze the distribution of the sample. Quantitative variables following a

normal distribution are expressed as mean and standard deviation (urinary osmolality). The quantitative variables, which did not have a normal distribution, were expressed as median and interquartile range (NAG/creatinine and albumin/creatinine ratios). ANOVA tests followed by Student's *t* test were used for the comparison of the differences between variables with a normal distribution. Kruskal–Wallis and Mann–Whitney tests were used for the comparison of the differences of the parameters analyzed when the distribution was not normal. The chi-square test was used to compare the frequency between groups of qualitative variables. Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated for the three functional markers analyzed. Parsimonious multivariate logistic regression models were fitted to identify variables associated with VUR. These analyses were performed using SPSS statistical software (SPSS v. 19.0, SPSS Inc., USA). A P value < 0.05 was considered to be statistically significant.

Normal values The normal values used as reference for urine osmolality after desmopressin test were defined in 125 healthy infants at the Hospital Universitario Nuestra Señora de Candelaria between 1984 [9] and 1988 [10] (Table 5 in Appendix).

Results

Patients were divided into groups according to renal ultrasound and renal scintigraphy results (Table 1). The group with renal parenchyma losses included patients with kidneys demonstrating one or more scars on DMSA renal scan ($n = 10$) and kidneys with atrophy, hypoplasia, or renal dysplasia ($n = 10$). Four of the children with mild hydronephrosis did also have ureteral dilatation.

Renal ultrasound and renal scintigraphy data as criteria to predict vesicoureteral reflux are shown in Table 2. Sensitivity, specificity, PPV, and NPV when including all normal vs. abnormal anatomical findings to detect VUR were sensitivity 90.5%, specificity 11%, PPV 39%, and NPV 64.7%: odds ratio 1.2 (IC 95% 0.4–3.4); p = NS.

Each of the renal abnormal findings to detect VUR was compared to normal kidneys.

When mild hydronephrosis was compared to normal kidneys, sensitivity to detection of VUR was 82.3%, specificity 13.1%, PPV 27.7%, and NPV 64.7%: odds ratio 0.7 (IC 95% 0.2–2.1); p = NS.

When moderate and severe hydronephrosis were compared to normal morphology, the sensitivity was 72.7%, specificity 55%, PPV 64%, and NPV 64.7%. In these groups, the odds ratio to detect VUR was 3.3 (IC 95% 0.9–11.8); p = NS.

When renal parenchymal loss was compared to normal morphology to detection of VUR, the sensitivity was 68.4%, specificity 61.1%, PPV 65%, and NPV 64.7%. The odds ratio was 3.4 (IC 95% 0.9–13.2); p = NS. Table 3 presents the urinary osmolality, albumin, and NAG excretion as predictors of VUR. The three tubular tests were related to the presence or absence of VUR in the VCUG of all patients studied. The value of urinary osmolality was significantly lower in the group of 63 children with VUR (524.4 ± 218.3 mOsm/kg, mean + SD) compared to 100 patients without VUR (611.5 ± 175.8 mOsm/kg, mean + SD, $p = 0.009$). Sensitivity was 74.6%, specificity of 56%, PPV 51.6%, and NPV 77.7%: odds ratio 3.73 (IC 95% 1.87–7.46); $p = 0.0002$. The three tubular tests were evaluated in patients with VUR according to the VUR grade (Table 4 and Fig. 1). Twenty-seven children with VUR grades IV and V have a statistically significant decreased urinary osmolality (375.3 ± 171.8 mOsm/kg, mean + SD) when compared to 100 patients without VUR (611.5 ± 175.8 ($n = 100$) mOsm/kg; $p < 0.001$) and 36 patients with VUR grades I to III (636.2 ± 180.2 mOsm/kg; $p < 0.001$). Moreover, none of the 27 patients with grades IV or V VUR had a normal urinary concentration test.

Twenty children with VUR grades IV and V have a statistically significant increase in urinary NAG excretion (26.4 (28.3) NAG/creatinine, median, and interquartile range) when compared to 67 patients without VUR (10.8 (17.9) U/g; $p = 0.003$) and 20 children with VUR grades I to III (7.6 (21.1) $p = 0.009$) (not shown in the table).

Values of both urinary osmolality and NAG/creatinine ratio in patients with grades I to III were not statistically different from those observed in patients without VUR.

Renal concentrating ability between the presence of severe VUR (grades IV and V) and absence of VUR showed a sensitivity of 100%, specificity of 56%, PPV 38%, and NPV 100%: odds ratio 69.8 (IC 95% 4.1–1176.7); $p = 0.003$.

NAG/creatinine ratio between the presence of severe VUR (grades IV and V) and absence of VUR showed a sensitivity of 40%, specificity of 88.1%, PPV 50%, and NPV 83.1%: odds ratio 4.9 (IC 95% 1.5–15.7); $p = 0.007$.

Table 1 Patient demographics

	Normal kidney	Mild hydronephrosis	Moderate–severe hydronephrosis	Renal parenchyma loss
Number	17	101	25	20
Sex	7 M, 10 F	72 M, 29 F	19 M, 6 F	13 M, 7 F
Age (months)	24.8 ± 32.1	7.9 ± 18.6	8.0 ± 13.2	18.0 ± 24.9

Table 2 Morphological findings on ultrasonography and/or renal scintigraphy and the presence or absence of VUR

	VUR ($n = 63$)	No VUR ($n = 100$)	Total 163
Normal kidney	6/17 (35%)	11 /17 (65%)	17
Mild hydronephrosis	28/101(28%)	73/101 (72%)	101
Moderate–severe hydronephrosis	16/25 (64%)	9/25 (36%)	25
Renal parenchyma loss	13/20 (65%)	7/20 (35%)	20

Moreover, in binary logistic multivariate regression, VUR grades IV and V associated significantly with decreased urinary osmolality with odds ratio of 4.3 ($p = 0.002$) which implies that patients with low urinary osmolality have 4.3 times greater chance of having severe VUR.

Discussion

It is known that the risk of developing chronic end-stage renal disease in a child with UTI is 1 per 10,000 [7]. Moreover, end-stage renal disease due to UTI represents 8% of children and adolescents that have undergone kidney transplantation [31]. Severe VUR (grades IV and V) in association with acute pyelonephritis is currently considered a risk factor for the development of chronic kidney disease [4, 25]. Voiding cystourethrogram (VCUG) is the technique used to diagnose and grade vesicoureteral reflux (VUR). The AAP has recently recommended that VCUG should not be performed routinely after the first febrile UTI unless renal ultrasound reveals hydronephrosis, scarring, or other findings that would suggest either high-grade VUR or obstructive uropathy [26]. Because severe VUR is considered a risk factor for renal failure, there is the need for tests that could help to differentiate severe from lower levels of VUR. In addition to the cost, discomfort, and radiation associated with VCUG, the AAP states that renal ultrasound is not effective to detect severe vesicoureteral reflux (VUR), as it may be normal even in some who have high-grade VUR [26]. Previous studies have shown that hydronephrosis is not a predictor of VUR. However, these studies have included in their evaluation mild to severe hydronephrosis. We expand this observation by demonstrating that all types of hydronephrosis (mild to severe), in addition to small kidneys, and renal scars are not predictors of VUR grades IV or V. In this report, we suggest that two renal tubular tests (urinary osmolality and NAG urinary excretion) may guide the decision to obtain a VCUG to rule out the presence of severe VUR (grades IV and V) even in the presence of a normal renal ultrasound. As shown in Table 3, when all types of VUR were included in one group, urinary osmolality after a desmopressin test was the only one of the three tubular tests studied that showed statistical significant difference between those patients with VUR and those without. When patients were grouped according to the degree of VUR, the urinary osmolality and NAG urinary excretion were significantly low and high respectively only in those patients with VUR grades IV and V when compared to values in patients without VUR and those with VUR grades I to III. Therefore, these two tubular tests may suggest the presence of severe VUR in UTI patients. Previous studies have shown conflicting results regarding the association of VUR and a reduced urinary osmolality [24, 30]. Uehling found no statistical significant difference between patients with or without VUR. The concentration test was limited to two samples, no grade of VUR was reported, and the author observed a low osmolality in those patients with "abnormal" excretory urogram but the abnormal finding was not defined [24]. Walker et al. using as Uehling did the same water deprivation test to assess urine osmolality divided 76 patients with VUR into four groups according to the degree of VUR. All VUR groups had a significantly lower urine osmolality than controls. The urine osmolality was the lowest in those patients with renal scars. In a follow-up study, they separated patients according the degree of VUR using the International criteria. Unfortunately, each group included patients with different degrees of VUR (grades I and II, grades II to III, grades III and IV, and grades II to V) [30]. In contrast, in this study, the groups with VUR are well defined and the urinary concentration test after desmopressin is more reliable than simply the water deprivation test. In addition, the desmopressin test was

Table 3 Comparisons of tubular markers in patients with or without VUR. All values are presented as mean ± SD

	VUR	No VUR	*P*
Urine osmolality (mOsm/kg)*	524.4 ± 18.3 ($n = 63$)	611.5 ± 175.8 ($n = 100$)	0.009
Albumin/creatinine (μg/μmol)**	3.9 (6.7) ($n = 57$)	3.1 (5.1) ($n = 90$)	NS
NAG/creatinine (U/g)**	13.7 (34.4) ($n = 40$)	10.8 (17.9) ($n = 67$)	NS

*Values for urine osmolality are presented as mean ± SD

**Values for albumin/creatinine and NAG/creatinine are presented as median/interquartile range

Table 4 Tubular markers and VUR grade

VUR	Grade I	Grade II	Grade III	Grade IV	Grade V	*P*
Urinary osmolality (mOsm/kg)§	625.7 ± 170.9 (*n* = 4)	631.7 ± 241.4 (*n* = 6)	638.9 ± 174.0 (*n* = 26)	426.1 ± 175.7 (*n* = 16)	301.4 ± 142.6 (*n* = 11)	< 0.001*
Albumine/creatinine (μg/μmol)§§	0.9 (–) (*n* = 3)	5.4 (24.5) (*n* = 5)	3.8 (5.5) (*n* = 24)	5.4 (7.1) (*n* = 15)	4.99 (8.14) (*n* = 10)	NS**
NAG/creatinine (U/g)§§	5.1 (–) (*n* = 2)	6.2 (–) (*n* = 3)	9.1 (33.9) (*n* = 15)	24.7 (29.4) (*n* = 12)	33.8 (94.3) (*n* = 8)	0.046**

Urinary osmolality. VUR grades IV and V vs. no VUR ($P < 0.001$) and vs. VUR grades I to III ($P < 0.001$)

NAG/creatinine ratio. VUR grades IV and V vs. no VUR ($P = 0.003$) and vs. VUR grades I to III ($P = 0.009$)

*P after ANOVA test

**P after Kruskal–Wallis test

§ Mean ± SD

§§ Median/interquantile range

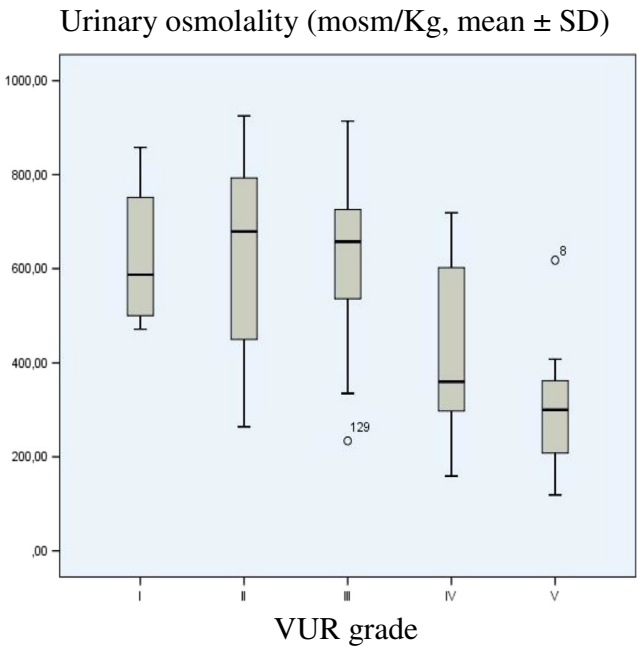

Fig. 1 Urine osmolality after a desmopressin test and VUR grade. Each box represents osmolality results for each VUR grade. The horizontal line in the box is the mean. The vertical lines represent 2 SD

done under medical supervision. The key finding in this study is that the urinary osmolality values and NAG urinary excretion were statistically lower and higher respectively when comparing patients with grades I to III to those with VUR grades IV to V. Moreover, the values in patients with grades I to III were not different from those observed in patients without VUR. It is particularly noteworthy that for both tubular tests, the sensitivity to detect severe VUR (grades IV and V) and the NPV were both 100%. The mechanism for a decreased urinary osmolality associated with severe VUR is unknown. The ability of the kidneys to concentrate urine deteriorates when the amount of functioning renal parenchyma is reduced [1, 8]. But scars are not the only explanation for our findings because patients with normal kidneys who did have VUR grades IV or V also presented decreased urinary osmolality in this study. Intratubular hyperpressure has been shown to produce a reduction in the activity of some tubular sodium transporters [22], in the expression of urea transporters [18], and in the activity of the aquaporins [17, 22]. Continuous hyperpressure as observed in obstructive uropathy in the experimental animal is associated with a decrease in urinary concentrating ability. However, this mechanism does not apply to our patients because cases with hydronephrosis associated with stenosis at the level of the pelvis or bladder were excluded. Nevertheless, the role of intermittent hyperpressure on the renal collecting tubule at the time of voiding in patients with VUR grades IV and V cannot be rejected as the reason for the observed decreased urinary osmolality.

N-Acetylglucosaminidase (NAG) is a lysosomal enzyme present in the proximal tubules normally secreted in small

concentrations as a consequence of a natural exocytosis process. In this study, there were no statistically significant differences in urinary excretion of NAG between patients with VUR of all grades and patients without VUR (Table 3). However, a significant difference was observed in cases of VUR grades IV and V with regard to VUR grades I to III and patients without VUR. Williams et al. also observed that the urinary NAG levels do not reliably detect the presence of reflux with the exception of grade V vesicoureteral reflux, and he concluded that NAG cannot be accurately applied as a screening test for detection of this common urologic problem [32]. In contrast, Carr et al. described a significant difference between patients with VUR and controls. The difference was more marked in two patients with intrarenal reflux [5]. Others have observed increased urinary NAG excretion related to the presence of renal scars rather than VUR [27–29]. We have studied the relationship between NAG levels and renal scars. In this study, we observed that the increased urinary NAG was related to the tubular high pressure. In all those patients presenting renal scars and elevated NAG levels, the levels normalized when the VUR was surgically corrected [12].

Limitations of the study This was a single-center study. The addition of other centers could have increased the number of patients studied likely allowing a stronger basis for conclusions. Impairment of the maximum concentrating ability after pyelonephritis and the time to recover (up to 3 months) have been shown by different authors [30–32]. We measured the concentrating renal ability at least 4 months after the episode of acute pyelonephritis. Therefore, it is unlikely that the renal parenchymal infection could have been the cause of the decreased urinary concentrating ability observed in this study.

In summary, abnormal findings in renal ultrasound and DMSA renal scan in patients with UTI are found not to screen for severe VUR. This study suggests that urinary osmolality and urinary excretion of NAG could be used as screening tests to predict the presence of severe VUR. A reduced urinary osmolality and an increased NAG/creatinine excretion are associated with VUR grades IV and V and justify a request for VCUG in an UTI patient showing even only mild abnormal findings on renal ultrasound or DMSA renal scan.

Authors' Contributions All authors contributed equally to this study and manuscript. All authors contributed to the final version of this manuscript.

Victor Garcia Nieto was involved in designing the study, writing the manuscript, and performing the statistical analysis.

Victoria E. Garcia-Rodriguez participated in designing the study and doing the statistical analysis.

Maria Isabel Luis-Yanez, Margarita Monge, and Pedro Arango-Sancho collected the data.

Eduardo H. Garin participated in designing the study and writing the manuscript.

Compliance with ethical standards The performed study is in accordance with the ethical standards and with the 1964 Helsinki declaration.

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 5 Normal values of urinary osmolality (mOsm/kg) obtained after desmopressin stimulus

Age	Number	Mean ± SD	Range
0–7 days*	17	542.8 ± 49.7	–
8–30 days*	11	619.5 ± 80.6	–
1–3 months**	17	698.8 ± 99.3	532–882
3–6 months**	31	722.9 ± 100.1	600–933
6–9 months**	32	805.2 ± 105.2	635–1048
9–12 months**	17	894.8 ± 131.3	740–1178
> 12 months***	–	> 800	–

*Reference [16]

**Reference [17]

***Reference [13]

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