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Urine Interleukin-8 as a Marker of Vesicoureteral Reflux in Infants

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ABSTRACT

OBJECTIVE. Vesicoureteral reflux (VUR) is a common finding in children presenting with urinary tract infection (UTI) and prenatally diagnosed urinary tract dilatation and in relatives of index patients. Children with VUR are at risk for ongoing renal damage with subsequent infections. Detecting VUR and renal scarring currently depends on imaging modalities with associated problems of radiation, invasiveness, and expense. Noninvasive methods would greatly facilitate diagnosis and would also help in identifying relatives of index cases who should be screened. Interleukin-8 (IL-8) is produced by epithelial cells of the renal tract in response to inflammatory stimuli and has been shown to increase during acute UTI. The objective of this study was to assess the urine levels of IL-8 as a noninvasive marker of VUR in infants in the absence of a recent UTI episode.

METHODS. We evaluated urine concentrations of IL-8 in 59 infants aged 1 month to 2 years. All infants were free of UTI for a minimum of 3 weeks before IL-8 evaluation. Infants were divided into 3 groups: group A, subjects with proven VUR (24 infants aged 0.15–1.95 years, median 0.43); group B, subjects with a history of UTI but negative investigation for VUR (14 infants aged 0.32–1.95 years, median 0.57); and group C, subjects without any history of acute or chronic condition that might impair renal function (21 infants aged 0.08–1.92 years, median 0.33). IL-8 concentrations were determined by a commercially available quantitative enzyme-linked immunosorbent assay. To avoid dilution effects, urinary levels of IL-8 were expressed as the ratio of cytokine-to-urinary creatinine.

RESULTS. Results were presented as medians and ranges. The Kruskal-Wallis test, the Mann-Whitney rank sum *U* test, and the Spearman rank order correlation test were performed for the univariate analysis. Two-tailed *P* values were calculated and the conventional level of significance *P* < .05 was applied in all cases. Infants in groups A and B had been free of UTI for a period of 3 to 52 weeks (median, 5.0 weeks) and 3 to 78 weeks (median, 4.5 weeks), respectively, before IL-8 determination. No significant difference was noted in the length of the UTI-free period between groups A and B (*P* = .469). Urine creatinine concentrations did not differ

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Key Words

diagnostic tests, interleukin-8, reflux nephropathy, urinary tract infection, vesicoureteral reflux

Abbreviations

VUR—vesicoureteral reflux
UTI—urinary tract infection
VCUG—voiding cystourethrography
DMSA—dimercaptosuccinic acid scintigraphy

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among groups A, B, and C (medians 1.15, 2.25, and 1.15 $\mu\text{mol/mL}$, respectively; $P = .080$). The median urine IL-8/creatinine concentrations ($\text{pg}/\mu\text{mol}$) were 40.5 (range, 2.04–3874) in group A, 1.91 (range, 0.001–386) in group B, and 2.47 (range, 0.002–55.6) in group C. Urine IL-8/creatinine concentrations were significantly higher in group A than both in group B ($P = .0003$) and in group C ($P < .0001$). No significant difference was observed between groups B and C ($P = .749$). In group A, no significant correlation was shown between IL-8/creatinine concentrations and the presence of renal parenchymal damage ($P = .506$), reflux grade ($P = .770$), or time from UTI ($P = .155$). A receiver-operator characteristic curve was constructed by plotting the sensitivity versus the specificity for different cutoff concentrations of IL-8/creatinine. With a cutoff concentration of urinary IL-8/creatinine at 5 $\text{pg}/\mu\text{mol}$, the sensitivity of this marker in diagnosing VUR was 88%, the specificity 69%, the positive prognostic value 66%, and the negative prognostic value 89%. In higher cutoff concentrations, specificity of the marker increased but sensitivity rapidly decreased.

CONCLUSIONS. We present evidence that urine IL-8 concentrations remain elevated in infants with VUR even in the absence of UTI and that a cutoff of 5 $\text{pg}/\mu\text{mol}$ IL-8/creatinine is of high sensitivity and adequate specificity for diagnosing VUR. Elevated urine IL-8 levels in VUR and renal scarring have already been reported; however, the present study is, to our knowledge, the first to confirm significant differences between infants with VUR and infants with a history of UTI alone and healthy controls, and to suggest a reliable cutoff concentration for diagnosing VUR. Our findings additionally suggest that inflammatory process in VUR is ongoing even after UTI has resolved, pointing against the currently held belief that sterile reflux cannot harm kidneys. The chronic inflammatory cell infiltrate associated with reflux nephropathy rather than VUR itself might offer an explanation for the secretion of IL-8, which may well be independent of reflux grade. Using urine IL-8 for diagnosing VUR is not free of limitations, because IL-8 may be elevated as a result of urinary tract manipulation or undetected UTI. In addition, this study focused on infants and not in older children with longstanding VUR. Increased urine IL-8 concentrations after UTI has resolved is a promising noninvasive marker for an initial screening for VUR in infancy with high sensitivity and adequate specificity.

VESICoureteral reflux (VUR) is a common finding in children presenting with urinary tract infection (UTI) and prenatally diagnosed urinary tract dilatation and in relatives of index patients.^{1,2} Children with VUR are believed to be at risk for ongoing renal damage with

subsequent infections, resulting in hypertension and reduced renal function.¹ VUR provides access for both infection and transmission of bladder pressure to the kidney; however, the progress from VUR and UTI to reflux nephropathy, renal parenchymal damage, and renal scarring has not been thoroughly elucidated.^{2–4} Detecting VUR and renal scarring currently depends on imaging modalities with associated problems of radiation, invasiveness, and expense.^{1,4,5} Noninvasive methods would greatly facilitate diagnosis and would also help in identifying relatives of index cases who should be screened.⁶

Cytokines are well known to modulate the inflammatory response in UTI and renal damage, but less is known on their role after acute infection has resolved.^{4,7–12} In an attempt to identify noninvasive markers of VUR, we evaluated urine levels of interleukin-8 (IL-8) in children with VUR in the absence of a recent UTI episode. IL-8, a proinflammatory mediator and a major chemoattractant for neutrophils, is produced by epithelial cells of the renal tract in response to inflammatory stimuli and has been shown to increase during UTI.^{6,9–10,12–17}

METHODS

Subjects

We evaluated IL-8 levels in the urine of 59 infants aged 1 month to 2 years. All infants selected for the study were free of UTI for a minimum of 3 weeks before IL-8 evaluation, as determined by clinical findings, normal blood white cell count, erythrocyte sedimentation rate, or C-reactive protein and urine microscopy and culture.^{8–10} Bacteriuria was determined by culture of urine obtained by suprapubic bladder aspiration (any growth), transurethral catheterization (growth of at least 10^4 bacteria/mL), or uniform growth of at least 10^5 bacteria/mL in 2 consecutive urine samples.^{1,10} Infants were divided into 3 groups: group A, subjects with proven VUR (24 infants aged 0.15–1.95 years, median 0.43); group B, subjects with a history of UTI but negative investigation for VUR (14 infants aged 0.32–1.95 years, median 0.57); and group C, subjects without any history of acute or chronic condition that might impair renal function (21 infants aged 0.08–1.92 years, median 0.33). The study was approved by the Hospital Research Committee.

Investigative Protocol

Patients in groups A and B were studied while undergoing urinary tract evaluation after documented UTI. Imaging protocol in general followed the guidelines of the American Academy of Pediatrics.¹ Urinary tract ultrasonography was performed within a week from acute infection to determine kidney size and outline, and to indicate any dilatation or anomalies. The presence and grade of vesicoureteral reflux were determined by voiding cystourethrography (VCUG), which was performed 6

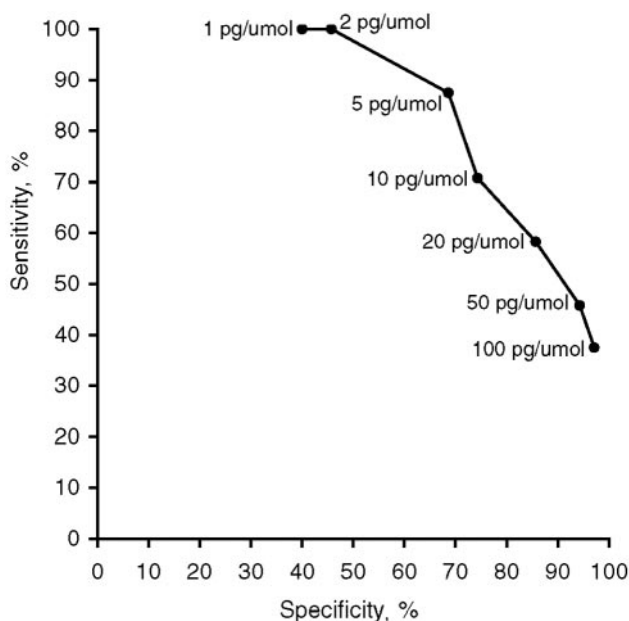


FIGURE 2 Sensitivity and specificity of urine IL-8/creatinine (pg/ μ mol) for diagnosing vesicoureteral reflux at different cut off values.

positive prognostic value was 66% and the negative prognostic value was 89%. In higher cutoff concentrations, specificity of the marker increased but sensitivity rapidly decreased. Thus, at the cutoffs of both 100 and 200 pg/ μ mol IL-8/creatinine, specificity was 97% and sensitivity 37.5%.

CONCLUSIONS

We present evidence that urine IL-8 levels remain elevated in infants with VUR even in the absence of a UTI. Elevation of urine IL-8 has been demonstrated in acute inflammatory renal disorders, including UTI,^{4,7,9,10,12} and a cutoff of 200 pg/mL has been proposed as a marker for diagnosing UTI.¹² Urine IL-8 secretion in UTI is quickly reduced after treatment, a finding confirmed in the group B of our study. Our findings suggest that the cutoff of 5 pg/ μ mol IL-8/creatinine is of high sensitivity and adequate specificity for diagnosing VUR. Higher cutoffs were associated with higher specificity but very low sensitivity. Studies focusing on serum IL-8 in patients with VUR have been inconclusive for such an association.^{10,16} Significantly elevated urine IL-8 levels in VUR and renal scarring have already been shown^{7,12}; however, the present study is, to our knowledge, the first to confirm significant differences between infants with VUR and infants with a history of UTI alone and healthy controls and to suggest a reliable cutoff concentration for diagnosing VUR.

Our findings additionally suggest that inflammatory process in VUR is ongoing even after UTI has resolved, pointing against the currently held belief that sterile reflux cannot harm kidneys.² The increase of IL-8 could

not be explained by the residual inflammation caused by UTI, because this increase was not noted in infants with UTI alone after UTI had resolved. Furthermore, it is of interest that among all the subjects of this study, the second highest IL-8 concentration was observed in a UTI-free infant with prenatally diagnosed VUR and normal DMSA. The chronic inflammatory cell infiltrate associated with reflux nephropathy rather than VUR itself⁴ might offer an explanation for the elevated levels of IL-8, which may well be independent of reflux grade.

The present study focused on young children, because this age group is at higher risk for developing renal damage.¹ Current imaging modalities, including VCUg and DMSA present with considerable limitations as screening tools, because they are associated with radiation and invasiveness.^{4,5} Ultrasound is a useful noninvasive tool for the definition of gross urinary tract anatomy; however, the modality's low sensitivity to detect reflux, which often is a dynamic condition, poses certain limitations to its use as a screening method.^{1,6,20} Using urine IL-8 seems to be a promising diagnostic marker for VUR. This marker is not free of limitations, because IL-8 may be elevated as a result of urinary tract manipulation, vaginitis, or balanitis,¹² or as a result of an undetected UTI. The latter scenario might explain the elevated IL-8 concentration in an infant in the non-VUR group B (Fig. 1). Nevertheless, determining urine IL-8 levels after UTI has resolved may provide substantial help as a screening test in evaluating high-risk patients for VUR and siblings of patients diagnosed with VUR.

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