

Urine Interleukin-8 as a Diagnostic Test for Vesicoureteral Reflux in Children

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Abstract

Background: Vesicoureteral reflux (VUR) is a common finding in children with urinary tract infection (UTI), mostly diagnosed by voiding retrograde cystogram (VCUG).. Children with VUR are at higher risk of renal damage with recurrent infections. Detecting VUR and renal scarring currently depends on imaging modalities with interventional invasive diagnostic methods. Noninvasive methods would greatly facilitate diagnosis and also help in identifying VUR in siblings of index cases who should be screened. Various imaging and biochemical methods with different specificity and sensitivity have been presented as substitute diagnostic tool for VCUG to identify VUR. Interleukin-8 (IL-8), a chemokine produced by damaged epithelial cells of the renal tract in response to inflammation, has been shown to increase during acute UTI. We have scarce data considering the cut point of urine IL-8 as a diagnostic method of VUR in children. 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: This cross sectional study was conducted on 28 patients with UTI and VUR (group 1), 28 patients with VUR and without UTI (group 2), and 28 healthy children/infants (control group) in St. Alzahra hospital, Esfahan, from January 2009 until March 2010.. Urine IL-8 level was measured for all children.. The data was analyzed by SPSS software version 17. The t-student test, χ^2 , and ANOVA were used as statistical method.

Results: The mean age of group 1, group 2 and control group were 4.3 ± 2.9 , 4 ± 2.6 and 4 ± 2.1 years respectively, $p > 0.05$. The mean level of IL-8 in group 1 was significantly higher than group 2 and control group 10 ± 14.8 versus 6.5 ± 8.4 , and 2.9 ± 4.5 respectively ($P=0.039$).

Conclusions: Although urinary IL-8 may be helpful in determining high grade VUR, but the results of this study showed that the sensitivity, specificity, PPV, and NPV of this marker were not satisfactory in cutoff point of 5 pg/ μ mol and other variables must be controlled.

Keywords: Urinary tract infection, Vesicoureteral reflux, IL-8, Children (JPMA 62: S-52; 2012).

Introduction

Vesicoureteral reflux (VUR) is one of the most common inherited disorders of the lower urinary tract.¹ The association between VUR, urinary tract infection (UTI), and reflux nephropathy has been well studied. Many recent studies have shown that VUR is an important risk factor for renal scarring after UTI. In addition UTI is a possible warning sign of the presence of the urinary tract anomalies. Hypertension and reduced renal function are the late consequences of kidney scars. However, in infants with high grade VUR, renal damage may be present at birth, even before developing any UTI.²

VCUG, as the gold standard method to diagnose VUR, is an invasive and expensive procedure that exposes gonads to high doses of radiation. Furthermore, it may cause iatrogenic UTI.^{1,5,6} Therefore, noninvasive methods must be considered to follow-up the patients with VUR.⁷

Chemokines, such as interleukin-6 and 8 are secreted by uroepithelial cells in response to the urinary tract infection. IL-8

is an important inflammatory mediator that can be produced by epithelial cells of the urinary tract in response to a variety of inflammatory stimuli. IL-8 has been shown to support neutrophil migration across infected epithelial cell layers.⁶ The correlation between urinary IL-8 and urine neutrophil numbers in individual patients has been shown.^{6,9,10,12-17} Elevated IL-8 levels have been reported in the urine of patients with VUR and renal parenchymal scarring (RPS). More recently it was reported that urine IL-8 levels remain elevated in infants with VUR even in the absence of UTI. The objective of this study was to assess the urinary level of IL-8 as a noninvasive marker of VUR in children and infants in the absence of a recent episode of UTI.

Subjects and Methods

This was a cross-sectional study that carried on 84 children aged 1 month to 10 years at St Al-zahra hospital from January 2009 until March 2010. The children were divided into 3 groups:

Group 1: 28 children with past history of UTI and VUR

(UTI+/VUR+)

Group 2: 28 children with past history of UTI but no proven VUR (UTI+/VUR-)

Control group: 28 children with no history of UTI

For every participant, according to the age of patients urine was collected by mid stream or urine bag and sent for culture before entering into the study. Participants with negative results were enrolled in the study. Considering the children's age, positive results were confirmed by repeated mid-stream sampling, transurethral catheterization, or suprapubic bladder aspiration to prescribe appropriate medications.

A non-centrifuged urine sample of each child was kept in -70°C for 3-6 months before measuring IL-8. The level of IL-8 was determined - in room temperature- with ELISA method [IL-8 ELISA from R&D Systems, Abingdon, U.K.]. To reduce the measuring error due to different concentrations of urine samples, we measured the ratio of .

VUR was graded from 1 to 5, based on the grading of the International Reflux Study Committee.¹ In this study, we contractually defined the severity of VUR as mild (unilateral grade I), moderate (bilateral grade I and grade II), and severe (grades III and IV).

Data were analyzed using SPSS-17 software. The t-test, χ^2 , and ANOVA tests were used as statistical method. P-values < 0.05 considered statistically significant.

The study was approved by the Research Ethics Committee of Esfahan University of Medical Sciences. The written informed consent was obtained from the parents and the children older than 6 years.

Results

Eighteen children out of 84 (21.4%) were male and the remaining were female 78.6%. Gender distribution was not

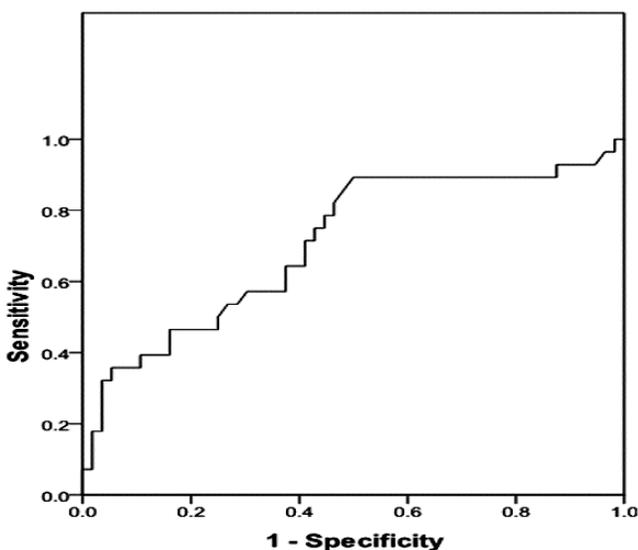


Figure-1: ROC curve for a cutoff point of IL8/Ucr at 10 pg/ μmol .

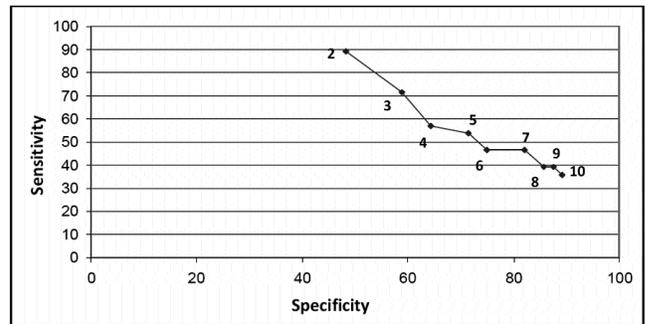


Figure-2: Sensitivity and specificity of IL8/Ucr (pg/ μmol) to diagnose VUR at different cutoff points.

significantly different among 3 groups, $p > 0.05$. The mean age of group 1, group 2 and control group were 4.3 ± 2.9 , 4 ± 2.6 and 4 ± 2.1 years respectively, and $p > 0.05$. In group 1 (UTI+/VUR+), three patients (10.7%) had grade I VUR, three (10.7%) had grade II, 15 (53.6%) had grade III, and seven (25%) had grade IV VUR.

The mean values of IL8/Ucr were 10 ± 14.8 pg/ μmol , 6.5 ± 8.4 pg/ μmol , and 2.9 ± 4.5 pg/ μmol in groups 1, 2 and control, respectively. One-way ANOVA test showed that the difference among the group was significant, $p = 0.002$. Using Tukey's post hoc test, the amounts of IL8/Ucr in groups 1 and 2 were significantly different ($P = 0.047$). In addition the values of IL8/Ucr in group 1 was significantly higher than control group, $P = 0.002$. However, no significant difference was found between group 2 and control group, $P = 0.47$.

The mean levels of IL8/Ucr in patients with mild, moderate, and severe VUR were 5.4 ± 2.5 , 14.8 ± 16.5 , and 10 ± 15.8 pg/ μmol , respectively. Using ANOVA test, no significant correlation was determined among the mean values of IL8/Ucr and the severity of VUR ($P = 0.75$). To determine the sensitivity and the specificity of urinary level of IL-8, receiver - operator characteristic (ROC) curve was drawn for different cut-off points of IL8/Ucr, Figure-2.

At cut-off point equals to 3pg/ μmol , the sensitivity and the specificity of this marker in diagnosing VUR were 71.4% and 58.9%, respectively. Furthermore, the positive prognostic value (PPV) and the negative prognostic value (NPV) were 66% and 89%, respectively. Therefore, at this cutoff point, IL8/Ucr is highly potent to distinguish the patients with the VUR from unaffected children.

In higher cut-off point amounts, the specificity of the marker increased but the sensitivity decreased sharply; i.e. at the cut-off point of 10pg/ μmol , the specificity was 89.3% and the sensitivity was 35.7%.

Discussion

To determine the diagnostic value of IL-8 as a predicting marker for the presence and the severity of VUR, 84 children/infants were studied. The mean of age of participants in our study was in agreement with the similar studies.^{4-6,13}

In different studies the role of IL-6 and IL-8 as inflammatory markers have been evaluated.^{5,13-15}

Sheu et al¹³ and Sharifian et al¹⁵ showed significant differences between the amounts of IL8/Ucr in children, before and after treatment of acute pyelonephritis.

Galanakis et al carried a study on infants aged 1 month to 2 years. They revealed a significant difference between the amounts of IL8/Ucr in patients with VUR and without it.⁶ In addition they showed no significant difference between the UTI+/VUR- and control groups.⁶ The higher level of interleukin 8 in UTI+/VUR+ suggested the role of VUR in kidney parenchymal damage even in the absence of current pyelonephritis. However, the young age of the participants was the limitation.

Increased level of serum and urine IL-8 and IL-6 have been demonstrated in acute phase of pyelonephritis.¹² This study in which pyelonephritis was proved by ^{99m}Tc-dimercaptosuccinic acid (DMSA) scan, 78 children aged 1-121 months were participated.

Tullus et al investigated urinary levels of the same cytokines during and after 6 weeks of acute pyelonephritis. They reported the higher level of IL-6 and IL-8 in children with pyelonephritis.¹⁶

Although higher level of urine IL-8 has been revealed in pyelonephritis comparing to asymptomatic bacteriuria, the urine IL-8 response was influenced by P-fimbriae and was associated with ESR, CRP, urine leukocytes and female sex.¹⁷

Faghihi et al reported persistent high amounts of IL8/Ucr in infants with VUR, even in the absence of UTI.¹⁴ These results may indicate that VUR causes inflammation even in the absence of UTI and this may change our current approach to the mild cases of VUR, without UTI.

We determined the sensitivity, specificity, PPV, and NPV of different IL8/Ucr cut-off points. In our study the cut-off point of 3 pg/ μ mol had the higher sensitivity and specificity. Another study conducted on children showed higher sensitivity and specificity of cut-off point 5 pg/ μ mol.⁶ In Shu et al study the sensitivity; specificity, PPV, and NPV have been shown to be equaled to 81%, 78%, 81%, and 78% respectively.¹⁴

Obviously the amounts of PPV and NPV in our study are different from the amounts reported from similar studies.^{6,14} The

difference in cut off points may rise from the time of sampling after the acute phase of pyelonephritis, the age of participants, the numbers of UTI attacks and the severity of previous scar formation. IL-8 we could demonstrated that IL-8/U Cr level even with a lesser cutoff point comparing to other studies (3 pg/ μ mo versus 5 pg/ μ mo) has been accompanied with VUR. This result may change our insight into the role of VUR in kidney damage.

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