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Is Urinary Interleukin-8 a Marker of Vesicoureteral Reflux in Children?

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Introduction: Developing non-invasive but accurate methods to diagnose vesicoureteral reflux (VUR) is in progress. Cytokines, such as interleukin-8 (IL-8), are important mediators in inflammatory responses and are demonstrated to change during UTI and pyelonephritis, as well. Therefore, we attempted to evaluate the differences of IL-8 in children with UTI compared to children with and without VUR to assess if it can be hypothesized to be an appropriate diagnostic marker in children with VUR.
Materials and Methods: We evaluated urine levels of IL-8 in 41 children aged 1 to 60 months who recovered from UTI for a minimum duration of 2-3 weeks. They were divided into 2groups: A and B (with and without VUR, respectively). Additionally, a group of normal children was considered as the control group (group C). Urine IL-8 levels were measured for the three groups and corrected for urine creatinine (Cr) (IL-8/Cr). Afterwards, they were compared using One-Way ANOVA test.
Results: The mean IL-8/Cr level was 81.7 ± 90.1 in group A, 289.8±640.2 in group B, and 9.6 ± 12.2 in group C with no significant difference (p=0.056).
Conclusions: Our finding suggests there is no significant difference in urine IL-8/Cr levels between patients with and without VUR and therefore, we cannot propose IL-8 as a diagnostic marker for VUR.

Keywords: Interleukin-8; Pediatrics; Pyelonephritis; Urinary Tract Infections; Vesico-Ureteral Reflux.

Running Title: Urinary Interleukin-8 a Marker of VUR

Introduction

Vesicoureteral reflux (VUR) is the most common urologic abnormality in children which causes recurrent and febrile urinary tract infections (UTI). It eventually results in renal scarring and insufficiency [1]. Therefore, as 30% of children with UTI are found to be involved with VUR, prophylaxis and treatment of these children and moreover, work up for VUR are mandatory [2]. When VUR is in, patients should be evaluated by various modalities such as voiding cystourethrography (VCUG) and dimercaptosuccinic acid (DMSA) renal scan, which are the most common worldwide. Their troublesome nature, however, considering radiation exposure, invasiveness, and cost are implicit [3], which therefore has drawn the attention of the investigators to non-invasive methods, such as cytokine assay. Hence, some studies have proposed the potential importance of cytokines such as interleukin-8 (IL-8), interleukin-6 (IL-6) and tumor necrosis factors in UTI, pyelonephritis, and reflux nephropathy (RN). In 1994, Tullus et al first demonstrated the increased levels of IL-6 and IL-8 in children with ongoing pyelonephritis in

comparison to children who had a febrile condition resulting from other causes and children recovered from pyelonephritis and the normal controls [4]. Moreover, Galanakis et al, in regards to IL-8 levels in the children's urine, hypothesized that renal insult continued regardless of the presence or absence of the infection in children with UTI [3]. It can however suggest the priority of cytokine assay as a diagnostic marker of VUR and RN. Thus, we performed this study to assess the possible difference in IL-8 levels in the urine of children with and without VUR compared to normal controls to evaluate its accuracy for the diagnosis of VUR.

Materials and Methods

This cross-sectional study was performed from September 2008 to September 2009 in Imam Reza Hospital affiliated with Kermanshah University of Medical Sciences, Kermanshah, Iran. The calculated sample size of 20 patients in each group was achieved. This study was approved by the institutional board of ethics of Kermanshah University and all the patients' parents signed the informed consent form. All children aged 1 to 60 months who had a proven fever (≥ 38.5 ºC), UTI, and normal renal function were considered for the study. The diagnosis of UTI was based on clinical manifestations, a positive urine culture ($\geq 10^5$ colonies with mid stream or urine bag sampling and ≥ 10^3 with catheterization) and suggestive urinalysis was defined as more than 10 white blood cells per high power field. Past medical history of the patients was recorded and ultrasound examination of the urinary system was performed. All patients with any uro-nephrologic disorder other than VUR, voiding dysfunction, and secondary VUR (abnormal neurologic signs and an abnormal bladder shape and wall thickness indicating neurogenic bladder in VCUG or positive VCUG findings in favor of the posterior ureteral valve) were excluded from the study. All patients who met the inclusion criteria were enrolled in this study and treated with antibiotics. After patients became culture negative, VCUG was performed and according to its result, patients were allocated in the following groups: Group A: patients with UTI and VUR. Group B: patients with UTI but not VUR. Two to 3 weeks after resolution, the study population was called back to the institution to collect the urine sample of each child and assess it for IL-8 using ELISA. A mid-stream urine specimen was used for evaluation of cytokine while the urine collection bag was used for infants.

In addition to the mentioned patient groups, a group consisting of healthy children of same age range with no history of UTI was used as the control group (group C) whose urine IL-8 levels were assayed to compare with groups A and B.
The specimen of each child was frozen at -20°C until all specimens were collected. After thawing in room temperature, the IL-8 level was measured in picograms per milliliter (pg/ml) and corrected for milligrams per deciliter (mg/dl) of urine Creatinine (Cr). ELISA (enzyme-linked immunosorbent assay) (colorimetric, Diazyme Laboratories, La Jolla, California, USA) was used for assessing the IL-8 levels and Jaffe reaction (auto-analyzer, RA 1000) was used for Cr (5). Chi-square was used to evaluate the correlation between qualitative variables, and independent sample t-test was used for quantitative variables in this study. For comparing the means between groups, one way analysis of variance followed by post-hoc was employed. We used the Pearson's test to calculate the correlation coefficient.

Results

Forty-five patients were enrolled in the study and underwent complete evaluation. Four patients were excluded because of anatomical abnormality of the urinary tract. Twenty and 21 patients were allocated to group A and B respectively and 20 normal controls were considered for group C. Group A consisted of 5 (25%) boys and 15 (75%) girls, Group B had 5 (23%) boys and 16 (77%) girls and group C was composed of 9 (45%) boys and 11 (55%) girls. Group A included 9 (45%) patients with unilateral (4 in grades II and III and 5 in grade IV) and 11 (55%) patients with bilateral VUR (all in grade II and III). Our groups had no significant difference in sex (p=0.23). However, they showed a significant difference in age ($p<0.001$); there was a significant difference in the mean ages between group C and each of the other two groups (A and B) ($p\leq0.001$ and p=0.001, respectively) whereas no significant difference was observed between groups A and B (p=0.939). Corrected IL-8 values were 81.7±90.1,

289.8±640.2 and 9.6±12.2 for groups A, B and C, respectively (p=0.056).

Discussion

The association between IL-8 and VUR in children remains controversial. Several studies have demonstrated elevated levels of procalcitonin and cytokines such as IL-8, IL-6, and TNF-α in UTI and particularly, pyelonephritis [6-10]. Nanda and Juthani-Mehta reviewed papers on this topic and proposed the effectiveness of serum procalcitonin to distinguish lower UTI and pyelonephritis [6]. Additionally, for the first time, Tallus et al [4] reported that both IL-6 and IL-8 were significantly higher in the urine of children with pyelonephritis whose disease went back to 6 weeks prior to cytokine assay, in comparison with normal healthy and non-renal infection groups. They found IL-6 in 52% and IL-8 in 98% of their patients. Moreover, in a similar study, they found increased levels of IL-6 but not IL-8 in patients with positive DMSA for renal scarring and therefore they hypothesized that IL-6 could be used as a predictor of renal insult [11]. However, they did not attempt to propose a marker for the diagnosis of VUR. Apart from that, the most common reason for childhood pyelonephritis is VUR that can result in end-stage renal disease if neglected. To diagnose VUR using biomarkers rather than potentially troublesome modalities such as VCUG, some studies have been performed to find non-invasive substitutes. In 1996, Haraoka et al [10] showed significantly higher urine amounts of IL-8 in children with VUR than children without VUR. However, this difference was not observed for IL-6. Their patients did not have UTI at the study time. Furthermore, Galanakis et al [3] conducted a thorough study evaluating urine IL-8 values in 38 infants aged 12 to 24 months who were infection-free for at least 3 weeks. Of this number, 24 patients had clear VUR as well as a history of UTI (group A). The remaining 14 had a history of UTI whit unknown VUR state (group B). After comparing with 21 normal control children (group C), they observed that the level of IL-8 in group A was significantly higher than groups B and C. Moreover, they reported that the cutoff of 5 pg/ mol of IL-8/Cr had a high sensitivity and 45% specificity for diagnosing VUR. Once cutoffs are increased, in order to achieve higher specificity, the sensitivity decreases inevitably. Interestingly, they hypothesized that the inflammatory process continued despite successful antimicrobial treatment. Gokce et al [12] analyzed 114 children for VUR and renal scarring (RS) using VCUG and DMSA, respectively. Urine IL-6 and IL-8 were measured using two-side chemiluminescent enzyme immunometric assay. They found significantly higher levels of IL-6 in both groups of patients (VUR + RS and VUR without RS) than controls. In addition, the IL-8 level was significantly lower in the normal group and patients with only VUR as compared to patients with VUR+RS. They concluded that VUR might participate in renal damage via immunological processes according to the highest levels of IL-8 in patients with both VUR and RS. In our study and in contrast with the above mentioned reports, although there was a difference in the urinary values of IL-8 between patients with and without VUR, this difference was not significant (p=0.056). This finding is similar to the results of a study by Sheu et al [7] who compared 70 children with pyelonephritis with 54 children with lower UTI in terms of serum and urinary amounts of IL-8, DMSA, and VCUG. In addition, they compared children with and without VUR (29 and 34 patients, respectively) and found no significant difference in serum and urinary values of IL-8 between them. Similarly, Badeli et al conducted a study on 16 children with VUR, 17 children with resolved VUR, and 18 normal children, and concluded that urinary IL-8 was not a good indicator of ongoing VUR and renal injury in children [13]. Although Merrikhi et al found higher levels of urinary IL-8 in patients with UTI and VUR in comparison with patients with UTI but not VUR and healthy control group, they finally concluded that the sensitivity, specificity, positive predictive value, and negative predictive value of this marker were not satisfactory in the cut-off point of 5 pg/μmol and other variables must be controlled [14]. They did not clearly describe the interval between UTI treatment and measurement

of urinary IL-8 in patients with UTI and VUR. A short interval between treatment and measurement could be the reason for the higher level of urinary IL-8 in their patients. So far, several studies have been performed to investigate the level of various cytokines in the serum and urine of the patients with UTI and/or VUR. In addition, some other researchers have attempted to propose biomarkers so that renal scarring can be diagnosed earlier [6, 8, 9, 15]. It may help investigators to explain the pathogenesis and progress of the upper UTI, as well [7,16]. The role of VUR in pyelonephritis is almost obvious [17]; however, literature review shows that few articles have assessed cytokines in VUR. Thus, we conducted this study to increase the knowledge of investigators worldwide concerning the diagnosis of VUR although more studies are required to suggest IL-8 as a diagnostic marker for VUR. Several studies have shown the correlation of elevated urinary biomarkers such as IL-8 and renal parenchymal damage rather than VUR per se [7,10,11,18]. As a limitation of this study, we did not evaluate the results of Dimercaptosuccinic acid (DMSA) renal scanning in our patients. Therefore, our study cannot correctly comment on the relationship between the urinary IL-8 level and renal parenchymal damage.

Conclusions

Our finding suggests there is no significant difference in urine IL-8/Cr levels between patients with and without VUR and therefore, we cannot propose IL-8 as a diagnostic marker for VUR.

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Conflict of Interest

None declared

Financial Support

None declared

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