Utility of Urine Interleukines in Children with Vesicoureteral Reflux and Renal Parenchymal Damage

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Purpose: Vesicoureteral reflux (VUR) is the most common risk factor of urinary tract infection in children. Currently, diagnosis of VUR depends on invasive imaging studies, with a high radiologic burden. Therefore, different biomarkers have been introduced for the evaluation of these patients. The objective of this study was to identify alteration of urinary interleukins (ILs) excretion in children with primary VUR and renal parenchymal damage, for further clinical application.

Materials and methods: Urinary concentrations of IL-1α, IL-1β, IL-6, and IL-8 were evaluated in 34 children with VUR (cases) and 36 without VUR (control), during 2018-2019. Urinary concentrations of IL-1, IL-1, IL-6 and IL-8 were measured, using polyclonal antibody ELISA kit, and standardized to urine creatinine (Cr). Patients with infectious or inflammatory disorders, urolithiasis, immune deficiency, acute or chronic kidney disease, and secondary VUR were excluded from the study.

Results: Mean age of cases (36.00 ± 27.66) had no significant difference with the control (32.86±29.31) group (p=0.44). The majority of patients had moderate VUR (58.8%), followed by severe (35.3%) and mild (5.9%) grades. Urinary concentration of all ILs/Cr were significantly higher in patients with VUR, compared with those without VUR. There was no significant correlation between urine ILs/Cr with age, gender, serum electrolytes, urine specific gravity, renal ultrasound, laterality or severity of VUR, and DMSA renal scan. All urine ILs/Cr had acceptable sensitivity and accuracy for workup of children with primary VUR.

Conclusion: Urine IL-1α, IL-1β, IL-6 and IL-8/Cr were sensitive and accurate additionary screening biomarkers in children with primary VUR.

Keywords: vesicoureteral reflux; interleukin; cytokine; renal damage

INTRODUCTION

Vesicoureteral reflux (VUR) accounts for 30–50% of urinary tract infections (UTI) in children. About 8.5–18% of chronic kidney disease occurs secondary to VUR in pediatrics. Therefore, early diagnosis and appropriate management of VUR might prevent its long-term complications, such as hypertension, proteinuria, and decreased renal function[1].

Nowadays, identification of VUR depends on invasive and expensive imaging modalities, with high radiologic exposure. Meanwhile, recently introduced noninvasive biomarkers such as urine interleukins (ILs) have been suggested as alternative diagnostic approaches in patients with VUR and their high risk siblings [2,3].

Cytokines are small soluble proteins, and regulate both humoral and cellular immunity[4]. IL-1α, IL-6, and IL-8 are proinflammatory cytokines, which stimulate peripheral neutrophilia, chemokine secretion, and scar formation in different tissues[5-7].

Lymphocyte and plasma cell infiltration is responsible for increased urinary IL excretion in patients with VUR or reflux associated nephropathy[7]. Although inflammatory processes and immune system dysfunction have been suggested in the pathogenesis of renal parenchymal damage (RPD), however, a correlation between VUR and urinary cytokines excretion remains controversial[7,8]. The purpose of this study was to identify alteration of urinary ILs excretion in children with primary VUR and RPD.

MATERIALS AND METHODS

This is a cross sectional multicentric case-control study on children admitted to 3 pediatric nephrology clinics during 2018-2019. It was approved by the institutional ethics committee (ethical code; ir.goums. rec.1396.02), and informed consent was obtained from legal guardians.

Children with a history of recurrent UTIs, urosepsis, UTI with abnormal ultrasound, atypical UTI, and asymmetrical kidneys who had a definite cystography and 99mTc-DMSA scintigraphy were included in this study. All of them were in healthy condition with normal body mass index at the time of the study.

Totally, 70 children (35 females, 35 males) were evaluated. Of them, 34 had VUR (case group) and 36 did not

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Table 1. Comparison of qualitative and quantitative variables in children with and without VUR.

Variables	VUR (mean±SD)	No VUR (mean±SD)	*P*-value
Age (m)	36.00 ± 27.66	32.86 ± 29.31	0.444
Gender (M/F)	17(50%)/17(50%)	18(50%)18((50%)	1
Serum Na	138.36 ± 6.40	137.22 ± 5.16	0.412
Serum K	4.17 ± 0.55	4.28 ± 0.48	0.172
Serum Cr	0.51 ± 0.10	0.54 ± 0.09	0.278
Serum HCO3	21.37 ± 2.45	21.46 ± 2.47	0.752
Urine SG	1014.12 ± 3.85	1013.67 ± 4.36	0.762
Ultrasound (N/H)	22(64.7%)/12(35.3%)	23(63.9%)/13(36.11%)	0.943
DMSA renal scan (N/D/S)	17(50%)/10(29.4%)/7(20.6%)	27(75%)/6(16.7%)/3(8.3%)	0.09

Abbreviations: m: month, M:male, F: female, Na: sodium, K: potassium, Cr: creatinine, HCO3: bicarbonate, SG: specific gravity, N: normal, H: hydronephrosis, D: decreased cortical uptake, S: scar

have VUR (control group). Conventional cystography (VCUG) or radioisotopic cystography (direct RNC) was done under prophylactic antibiotic treatment in all patients. 99mTc-DMSA renal scan was performed in patients with documented VUR or other inclusion criteria 9.

Patients with a history of UTI in the preceding 3 months, inflammatory disorders, active infections, ongoing antibiotic treatment, secondary VUR, neurogenic bladder, obstructive uropathy, urolithiasis, immune deficiency, malnutrition, obesity, hypertension, and chronic kidney disease were excluded from the study.

Based on imaging studies, VUR was classified as mild (I, II), moderate (III), and severe (IV, V) grades. The highest grade was taken in to consideration in patients with bilateral VUR. Parenchymal damage was defined as decreased cortical uptake or renal outline defect in 99mTc-DMSA scintigraphy.

A spot morning urine sample was obtained from all individuals and frozen at _80°_ C within 3 hours of collection. Urinary level of cytokines was measured using polyclonal antibody ELISA kit. To avoid dilutional effects, urinary ILs were expressed as the ratio of cytokine-to-urine creatinine (Cr) excretion (pg/mg).

Statistical analysis was performed using SPSS ver. 24.0 and 15.4 Med calc. Values are presented as mean±SD. Student's t test, nonparametric Mann-Whitney test, and Chi2 were used for comparison of variables between two groups. Correlations between urine ILs with other variables were determined, using the Spearman's, Mann-Whitney, and Kruskal Walis tests. A receiver operating characteristic (ROC) curve was constructed to determine the cutoff values of each cytokine with the best sensitivity, specificity, and accuracy. *P* values < 0.05 considered to be statistically significant.

RESULTS

A total of 34 children with VUR (M/F=1), and 36 without VUR (M/F=1) were enrolled in this study. VUR was bilateral in 21 (61.8%) patients. Two patients (1 unilateral, 1 bilateral) had mild VUR, followed by 20 with moderate (7 unilateral, 13 bilateral) and 12 with severe (5 unilateral, 7 bilateral) grades.

Laboratory findings including renal function, serum electrolytes, serum bicarbonate, and urine specific gravity had no significant difference between the two groups. Renal ultrasound and 99mTc-DMSA renal scan were normal in the majority of patients, with no significant difference between the two groups (Table 1).

Mean urinary concentration of all ILs/Cr were significantly higher in children with VUR, compared with those without VUR (**Table 2**).

None of the urine ILs had a significant correlation with quantitative (age, sodium, potassium, bicarbonate, urine SG) and qualitative (gender, ultrasound, VUR laterality, VUR grade) variables, except for a direct correlation between urine IL-1β/Cr ($p = 0.033$) and IL-6/Cr ($p = 0.037$) with DMSA renal parenchymal damage. However, Multivariate analysis showed no association between DMSA scan, unilateral or bilateral VUR, and severity of VUR with urine ILs/Cr excretion (**Table 3**).

The optimal cutoff values of urine ILs/Cr with the highest sensitivity, specificity, and accuracy are shown in **Table 4** and **Figure 1**. Accordingly, all ILs/Cr had acceptable sensitivity and accuracy for the workup of children with VUR.

DISCUSSION

Increased urinary cytokine excretion occurs secondary to tubular damage and interstitial fibrosis in patients with reflux associated nephropathy[3,7]. Therefore, measurement of urine ILs has been suggested for early identification of VUR, prior to the development of RPD and its serious complications[4,10]. This study was performed to identify alteration of urine ILs excretion in children with primary VUR and RPD.

IL-6 is a proinflammatory cytokine which is produced by endothelial and mesangial cells, fibroblasts, activated T cells and B cells, macrophages, and destructive renal tubular cells. Urinary concentration of IL-6 might reflect intrarenal production of this cytokine[7,10]. It has a central role in T cell and B cell differentiation, mesangial cell proliferation, and promotion of tubulointerstitial damage. Urine IL-6 has been considered a noninvasive

Table 2. Comparison of urine ILs/Cr in children with and without VUR.

Variables	VUR (mean±SD)	No VUR (mean±SD)	*P*-value
IL1α/Cr	5.88 ± 7.62	3.52 ± 10.28	< 0.001
IL1β/Cr	343.44 ± 462.37	72.66 ± 193.24	< 0.001
IL6/Cr	8.19 ± 11.01	1.90 ± 7.32	< 0.001
IL8/Cr	14.94 ± 21.78	0.8 ± 1.51	< 0.001

Table 3. Multivariate analysis of urine ILs/Cr excretion (Regression model).

Variables	DMSA scan		Severity of VUR		uunilateral or bilateral VUR	
	Regression coefficient	*Pv*	Regression coefficient	*Pv*	Regression coefficient	*P*-value
IL1α/Cr	-0.430	0.793	-.229	0.923	1.219	0.654
IL1β/Cr	-14.309	0.886	-52.854	0.717	-61.644	0.710
IL6/Cr	-0.538	0.822	-.597	0.894	1.012	0.798
IL8/Cr	-1.351	0.788	-1.414	0.839	1.859	0.815

biomarker for monitoring the progression of RPD in patients with reflux associated nephropathy[4,6,10].

Urine IL-6/Cr was higher in our children with VUR, with acceptable sensitivity and accuracy. Similarly, Krzemier et al showed increased urine IL-6/Cr in 8/33 children, aged 1-24 months with first time febrile UTI and mild- moderate VUR[8]. In addition, Gokce found increased urine IL-6/Cr in a study on 114 patients in 4 groups with or without VUR and RPD[7]. However, urine IL-6 level was below the lower detection limit with no clinical importance in Haraoka et al. study on 17 renal units with VUR (2 mild, 12 moderate and 3 high grade)[11]. Fernández et al. found no significant difference of urine IL-6/Cr excretion in a case control study on 40 children with documented VUR[12].

Urine IL-6 had no significant correlation with RPD in multivariate analysis and seems to be an unreliable biomarker for the prediction of RPD in our study. Similarly, Renata et al. found no correlation between urine IL-6 concentration and renal scarring, and urine IL-6 was not higher in those who developed renal scar than those without scar[13]. However, urine IL-6 was higher in children with severe renal damage than those without renal scar in Wang et al study on 66 patients aged 10-18 years with a history of antireflux surgery[10]

IL-8 is a major proinflammatory chemokine, which is produced by mesangial and destructive renal tubular epithelial cells in patients with RPD,[7] and consider a useful biomarker for localization and determination of the severity of urinary tract inflammation. It has an important role in neutrophil chemoattraction and IL-6 secretion[13]. Increased urinary IL-8 concentration has been reported in patients with urinary tract infection, VUR, and congenital kidney urinary tract abnormalities (CAKUT). It has been suggested a sensitive and nonspecific screening test for diagnosis of VUR and RPD in the previous studies[3,7].

Urine IL-8/Cr level was higher in our children with VUR, compared with the control group. Urine IL-8/Cr>0.6 pg/ml was a sensitive, specific, and accurate biomarker for evaluation of VUR in our patients. Therefore, we suggested evaluation of urine IL-8 as a valuable test for prediction of VUR. Similarly, urine IL-8 was a noninvasive diagnostic biomarker of isolated VUR in some of the previous studies, which suggested mild inflammatory process in these patients, and independent to the severity of VUR[3,11,12,14]. Galanakis et al. performed a study on 59 infants in 3 groups (24 with VUR, 14 with a history of UTI and no VUR and 21 with a history of impaired renal function), and recommended screening of VUR in patients with increased urine IL-8 excretion[2]. Urine IL-8 was higher in patients with VUR and RPD or isolated renal scar in the other studies, which suggested urine IL-8 as a predictive biomarker of RPD with a direct correlation to the severity of renal damage[7,11,13].

However, we showed no correlation between urine IL-8 excretion and DMSA uptake defect in our patients.

IL-1 is the first line cytokine of antigen recognition and anti-inflammatory function, which has been considered for prediction of late renal scar in children with acute pyelonephritis[5].

Both urine IL-1α/Cr and IL-1β/Cr were significantly higher in our children with VUR, irrespective to its severity, with acceptable sensitivity and accuracy for the prediction of VUR. Therefore, alteration of urine IL-1α and IL-1β were valuable biomarkers for the prediction of VUR in our patients. In addition, urine IL-1α/Cr or IL-1β/Cr had no significant correlation with renal damage in multivariate analysis. Sheu et al. in a study on 69 children, aged 1-121 months, found no alteration of urine IL-1β excretion in patients with VUR, but lower level in those with renal cortical scarring, and suggested protective effect of urine IL-1β against renal scarring during the active phase of acute pyelonephritis[5].

We found no significant correlation between urine ILs with age, gender, serum electrolytes, renal function, laterality or severity of VUR, and imaging studies (ultrasound, DMSA scan) in our patients. Similarly, age and gender had no significant effect on urine IL-1, IL-6, and IL-8 excretion in the previous studies[5,7].

We concluded that urine IL-1α/Cr, IL-1β/Cr, IL-6/Cr, and IL-8/Cr were sensitive and accurate noninvasive additionary biomarkers in children with primary VUR. In addition, none of these ILs had significant value for the prediction of renal damage in these patients.

The major limitation of this study was the low number of patients with RPD, which needs further studies for accurate diagnosis. Meanwhile, multiple collections of urine ILs over years might benefit the prediction of late renal scar in these patients.

In addition, future studies with a larger patient population are recommended to confirm the potential application of these biomarkers in suspected patients to primary VUR, especially those with negative imaging studies, and siblings of an index case. Further studies

Table 4. Sensitivity, specificity and accuracy of different urine ILs/Cr in patients with VUR.

Variables	Cut point	Sensitivity	Specificity	AUC	CI (95%)	SE	*P*-value
IL1α/Cr	>0.83	78.79	69.44	0.744	0.625-0.842	0.0617	< 0.001
IL1β/Cr	>12.38	97	63.90	0.854	0.749-0.928	0.0456	< 0.001
IL6/Cr	>0.49	90.91	72.22	0.875	0.773-0.942	0.0428	< 0.001
IL8/Cr	>0.6	97	77.80	0.929	0.841-0.977	0.0440	< 0.001

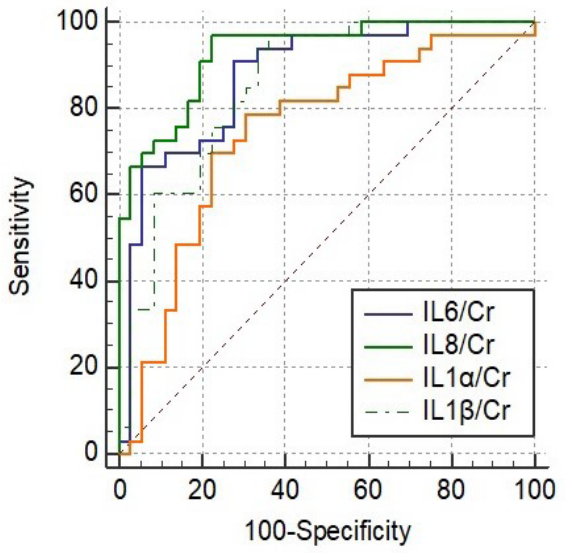

Figure 1. ROC analysis demonstrated an overall accuracy of different ILs /Cr for diagnosis of VUR.

are recommended for differentiation of primary VUR from secondary suspected patients with controversial results.

CONFLICT OF INTEREST

None declared by the authors.

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