Increased Urine Interleukin-17 and Interleukin-22 Levels in Patients With Candidal Urinary Tract Infection

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Introduction. Candiduria is common in the hospitalized patients. This study aimed to quantify interleukin (IL)-17 and IL-22 levels in urine of candiduric patients.
Materials and Methods. A case-control study was conducted on inpatients at Hashemi Nejad Kidney Center. Thirty-four patients were identified with *Candida* species in their urine samples (> 10^3 colony-forming units per milliliter and presence of *Candida* species only). Urine samples with concomitant infections were excluded. Thirty-four patients with negative direct examination and culture were included as the control patients. Interleulin-17 and IL-22 levels were measured in the lyophilized and nonlyophilized urine. The relevant cytokine titers of the two groups were compared, and the association of cytokine elevation and candiduria was investigated.
Results. The majority of the candiduric patients were from the intensive care and urology units of women. Only 4 patients (11.7%) manifested fever and dysuria. Massive leukocyturia was observed in 4 patients. *Candida glabrata* was the most commonly isolated species (44%). Levels of the urine IL-17 and IL-22 were significantly elevated in the candiduric patients, when compared to the noncandiduric controls. While an increased IL-17 level was significantly associated with candiduria (odds ratio, 1.09; 95% confidence interval, 1.003 to 1.17; P = .04), an increased IL-22 level was not. The results showed that lyophilized urine samples maximized the detection power of urinary cytokines.
Conclusions. Our results indicated that direct examination, fungal urine culture, and investigation of urine IL-17 and IL-22 levels are useful tools for diagnosis of *Candida* urinary tract infection.

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INTRODUCTION

Candiduria, the presence of *Candida* species in urine specimen, is a common finding in hospitalized patients.[1,2] *Candida albicans* is the most frequent causative organism of candiduria.[3,4] The host risk factors to develop candiduria include urinary catheter or urinary tract abnormality, older age, female sex, prior antibiotic use, hospitalization in intensive care units and diabetes mellitus.[2,4,5] The majority of candiduric patients reveal no symptoms, while few numbers of them present symptoms such as fever, dysuria, and flank pain.[2,5,6] Although most patients with candiduria reflect only colonization of urinary tract,[7] candiduria can be a result of cystitis and pyelonephritis in some.[1,5] The presence of *Candida* species in urine may be the

first marker of disseminated candidiasis, especially in critically ill patients[8]; only low percentages of patients with candiduria develop candidemia.[2,9,10] Although diagnosis of bacterial urinary tract infections (UTIs) can be established by observation of leukocyturia and bacteriuria, colony counting also can be helpful.[1]

Unfortunately, there are no reliable diagnostic procedures to differentiate colonization from *Candida* UTI,[1] because the urinary sediment of candiduric patients is nonspecific,[3,11] and pyuria is rarely observed in urine of candiduric patients.[2,5] Quantification of grown colony has proved to be less diagnostically useful to differentiate colonization from urinary true infection of *Candida*.[12] Moreover, although inflammatory cytokines assay can lead to differentiate bacterial colonization in the urinary tract from true infection, there is lack of such criteria to determine whether these biomarkers can be applied to contribute differentiation of colonization from urinary tract candidiasis. Since, T helper 17 (Th17) response via production of inflammatory cytokines is crucial for the defense against mucosal candidiasis at mucosal levels; therefore, the goal of this study was to evaluate local immunity of host Th17 inflammatory response to candiduria and to detect interleukin (IL)-17 and IL-22 levels in urine of candiduric patients in comparison with a control group.

MATERIALS & METHODS

Study Population

A case-matched control study was conducted. All patients provided written informed consent. Medical and demographic data, including age, sex, underlying disease, symptoms (such as fever and dysuria), having urinary catheter, and history of steroid, antibiotic, and immunosuppressive therapy, were recorded. Clean voided midstream morning urine specimens were taken from 338 patients hospitalized at Hashemi Nejad Kidney Center over 9 months (from September 2014 to June 2015).

Methods

In the case of catheterized patients, the catheter was clamped till the patient sensed to urinate, then the port of the catheter was sterilized with 70% alcohol and 10 mL of urine was collected using a needle and syringe. Then, the samples were transferred to the seromycology laboratory, School of Public Health, Tehran University of Medical Sciences, for microscopic examination and culture. If yeasts were seen by microscopy visualization, the samples were assigned to the case group. If no microorganisms were detected then the samples were assigned to the control group.

Microscopic Examination

Fungal urine culture and colony counting of yeast-positive samples were performed by inoculation of 10 µL of urine on Sabouraud dextrose agar (Merck, Germany) and CHROMagar Candida plates (MicroMedia, France) according to laboratory-based guidelines. We used the lower colony-forming unit (CFU) cutoff (10^3) as an inclusion criterion in candiduric patients.[10] Thirty-four patients who fulfilled the inclusion criteria (> 10^3 CFU/mL and presence of only *Candida* species in urine specimen) were enrolled as the case group and urine samples with concomitant bacterial infections were excluded. Thirty-four patients with negative direct examination and culture were included in the control group.

Species Identification

Yeast species were identified by culture on CHROMagar Candida and Cornmeal agar. The plates were incubated at 35°C and 30°C, respectively.

Urinary Cytokine Assay

The urine samples were centrifuged at 2000 rpm to remove the yeast, and supernatant were stored at -80°C until cytokines assessment. The eBioscence enzyme-linked immunosorbent assay kit (USA) was used according to the manufacturer's instructions to evaluate the concentration of urine IL-17 and IL-22 levels in duplicates. We explored IL-17 and IL-22 in urine of both groups before and after lyophilization of urines. Quantified cytokine levels were reported as pg/mL. Lower limit of detection for IL-17 and IL-22 was 4 pg/mL and 8 pg/mL, respectively. Values of noncandiduric urines were served as controls for IL-17 and IL-22 levels.

Lyophilization of Urine

Each urine specimen was quickly frozen in individual vials at -70°C. Then, vials were transferred to lyophilizer (VIRTIS, USA), flushed with vacuum, and lyophilized at about -40°C. For

assay, each vial was reconstituted by adding 500 µL of distilled water with a precalibrated sampler.

Statistical Analysis

For comparison of categorical variables between the two groups, the chi-square or the Fisher exact test, and for continuous variables, the independent *t* test was performed to determine statistical significance ($P < .05$). Multivariable logistic regression models were constructed to estimate odds ratios and the associated 95% confidence intervals. The Pearson correlation coefficient was then used to evaluate the correlation between the cytokine titers and the colony forming. All statistical analyses were carried out using the SPSS software (Statistical Package for the Social Sciences, version 24.0, IBM Corp, New York, NY, USA).

RESULTS

Characteristics of Candiduric Patients

Candiduria was commonly detected in the women (58.8%). Candiduric patients were commonly from intensive care unit and urology service for women. Up to 61.8% of candiduric patients were greater than 60 years old. Only 4 patients (11.7%) manifested fever and dysuria (these patients also had pyuria and high erythrocyte sedimentation rate; Table 1).

Candida Species

A total of 34 urine samples with yeast on direct examination were inoculated on fungal culture media. These media recovered *Candida* species in 34 (100%) urine samples. *Candida glabrata* was the most common isolated *Candida* species (44.0%), followed by *Candida albicans* (26.4%), *Candida tropicalis* (9.0%), and C*andida krusei* (3.0%). Six urine samples (17.6%) had more than one species (Table 2). Colony count varied between 10^3 and 10^5 yeast cells per milliliter of urine.

Nonlyophilized Urine Levels of Interleukin-17 and Interleukin-22

The median IL-17 level was 12 pg/mL (range,

Table 1. Characteristics of Patients With Candiduria and Control Group*

Characteristic	Candiduric Patients (n = 34)	Control Group (n = 34)	*P*
Age, y	59.5 (17 to 89)	50.7 (21 to 65)	.04
Sex			
Male	14	16	
Female	20	18	> .05
Foley catheter	21 (61.8)	16 (47.1)	> .05
Diabetes mellitus	19 (55.9)	2 (5.9)	< .001
Prior antibiotics use	12 (35.2)	1 (2.9)	.001
Hospitalized within at least 6 months	4 (11.7)	0	> .05
Mortality	7 (20.5)	0	.01
Central venous line	8 (23.5)	0	.005
Hospitalized in intensive care unit	12 (35.2)	0	.001
Dialysis	1 (2.9)	0	> .05
Kidney transplantation	2 (5.9)	0	> .05
Heavy leukocyturia (> 50/HPF)	4 (11.7)	0	> .05
Fever and dysuria	4 (11.7)	0	> .05
Mental confusion	5 (14.7)	0	.05
Surgery	8 (23.5)	10 (29.4)	> .05

*Values are mean (range) for age and frequency (percentage) for others.

Table 2. *Candida* Species in Subgroups of Patients With Candiduria

Patient Group	C *albicans*	C *glabrata*	C *tropicalis*	C *krusei*	More Than 1 Species
All Candiduric patients (n = 34)	9 (26.4)	15 (44.0)	3 (9.0)	1 (3.0)	6 (17.6)
Diabetic patients (n = 19)	4 (22.0)	9 (47.0)	2 (10.0)	0	4 (21.0)
Catheterized patients (n = 21)	4 (19.0)	10 (47.5)	2 (10.0)	0	6 (23.5)
Patients with prior antibiotic use (n = 12)	3 (25.0)	3 (25.0)	2 (16.3)	0	4 (33.7)
Women with candiduria (n = 20)	4 (20.0)	9 (45.0)	3 (15.0)	1 (5.0)	3 (15.0)

6 pg/mL to 198 pg/mL) in the case group and comparably 13 pg/mL (range, 6 pg/mL to 22 pg/mL) in the controls. The IL-22 levels did not show any significant difference between the two groups either.

Lyophilized Urine Levels of Interleukin-17 and Interleukin-22

The IL-17 and IL-22 levels were significantly elevated in the patients with candiduria when compared with the noninfected patients; the median IL-17 level was 17.5 pg/mL (range, 7 pg/mL to 200 pg/mL) in the case group and 14 pg/mL (range, 6 pg/mL to 21 pg/mL) in the controls. The median IL-22 level was 21 pg/mL (range, 8 pg/mL to 318 pg/mL) in the case group and 16 pg/mL (range, 9 pg/mL to 45 pg/mL) in the controls.

A logistic regression model was constructed for the increased levels of IL-17 and IL-22 in lyophilized urine samples, adjusted for age, sex, and indwelling Foley catheter, which showed an association between an elevated IL-17 and candiduria; the receiver operator characteristic curve was constructed for determining the cutoff point, which demonstrated 80% sensitivity and 50% specificity. In contrast, an increased level of IL-22 was not associated with candiduria (Table 3).

We found a significant negative correlation between IL-17 levels and colony formation ($r = -0.331$, $P = .05$). In addition, there was no relationship between elevated IL-17 levels and the *Candida* species isolated from candiduric patients (data not shown). Furthermore, significant differences were observed in the mean levels of IL-17 and IL-22 before and after lyophilization.

DISCUSSION

Our data demonstrated that urine IL-17 levels were elevated more than IL-22 levels in candiduric inpatients. Although most candiduric patients in this study were asymptomatic, they significantly showed high urine levels of IL-17 in comparison to noncandiduric control patients. In the multivariable analysis, an elevated IL-17 level remained significantly associated with candiduria when adjusted for age, sex, and Foley catheter. These results should encourage future multicenter studies to be undertaken with larger numbers of patients to determine whether these biomarkers can contribute to differentiate *Candida* colonization from true infection of urinary tract.

Table 3. Associations Between Increased Lyophilized Urine Levels of Interleukins and Candiduria*

Cytokine (pg/mL)	Odds Ratio (95% Confidence Interval)	*P*
Interleukin-17	1.09 (1.00 to 1.17)	.04
Interleukin-22	1.03 (0.99 to 1.07)	.19

*Adjusted for age, sex, and indwelling Foley catheter

Because the former studies have demonstrated that neither direct examination nor culture alone can diagnose candiduria,[5] we employed both microscopic visualization and fungal urine culture. According to previous studies,[10] we used the lower colony-forming unit cutoff (10^3) as an inclusion criterion in candiduric patients. Symptomatic catheter-related bacterial UTIs defined as positive urine culture (10^4) appear by any of the following symptoms: fever, urgency, frequency, dysuria, suprapubic tenderness, altered mental status, or hypotension.[13] It is striking that the defining clinical symptoms could not readily be recorded in our candiduric population because they commonly had indwelling Foley catheters or were hospitalized in intensive care units (heart failure, brain tumor, kidney failure, and trauma) and intubated.

In our study, like other studies, only a few number of the candiduric patients manifested symptoms such as fever, urgency, and dysuria (11.7%). Therefore, evaluation of symptoms in candiduria is not definitely helpful in differentiating colonization from true infection. The usefulness of findings such as leukocyturia in candiduric patients has not been established.[1,2] Massive leukocyturia was observed in only 11.7% of the case group. We evaluated immunological biomarkers in the urine specimens of patients with candiduria because animal model study implied that host mucosal surface defense against pathogen determine the outcome of UTI.[14] Our attempt was to gather if there was sufficient evidence to finally convince a larger multicenter study to find out if immunological biomarkers assay can be hired in differentiating colonization from true urinary *Candida* infection that should be treated. We expected those inflammatory markers to show an increase that is recognizable from control patients and also diverse in candiduric patients as the great numbers of candiduric patients are merely colonized with no damage.[2]

In this study we did not choose to evaluate

the concentration of IL-1 and IL-8, as their assessment in urine is mostly associated with existence of leukocyturia which is uncommon finding in candiduria.[2] We also did not explore the immunoglobulins because in former reports these were shown to be nonspecific finding.[1,8] The recent studies have illustrated a crucial function of Th-17 cytokines in host defense at mucosal surfaces such as lungs, gut, and mouth against fungi mediated by recruitment of neutrophils to infected tissues, induction of antimicrobial peptides (defensins, mucins, and S100 protein).[15-17] Therefore, we measured the concentrations of Th-17 cytokines (IL-17 and IL-22) in the patient's urine of both groups. Elevation of IL-17 (an immunomodulatory cytokine inducing multiple proinflammatory mediators such as CXC chemokines,[18] granulopoetic cytokines,[19] and metalloproteinases from epithelial and fibroblast cells[19]) has been reported in previous study of candiduric urine.[2] Both protective role and inflammatory pathology have been attributed to IL-17 in fungal infections.[18-20] Interleukin-17 induces migration of neutrophils to infected tissues,[21] and also IL-17 has been thought to interact by direct binding to *Candida*-stimulating nutrient starvation status in the organism.[22] Dectin-1 attachment to B-glucan of *Candida* cell wall enhances IL-17 response in disseminated candidiasis model.[23,24] In recent studies, critical defending role of IL-17 or its receptor against *Candida* has been demonstrated,[18,20,21] and it has been shown that IL-17 receptor-deficient mice are susceptible to disseminated candidiasis and exhibited decreased survival.[18] In the present study, significant levels of IL-17 were documented in urine of a subset of candiduric patients, but were not constricted to leukocyturic patients or patients with urinary Foley catheter.

In spite of the lower odds ratio for IL-17 in our study than Helbig and colleagues',[2] we found an elevated IL-17 level was significantly associated with candiduria. Our results were consistent with this recent report on candiduric patients' urine cytokine that revealed high titers of IL-17.[2]

Given the damage expected to occur during true infection by *Candida* species to renal epithelium,[1] and also IL-22 which is recognized to mediate antifungal resistance through controlling the integrity of epithelial surfaces,[25-27] we investigated urine levels of IL-22 in this study as well. Interleukin-22, a member of the IL-10 family cytokines, plays a more critical antifungal role than IL-17 in mucosal surfaces against *Candida*,[28,29] by inducing production of antimicrobial peptides and warranting epithelial homeostasis.[25,30]

A previous study has shown in addition to secretion of IL-22 by Th-17 cells, other cells including NKp43+ and dendritic cells are able to produce it as well.[25] Interleukin-22 level has not been determined in candiduric patients previously. In our study, although urine levels of IL-22 in candiduric patients were significantly elevated when compared with those of noninfected urines, an elevated IL-22 level did not remain significantly associated with candiduria in the multivariable analysis. Another previous study implied that IL-22 constrict early growth of the infecting yeast in the early stage of infection via various mechanisms including induction of antimicrobial peptides production with anticandidal activity and epithelial integrity maintenance,[25] and thereby, limiting inflammation and injury during mucosal candidiasis.[25,31] Another report has shown that IL-22 contributes to resistance to *Candida albicans* in the vagina, and it was detected with high expression in the early stage of infection.[31] Another former study has reported time-dependent model of IL-22 gene expression during an experimental model of gram-negative pneumonia that highly expressed at 16 hours after infection and prevented from bacterial dissemination outside of the lung in the early stage of infection.[28] Therefore, these reports indicate that IL-22 secretion is time dependent and highly elevated in the early stage of the mucosal infection. The fact that an elevated IL-22 level was not significantly associated with candiduria may result from the variable timing between affliction and sampling in our patients.

As noted, due to lack of standard diagnostic criteria to confirm true infection of candiduria, we could not compare the results of our study with a reference confirmatory diagnostic method for candiduria. In addition, it is worth noting that some of candiduric patients may have immunodeficiency as a consequence of human immunodeficiency virus status or immunosuppressive medications that cause defect in immune response and compromise antifungal immunity of Th17 pathway. Thus, investigation of inflammatory cytokines in these patients would not be helpful.

CONCLUSIONS

Since symptomatic candiduria and leukocyturia were rarely diagnosed in this study, evaluation of these criteria cannot surely diagnose *Candida* UTI. In conclusion, through urinary cytokine investigation, our results again indicate that the elevated level of IL-17 in the urine of candiduric patients can be hired as a key factor in distinguishing the *Candida* UTI from colonization. We also showed that lyophilization of the urine samples would maximize the detection power of urinary cytokines.

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CONFLICT OF INTEREST

None declared.

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