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BRIEF REPORT

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Persistence of candiduria in ICU catheterized patients is not linked to adherence and proteolytic activities of *Candida* strains

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Abstract *Objective:* In this study, the patients who developed asymptomatic candiduria in the intensive care unit were followed prospectively for the persistence of candiduria after the replacement of indwelling urethral catheter and the correlation between persistence and virulence factors (proteinase enzyme activity and epithelial adhesion) was assessed. *Design:* Prospective study. *Setting:* Intensive care unit and mycology laboratory at a university hospital. *Patients:* Thirty-four patients with asymptomatic candiduria were included in the study. *Results:* Candiduria persisted in 19 of 34 patients(56%; group 1) and cleared in 15 of 34 patients(44%; group 2) after urinary catheters were changed. When the virulence factors (epithelial adhesion and proteinase activity) and distribution of *Candida spp.* were compared between two groups, no statistically significant correlation was found. *Conclusion:* The host immune response might be more important than virulence factors of *Candida spp.* for persistence of candiduria.

Keywords Candiduria · *Candida* · Proteolytic activities

Introduction

Nosocomial urinary tract infections that are caused by *Candida* species have increased dramatically in recent years. Risk factors for candiduria include the extensive use of broad-spectrum antimicrobial agents, corticosteroids, immunosupressive agents; cytotoxic chemotherapy complicated by mucositis and neutropenia; elderly age, diabetes mellitus; and structural or functional abnormalities of urinary tract with indwelling urinary catheter or nephrostomy [1].

The presence of candiduria may signal a diverse variety of different pathologic states including invasive renal parenchymal disease, fungal balls and obstructed ureters, superficial lower urinary tract infections, urinary tract *Candida* colonization associated with urinary catheterization, and contamination [2].

The differential diagnosis of infection (cystitis) from colonization (asymptomatic candiduria) of urinary tract is extremely difficult. For example, most of the catheterized critically ill patients do not develop specific symptoms such as dysuria, urgency, and frequency in the intensive care units (ICUs). Also, neither the quantitative urine colony counts, nor the fungal morphology, such as the presence of hyphae, possess a diagnostic value in catheterized patients [3].

Asymptomatic candiduria can be resolved by the removal of risk factors, especially the broad-spectrum antimicrobial therapy and indwelling urinary catheters; however, this is not possible for critically ill ICU patients. If there is an existence of multiple risk factors, then persistence of candiduria can occur despite the change of the urethral catheter. It is shown that candiduria could not be cleared when no therapy is given, especially for the patients with diabetes mellitus [4].

On the other hand, *Candida* species possess several virulence factors that can effect the progression of infections [5]. Adhesion to epithelial and/or endothelial cells is the first step to initiate an infection and the secretion of hydrolytic enzymes(especially proteinase) is

necessary for the invasion into the tissues. This suggests that the more virulent isolates would cause persistence; hence, the aim of this study was to investigate the correlation between virulence factors (adhesion and proteinase secretion) of *Candida spp.* and the persistence of candiduria despite the change of the urethral catheter.

Materials and methods

Patients population and clinical design

In this study patients who developed asymptomatic candiduria in the ICU (combined medical–surgical) were prospectively followed up for the persistence of candiduria after changing the indwelling urethral catheters.

This study was conducted from January 1997 to December 1998. Thirty-four patients with candiduria were included in the study and candiduria persisted in 19 of these patients (56%). Inclusion criteria for the study were: the presence of indwelling urethral catheter while receiving broad-spectrum antibiotic therapy during candiduria; no antifungal, corticosteroid or immunosupressive therapy before enrollment; the absence of HIV infection, diabetes mellitus or neutropenia; and the absence of systemic inflammatory response syndrome (SIRS).

Candiduria was defined as the presence of *Candida* species in urine cultures of $\geq 10^3$ CFU/ml. When the candiduria was detected, the catheter was changed and a second urine culture was performed within 24–72 h. The clearance was defined as the absence of the growth in the second urine culture and persistence was defined as the presence of the same *Candida spp.* ($\geq 10^3$ CFU/ml) in the second urine culture.

In vitro virulence factors

Isolates

The isolated yeasts were presumptively identified as *Candida albicans* by morphological criteria (germ tube and chlamydospore formation) and definitive identification was made by using API ID 32 C system (BioMerieux Diagnostic System, France).

Adhesion and proteinase activity

Adhesion to buccal epithelial cells

Yeast adhesion to exfoliated buccal epithelial cells was determined by light microscopy as described previously [6]. Standardized suspensions of human buccal epithelial cells (1×10^5 cells ml^{-1} in PBS; 0.1 ml) and yeasts (1×10^7 organisms ml^{-1} in PBS; 0.1 ml) were mixed and incubated at 37°C for 45 min, with gentle shaking. Epithelial cells were then collected by filtration, washed, and stained. Adherent yeasts on each of 100 epithelial cells were counted on every filter. Epithelial cells were obtained from the buccal mucosa of a single donor at the same time of the day to minimize variability. All adhesion values quoted represent mean figures derived from two independent assays done in duplicate observation.

Proteinase production in solid medium

Extracellular proteinase production by *Candida* isolates was induced with BSA, according to the technique described by Staib [7] and Wu et al. [8]. Briefly, an 18-h yeast suspension of 1×10^6 cells/ml was prepared, and 5 ml of this yeast suspension was spot inoculated onto a BSA (%1) agar plate. The plate was incubated aerobically in a humidified container for 7 days at 28°C. After staining with amido black, the isolates that had clear proteolysis zones around the colonies were considered as enzyme producers.

Statistical analysis

Student's *t* test was used to compare the groups with respect to age, duration of antibiotic therapy, and stay in ICU before development of candiduria. Mann-Whitney U test was used to compare the groups with respect to epithelial adhesion. Fisher's exact test was used to compare the groups with respect to proteinase activity, distribution of *Candida spp.*, sex, and surgery.

Results

Thirty-four patients with asymptomatic candiduria who met the inclusion criteria of the study were included. There were 16 females and 18 males. The mean age was 58±17 years (age range 17–87 years). Candiduria persisted in 19 of 34 patients (56%; group 1) and cleared in 15 of 34 patients (44%; group 2) after urinary catheters were changed. The detection of candiduria varied from 3 to 75 days of stay in ICU (median±SD, 13±19 days) in group 1, and 6–47 days (median±SD, 12±11 days) in group 2. The duration of antibiotic treatment and other characteristics of the patients are shown in Table 1. The persistence was significantly higher in males than in females ($p<0.05$).

Epithelial adhesion, proteinase enzyme activity, and distribution of *Candida spp.* are shown in Table 2. No statisticaly significant differences were found when the persistence and virulence factors were compared, and in addition, species distribution of *Candida* isolates between two groups was not different ($p=0.097$ and $p>0.05$, respectively).

Discussion

The persistence of candiduria despite the replacement of a new urethral catheter presents a therapeutic dilemma to the clinician. Nevertheless, clinicians tend to treat the persistent candiduria after changing the indwelling urethral catheter in critically ill patients in ICUs since the oral fluconazole is readily available, safe, and possesses good pharmacokinetic profile. Sobel et al. [9] demonstrated that oral fluconazole is safe and effective for short-term eradication of candiduria, especially following catheter removal. Long-term eradication rates were similar for both fluconazole and placebo groups. It was also demonstrated that candiduria was resolved in about 20% of chronically catheterized patients when only their catheter was changed, and eradicated in 41% of untreated patients when the catheter was removed [9].

Table 1 The demographic and clinical characteristics of patients. *CAP* community-acquired pneumonia, *CVD* cerebro-vascular disease, *COPD* chronic obstructive pulmonary disease, *VAP* ventilator-associated pneumonia, *UTI* urinary tract infection, *SSI* surgical site infection, *BSI* bloodstream infection

Characteristics	Group 1 (persisted)	Group 2 (cleared)
Age (mean±SD, range)	57±17 (17–87)	60±18 (22–78)
Male/female	13/6	5/10
Surgery/no surgery	9/10	3/12
Antibiotic duration (day; mean±SD, range)	10.6±0.99 (4–18)	10.6±1.5 (2–24)
Stay in ICU before candiduria (day; median±SD, range)	13±19 (3–75)	12±11 (6–47)
Diagnosis on ICU admission (no. of patients)	Trauma (2) CAP (2) CVD (2) Malignancy (2) Tetanus (1) COPD (2) Surgical procedure (5) Myasthenia gravis (1) Intracranial mass (2)	Trauma (3) CAP (1) CVD (3) Encephalopathy (1) Tetanus (1) COPD (3) Surgical procedure (3)
Concominant or preceding infection during candiduria	VAP (8) BSI (4) UTI (4) SSI (1) Nosocomial meningitis (1) CAP (1)	VAP (9) BSI (2) UTI (4)

There are several studies which demonstrate the importance of virulence factors in establishing the infection [5]. But in this study, correlation between virulence factors and persistence of candiduria could not be determined. It is known that different environmental factors, such as pH, iron concentration, sugar concentration, and temperature, can affect the expression of virulence factors and the virulence factors expressed or required by *Candida* species vary, depending on the site (e.g., uroendothelial cells), stage of invasion, and the nature of the host response. It is also known that more stable in vitro condition may not be similar to the in vivo conditions and different behavior of the yeasts. On the other hand, uroendothelial cells may have different anti-*Candida* activities [10].

Table 2 Epithelial adhesion, proteinase enzyme activity, and distribution of *Candida spp.* in groups 1 and 2

Epithelial adhesion (*n*, mean±SD)	Group 1 (persisted)	Group 2 (cleared)
	211±169 (17)	146±220 (12)
Proteinase activity (*n*, –/+)	12/6 (18)	11/2 (13)
C. albicans	6	6
C. tropicalis	8	6
C. parapsilosis	5	1
C. kefyr	–	1
C. lusitaniae	–	1

C. tropicalis and *C. albicans* were found as the most frequent species in this study. The absence of *C. glabrata* is not easy to explain, but it can be suggested that this might be due to the variety of regional localization of patients from different studies. This idea is also supported by the findings of our as well as others' previous studies which detected very few *C. glabrata* isolations from blood specimens [11, 12].

In the light of the results of this and other studies [13, 14, 15], it is suggested that host immune response might be more important for persistence of candiduria and progress to candidemia. Direct association between Th2 cytokines and susceptibility to candidemia has been demonstrated in murine models [13, 14]. In addition, abnormal serum levels of anti-inflammatory cytokines, such as IL-4 and IL-10, were demonstrated in patients with candiduria and to a greater degree in those with candidemia [15].

Conclusion

In conclusion, we were unable to detect a correlation between the virulence factors and persistence of candiduria in our study; however, it is clear that larger studies are necessary to evaluate both the host immune response and candida virulence factors in order to estimate the possible risks of candiduria persistence.

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