Original Research Article

Relationship between virulence factors of *Candida* species with candiduria and myeloperoxidase concentrations

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A B S T R A C T

K e y w o r d s	Studies were carried out on *Candida* species which includes isolation and identification from urine specimens of 200 hospitalized patients who used urinary catheter (group I) and non catheterized patients (group II) as well as studies on susceptibilities to antifungal agents and secretory aspartic proteinase (ASP), phospholipase (LP) activities, biofilm (BF) formation as virulence factors that were assessed by the standard methods. Neutrophil counts and myeloperoxidase concentrations in patients' sera were determined using ELISA. Sixty two *Candida* isolates were isolated and among them *Candida albicans* is 56.5%, *C. glabrata* is 41.9% and *C. krusei* is 1.6%. The resistance levels to itraconazole and flucytosine were relatively high and susceptible to amphotericin B for all *Candida* isolates. *Candida albicans* isolates were positive for all the virulence factors while *C. glabrata* and *C. krusei* were negative for SAP and LP activities of both groups and BF formation was detected in *C. krusei* of group I. Although, catheterized and non-catheterized patients have significant increase in neutrophil counts than healthy controls yet myeloperoxidase concentrations were significantly reduced in catheterized patients. Significant negative correlations between myeloperoxidase concentrations and phospholipase and secretory aspartic proteinase virulence factors were detected in catheterized patients. This may be exaggerated by decreased myeloperoxidase concentrations which should be considered and improved by effective treatment.
Candiduria, Antifungal susceptibility, Virulence factors, Neutrophil count, Myelo-peroxidase concentration	

Introduction

Urinary tract infection (UTI) is the most common type of nosocomial infections and 10 to 15% of UTIs are caused by *Candida* species. *Candida* UTIs are potentially

serious with a reported mortality up to 61% (Agrawal *et al*., 2010). Yeasts are becoming important causes of morbidity and mortality in many patients, because of alternations in the immune system and invasive hospital procedures (White *et al.*, 1998). Candiduria may represent contamination, colonization of the urinary catheter or of the bladder. *Candida albicans* has been the most common species isolated from urine samples, followed by *C. glabrata* and *C. tropicalis* (Kauffman, 2005).

Candida albicans, cause an increasing number of severe infections with high mortality rates (Hajjeh *et al.*, 2004). In immunosuppressed patients, most pathogens including *Candida* species have developed an effective battery of virulence factors and specific strategies that contribute in pathogenesis and to cause different diseases ranging from mucocutaneous to systemic infections. The virulence traits that are expressed by *Candida* species may vary depending on the type of infection, the site and stage of infection and the nature of the host responses (Mohandas and Ballal, 2011).

Direct host cell damage and lysis are the main mechanisms contributing to fungal virulence. Secreted aspartic proteinases (SAPs) are principle among hydrolytic enzymes and degrade proteins related to structural and immunologic defences such as collagen, keratin, mucin, antibodies, complement and cytokines during tissue invasion (Borst and Fluit, 2003). Phospholipases (PLs) enzymes are also associated with adherence, penetration and membrane damage of outer cell envelop of the host cells. Biofilms (BF) are structural microbial communities that are attached to surfaces. Individual microorganisms in BF are embedded within a matrix of often slimy extracellular polymers. Crucially, they are significantly less susceptible to

antimicrobial agents (Mohandas and Ballal, 2011).

The physiological condition of the host is the main factor governing the etiology of candidiasis. Slight alterations in this status can turn the harmless commensal microorganism into a pathogen. The transition is attributed to the suitable predisposing conditions that occur in the host (Bhat *et al.*, 2011). Individual requiring indwelling catheter are susceptible to the development of *Candida* associated UTIs especially by potentially pathogenic multidrug-resistant spp. in hospital setting. Urinary catheterization may predispose to candiduria with the absence or presence of underlying disease and high counts would represent the amount of yeasts in the bladder which requires appropriate aggressive treatment to improve the outcome (Behiry *et al.*, 2010).

Cell mediated immunity mediated by T cells and immunity mediated by macrophages, neutrophils and natural killer cells are considered the most important lines of defence against candidiasis (Lopez-Ribot *et al.*, 2004). Neutropenia and/or decrease in function of circulating neutrophils in immunocompromised hosts may be responsible for reduced resistance to infection, which may cause invasive candidiasis, with a high mortality rate up to 90% even with maximal antifungal treatment (Nathan, 2006).

Myeloperoxidase (MPO) is one of the most abundant proteins in neutrophils that mediates efficient antimicrobial action and participates in the early inflammatory process (Metzler *et al.*, 2011). Myeloperoxidase catalyzes the oxidation of chloride and other halide ions in the presence of hydrogen peroxide to generate hypochlorous acid and other highly reactive

products that mediate efficient antimicrobial action. Oxidative radical-forming mechanisms appear to damage fungi by producing protein modifications, nucleic acid breaks and lipid peroxidations. Its measurement can be used as an index of neutrophil activation and function (Koziol-Montewka *et al.*, 2006).

The objectives of this study were to investigate *Candida* species isolated from urine specimens of hospitalized patients used urinary catheter and do not use urinary catheter, study their identification, the virulence factors (secretory aspartic proteinase, phospholipase, biofilm formation) of *Candida* species and antifungal susceptibility patterns. Neutrophil counts and myeloperoxidase concentrations in sera of the patients was also studied to identify the appropriate management.

Materials and Methods

This study was done at nephrology department or in intensive care unit at Theodor Bilharz Research Institute Hospital, Egypt between June 2011 and April 2012 included 200 patients admitted with suspected hospital acquired UTI. For patients with UTI, detailed history was estimated, including old age, gender, stayed in intensive care unit, use of indwelling Foley catheter, antibiotics, diabetes mellitus, renal impairment, haemodialysis and hospitalization. The patients were grouped into 2 groups according to the presence or absence of urinary catheter: group I, included 62 patients with urinary catheter (29 were males and 33 were females, their age ranged from 30 to 80 years) and Group II, included 138 patients without urinary catheter (67 were males and 71 were females, their age ranged from 16 to 83 years). In addition to 19 healthy controls (group III) (6 males and 13 females;

their age ranged from 21 to 78 years) were included in this study to determine the normal levels of laboratory investigations. All patients or their legal guardians provided written informed consent according to local institutional review board guidelines by Theodor Bilharz Research Institute Hospital.

Microbiological study

Urine specimens were collected as midstream morning sample or from the port of the catheter and processed for microbiological examinations, culture, identifications and antimicrobial sensitivity. Specimens were cultured on Sabouraud's Dextrose (SD) agar (Oxoid, UK) with chloramphenicol and incubated at 37°C for 24–48 hr. Growth concentration $\geq 10^3$ CFU/ml is considered significant for candiduria. All the isolates were stored in vial tubes containing SD broth plus 10% glycerol in a freezer at 80°C. *Candida* identification were done by Gram stain, subculture of the yeast isolates from SD agar for purity, and followed by inoculation on the chromogenic medium CHROMagar *Candida*, (BioRad France). Plates were incubated for 24 to 48 hr at 37°C.

Yeast were identified according to colour and morphological appearance of the colonies at 24 hr and 48 hr. Pink to purple coloured colonies indicating presence of *Candida albicans,* intense turquoise mat convex colonies for *Candida tropicalis,* pale turquoise coloured colonies with flat, shiny, smooth morphology (fish eye) for *Candida glabrata* and turquoise-blue colonies with rough irregular outline for *Candida krusei.* Microscopy features on Cornmeal-Tween 80 agar (Oxoid, UK) slide culture and germ tube formation test were done to confirm the *Candida* species identification (Kurtzman and Fell, 1998).

Susceptibility test

Reference antifungal susceptibility testing of all *Candida* isolates was performed using the disk diffusion method, Mueller-Hinton agar plate and opacity control 1 McFarland as described in Clinical Laboratory Standards Institute (CLSI, 2004; formerly NCCLS) document M44-A and the manufacturer's instructions against 8 antifungal agents. After dilution of the initially prepared inoculums and inoculation of 1×10^3 yeast/ml on SD agar, plates were incubated at 37°C for 24 hr. The growth assessment is based on observation of inhibition zone. The antifungal included were; amphotericin B (100 µg), flucytosine (1 µg), econazole (10 µg), fluconazole (25 µg), itraconazole (10 µg), ketoconazole (50 µg), miconazole (10 µg) and voriconazole (1 µg)). The interpretation follow for flucytosine (>20 µg/ml, susceptible; 19–12 µg/ml, DD; ≤11 resistant), fluconazole (≥19–25 µg/ml, susceptible; 18–15 for 25 µg/ml, DD; ≤14 resistant), itraconazole (≥23-10 µg/ml, susceptible; 22–14 for 10 µg/ml, DD; <13 resistant), miconazole (≥ 20-10 µg/ml, susceptible; 19–12 for 10 µg/ ml, DD; ≤11 resistant), voriconazole (≥17 µg/ml, susceptible; 16–14 µg/ml, DD; ≤13 resistant), amphotericin B (>10–100 µg/ml, susceptible; ≤10 resistant), econazole (≥20–10 µg/ml, susceptible; 19–12 10 µg/ml, DD; ≤11 resistant), ketoconazole (>20–50 µg/ ml, susceptible; 20–10 10 µg/ml, DD; <10 resistant).

Detection of virulence factors of *Candida* isolates

Secretory aspartic proteinase activity test: *Candida* isolates were screened for production of SAP enzyme using bovine serum albumin agar which is considered as the "gold standard" in detection of this extracellular enzyme (Cassone *et al.*, 1987*).*

Extracellular phospholipase activity test: *Candida* isolates were screened for production of PL enzyme by measuring the size of the precipitation zone around the fungal growth on Sabouraud's egg yolk agar (Oxoid), which is considered as the "gold standard" in detection of this extracellular enzyme (Samaranayake *et al.*, 1984*).*

Biofilm formation: Biofilm formation was carried out using the tube adherence method proposed by Shin *et al.* (2002), each *Candida* isolate that was grown for 24 hr at 35°C on SD agar was suspended in 5 ml of sterile saline, centrifuged and the pellet of *Candida* cells was resuspended in SD broth in a turbidity corresponding to 1 McFarland. One ml of the suspension was inoculated into a polystyrene tube containing 9 ml of SD broth. After incubation at 35°C for 24 hr the culture broth was aspirated, the internal wall of the tube was washed then stained with 0.1% safranin dye (Oxford, India). The adherent slime layer of BF was visually examined and scored as described by D'Antonio *et al.* (2002): (-): Negative; no biofilm formation. (+): Weak formation; presence of biofilm at the bottom of the tube. (++): Moderate formation; presence of biofilm at the bottom and the internal wall of the tube. (+++): Strong formation; presence of biofilm at the bottom, on the internal wall and as a ring at the top of the tube.

Neutrophil counting and measurement of myeloperoxidase concentration in serum

Two ml of venous blood were taken from all patients and healthy control on Ethylene diamine tetra acetic acid (EDTA) for neutrophil count using Adx Micros 60 device (Horiba Medical, France), also taking 3ml of venous blood for determination of MPO concentration using Quantikine ELISA kit from R&D Systems, Inc. USA. The manufacturer's instructions

for ELISA procedure were followed. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for MPO has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MPO present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for MPO is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added. The colour develops is proportional to the concentration of MPO bound in the initial step. The colour development is stopped and its intensity is measured using microplate ELISA reader at wavelength of 450 nm.

Statistical analysis

Data were analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16. Parametric data were expressed as mean ± standard deviation and non parametric data were expressed as number and percentage of the total. Determining the extent that a single observed series of proportions differ from a theoretical or expected distribution was done using the Chi-square test. linear correlation coefficient was used for correlation between two quantitative variables in one group. P values were insignificant when >0.05, significant when ≤0.05, highly significant when <0.01 and Marked significant when P<0.001(Sokal and Rohlf, 1995).

Results and Discussion

Of the two hundred patients with suspected *Candida* UTI, 62 patients with urinary catheter (group I) and 138 patients without catheter (group II) were developed. The most common predisposing factors in group I were old age >40 years (97.4%) with mean

± SD of 58.2±9.4, ICU stay (89.7%) duration of stay at ICU ranged from 1 to 13 days with mean ± SD of 5.2±3.2, antibiotic use (79.5%) duration of antibiotic use ranged from 2 to 13 days with mean ± SD of 5.9±3.1, renal impairment (25.6%), female sex (61.5%) while the most common predisposing factors in group II were age >40 years (59.1%) with mean ± SD of 47.6±19.8, hospitalization (90.9%) duration of hospitalization ranged from 1 to 9 days with mean ± SD of 3.7±2.4, antibiotic use (59.1%) with mean ± SD of 5.3±2.3, renal impairment and haemodialysis (50%), female sex (90.9%). Significant differences were detected between both groups in the frequency of all predisposing factors for candiduria (P<0.01) except for diabetes mellitus (p□0.05).

Sixty two (31%) of the patients were proved to high significant candiduria. In catheterized patients, the isolation rate of all *Candida* species was 39 (63%), it was significantly higher (P<0.001) than in non catheterized patients 23 (16%) whereas one patient in group II was infected with *C. albicans* and *C. glabrata.* Of our 62 *Candida* isolates identified were 35 (56.5%) *C. albicans* which is the most common species with significant candiduria followed by *C. glabrata* 26 (41.9%) then *C. krusei* 1 (1.6%). In frequency of *Candida* species among patients groups with candiduria, the 35 isolates of *C. albicans*, 51.3% and 65.2% were found in group I and group II respectively with a significant difference between the two groups (P<0.05) while, 46.1% and 34.8% of *C. glabrata* were found in both groups in respect (p□0.05), and only one (1.6%) isolate of *C. krusei* was isolated from a catheterized patient.

Antifungal susceptibility patterns of the isolated *Candida* species

Susceptibility tests for flucytosine,

fluconazole, itraconazole, miconazole, voriconazole, amphotericin B, econazole and ketoconazole were performed on 62 isolates of *Candida* species. Among all evaluated isolates, *Candida albicans* isolates were resistant to itraconazole (71.4%), flucytosine (28.7%) and fluconazole (8.6%) while (100%), (42.3%) and (26.9%) of *C. glabrata* isolates were resistant to itraconazole, flucytosine and fluconazole respectively. *C. krusei* isolate was resistant to itraconazole, flucytosine and fluconazole (100%). All *Candida* isolates were susceptible to amphotericin B, econazole, ketoconazole and voriconazole while miconazole was effective against *C. glabrata*, *C. krusei* and the majority (62.8%) of *C. albicans* isolates (Table 1). Generally, high rates of resistance were observed with itraconazole and flucytosine.

Virulence factors of *Candida* species among patient groups

Out of 62 *Candida* isolates, 32 (51.6%) were positive for SAP production, all of them were *C. albicans* while *C. glabrata* and *C. krusei* isolates were negative for SAP production. Out of 35 *Candida albicans*, 32 (91.4%) were positive; 19 (95%) of them were in group I and 13 (86.7%) were in group II with a significant difference between the patient groups (P<0.05) (Table 2). Also the results showed that 24 (38.7%) of *Candida* isolates were positive for LP production, all of them were *C. albicans* while *C. glabrata* and *C. krusei* isolates were negative for LP production. Out of 35 *Candida albicans*, 24 (68.6%) were positive; 15 (75%) of them were in group I and 9 (60%) were in group II with a significant difference between the patient groups (P<0.05) (Table 3). On the other hand 10 (16.1%) of *Candida* isolates were positive for BF formation; 5 (50%) in group I and 5 (50%) in group II. Out of 35 *Candida albicans*, 9 (25.7%) were positive;

4 (20%) of them were in group I and 5 (33.3%) were in group II with a significant difference between the patient groups (P<0.05) (Table 4).

Neutrophil counts and myeloperoxidase concentrations in the studied groups

There was a highly significant increase in neutrophil counts in catheterized patients as compared to non catheterized patients (P<0.01) and healthy controls (P$_1$<0.01). Significant higher counts in group II was found as compared to group III (P$_2$<0.01). Significant higher concentrations of MPO in both patient groups than healthy controls (P<0.01) while significant lower MPO concentrations in group I than group II (P<0.01) (Table 5).

Correlation coefficient study

Correlation coefficient between MPO concentrations and neutrophil counts, LP and SAP activity in catheterized and non catheterized patients were studied. In catheterized patients, a positive correlation was detected between MPO concentrations (ng/ml) and neutrophil counts (%) in group I (P<0.01) (Fig. 1A). Negative correlations were detected between MPO concentrations (ng/ml) and PL activity (P<0.01) (Fig. 1B) and SAP activity (P<0.05) (Fig. 1C) in group I, while insignificant correlations were found in non catheterized patients (P>0.05).

Recent studies highlight the changing epidemiology of candiduria in hospitalized patients (Baysan *et al.*, 2012) and catheter associated urinary tract infection is the most common nosocomial infection (Behiry *et al.*, 2010). The current study revealed that the prevalence of *Candida* UTI is 62 isolates which isolated from 200 urine specimens obtained from catheterized and non catheterized patients suspected to have

UTI. The most common predisposing factors in catheterized patients were old age >40 years, ICU stay, renal impairment, antibiotic use, female sex while the most common predisposing factors in non-catheterized patients were old age >40 years, hospitalization, renal impairment, antibiotic use, female sex and haemodialysis, da Silva *et al.* (2007) in Sao Paulo reported the same predisposing factors. Behiry *et al.* (2010) reported that the age of patients with candiduria ranged from 28 to 84 years and Jain *et al.* (2011) observed that nosocomial UTI in catheterized patients due to *Candida* species was more common (80%) in extremes of age. This could be due to lowered host defence at extremes of age (Passos *et al.*, 2005). Kauffman (2005) explained the high incidence of candiduria in females than males because of shorter urethra and frequent vulvo-vestibular colonization with *Candida*. The findings that the catheterized and non-catheterized patients were using antibiotics are in agreement with Chen *et al*. (2008) who found that 74.1% of the patients with candiduria were using broad spectrum antibiotics which play a critical role in the pathogenesis of candiduria by suppressing susceptible endogenous bacterial flora in the gastrointestinal and lower genital tracts. Antibiotic favours epithelial surface fungal colonization with ready access to the urinary tract especially in the presence of indwelling bladder catheter. It has been shown that antibiotics impaired phagocytic activity and antibody synthesis and consequently decreased the resistance of the host against candidal invasion.

The findings that the frequency of *Candida* species among catheterized patients was 63% which is significantly higher than in non-catheterized patients (16%) is going with the concept that catheterization predisposes to *Candida* infection. Similarly, Rajeswari *et al.* (2012)

and Behiry *et al.* (2010) reported that 68% and 50% respectively of patients with candiduria had Foley catheter. An important problem identified with this kind of infection is the change in microbiological and antibiotic sensitivity pattern of the pathogen and during catheterization process. With the use of indwelling medical devices, *Candida* infections are on the rise. The infection rate is directly proportional the number of days during which the catheter was present in the patient (Chen *et al.*, 2008).

Although, *C. albicans* was the most common species isolated in this study from patients with candiduria; yet the *Candida non albicans* (*CNA*) species were found to be emerging in the UTI cases. *C. albicans* was found in 51.3% of catheterized patients and 65.2% in non-catheterized patients, *C. glabrata* were found in 46.1% and 34.8% of catheterized and non-catheterized patients respectively and *C. krusei* in 1.6% of catheterized patients. Pakshir *et al.* (2004) found that *C. albicans* accounts for 50-70% of all candiduria followed by *C. glabrata* 20%. Recently, Behiry *et al.* (2010) found that 66% of the isolates were *CNA* compared to 34% of *C. albicans.* Similar findings were reported by Akortha *et al.* (2009) who found that *C. albicans* and *C. glabrata* were the only species that were isolated in candiduria. Bishop *et al.* (2008) revealed that the recorded incidence of *CNA* was 42% which indicates that geographical and time-associated variations are highlighted. Moreover, our findings that *C. krusei* is the least species that are isolated from patients with candiduria is similar to that of Pfaller *et al.* (2008) who reported that in catheterized patients with candiduria, *C. krusei* remains a relatively uncommon clinical isolate throughout the world.

The findings that most of *C. Candida* have high rates of resistance to itraconazole

and all *CNA* species have high rates of resistance to itraconazole, flucytosine and fluconazole. Resistance to fluconazole and itraconazole was observed relatively high, mainly in isolates of *C. glabrata, C. tropicalis* and *C. albicans* (Bruder-Nascimento *et al.*, 2010) while Baysan *et al.* (2012) revealed that *C. krusei* is intrinsically resistant to fluconazole. The percentage of isolates resistant to fluconazole was smaller than to itraconazole (Laverdiere *et al.*, 2007). As expected, high secondary resistance rates were observed in *C. glabrata* to fluconazole (68%) and itraconazole (88%); this resistance to multiple azoles has been explained by an upreglutation of CDR genes that encode the CDR efflux pumps (Pfaller and Diekema, 2007). Fungi and yeasts frequently develop resistance to flucytosine, so it is rarely administered by itself. *Candida glabrata* strain resistant to itraconazole was recovered in Brazilian tertiary care hospital and similar results with *C. krusie* and *C. tropicalis* isolates were reported in Greece (Behiry *et al.*, 2010).

The findings that all 62 *Candida* isolates were susceptible to amphotericin B, with similar result reported in Behiry *et al.* (2010) who found that all 40 *Candida* isolates were susceptible to amphotericin B. Nevertheless, in clinical practice amphotericin B should be administered with caution, and it is only recommended for cases that are resistant to other drugs, due to the risk of toxicity (White *et al.*, 1998).

Moreover, 40% *C. albicans*, 62.8% *C. glabrata* and 100% *C. krusei* in this study were susceptible to voriconazole with similar result reported in Baysan *et al.* (2012) who found that all *Candida* species were susceptible to voriconazole except one *C. glabrata* strain which was intermediate susceptible.

The predisposition to candiduria is multifactor and is attributed to both pathogen virulence and host factors. *Candida* is a part of the microflora of healthy individuals. The capacity to shift from a commensal to pathogenic state requires a coordinated metabolic response that induces the expression of specific virulence traits (Rozell *et al.*, 2006). The present study has shown that BF, PL and SAP production are involved in virulence. Biofilm formation was detected in 25.7% of *C. albicans*, 20% and 33.3% of them were in catheterized patients and non-catheterized patients respectively and 100% of *C. krusei* isolates in catheterized patients, while all *C. glabrata* isolates were negative. These results are in agreement with that of Omar *et al.* (2008) who found that BF was detected in (39.3%) of the isolated *Candida* species from patients with candiduria and that BF was significantly higher in *C. krusei* (81.8%) and *C. tropicalis* (63.6%) than *C. albicans* (28.6%) and *C. glabrata* (11.1%). Previously, Basu *et al.* (2003) reported that *CNA* spp. particularly *C. tropicalis* can produce significant amounts of BF but not *C.glabrata*. Biofilm formation varies greatly among clinical isolates of *Candida* strains and promotes adherence to surfaces like catheters. However, about 100% of the biofilm forming isolates were sensitive to amphotericin B, econazole and ketoconazole while miconazole was effective against all *C. krusei, C. glabrata* (31.4%) and the majority (62.8%) of *C. albicans* isolates. Andes *et al.* (2004) demonstrated that one of the best known distinguishing characteristics of biofilm is the development of antimicrobial resistance that can be up to 1,000-fold greater than that of planktonic cells. These data support the strategy to treat candiduric patients with brief courses of intravenous amphotericin B or other azoles as econazole, ketoconazole

and miconazole rather than with fluconazole. Health professionals should take special care when managing urinary catheters to prevent biofilm formation, since one of the main reasons for treatment failure stems from this capacity of fungi to produce biofilms on the surface of foreign bodies (Kuhn *et al.*, 2002).

In this study, PL activity was detected in 68.6% of *C. albicans* isolates (75 and 60% of them were in catheterized and non-catheterized patients respectively) while all *C. glabrata* and *C. krusei* isolates were negative for PL production. Our results are in agreement with those of Basu *et al.* (2003) who demonstrated that 48.7% of *C. albicans* clinical isolates and only two isolates from healthy people were positive for PL. The results of Mohandas and Ballal (2011) support our results that *CNA* secreted smaller amounts of PL. In contrast, Costa *et al.* (2010) reported that 15.4% and 35% of *C. albicans* and *CNA Candida* isolates respectively from catheterized patients with candiduria were positive for PL production.

Secretory aspartic proteinase activity was demonstrated in 91.4% of *C. albicans* isolates (95 and 86.7% of them were in catheterized and non-catheterized patients (respectively) while all *CNA* isolates were negative for SAP production. Kantarcioğlu and Yücel (2002) found that 95% of the isolates of *C. albicans* produced SAP. In addition, Kalkanci *et al*. (2003) reported that all *C. albicans* isolates expressed an enzymatically active SAP and no production was observed in the *CNA* species. In contrast, Costa *et al.* (2010) who reported that 61.6% and 95% of *C. albicans* and *CNA* respectively isolated from catheterized patients with candiduria were positive for SAP production.

Several evidences indicate that the host immune responses are critical in the defence against fungal infections which requires a well-coordinated innate and adaptive response. The current study revealed a significant increase in neutrophil counts in all patients with candiduria with more increase in catheterized patients. Behnsen *et al.* (2007) reported that neutrophils play a central role in antifungal immunity because they possess a wide range of antimicrobial activities to destroy pathogens efficiently, including physical removal or trapping of the pathogens, production of reactive oxygen species, proteinases and antimicrobial peptides. In addition to their direct killing of *C. albicans*, it was demonstrated that polymorph nuclear neutrophils are the only cell type in blood which can inhibit *C. albicans* germ tube formation (Fradin *et al.*, 2005). However, neutropenia was found to be associated with high incidence of *candida* infections in immunocompromized patients such as cancer patients with indwelling catheters and prolonged use of antibiotics and chemotherapy that may have a bad impact on neutrophils (Dimopoulos *et al.*, 2009). The findings that there are significant higher concentrations of neutrophil MPO in sera of catheterized and non catheterized patients as compared to healthy controls is explained by the report of Kojic and Darouiche (2004) that the most efficient microbicidal system employed by neutrophils depends on the reactive oxygen variants generated by NADPH-oxidase and MPO. These oxidants play a critical role in the destruction of invading pathogen.

Although, catheterized patients have a significant increase in neutrophil counts yet, they demonstrated a significant reduction in MPO concentrations in their sera than non catheterized patients which may explain the increased incidence of candiduria with

urinary catheter and may indicates that those patients may have some immune disorder in neutrophil function. Kojic and Darouiche (2004) reported that impairment in lymphocyte and granulocyte functions, circulating inhibitors to chemotactic factors, defect in mucosal barriers and metabolic acidosis may contribute to increased incidence of candiduria. It has been shown also that MPO-deficient polymorph nuclear neutrophils lose their candidicidal property. Although, phagocytes deficient in MPO express a mild to moderate defect in bacterial killing but a marked defect in fungicidal activity *in vitro* (Aratani *et al.*, 2006). The findings that there are significant negative correlations between MPO concentrations and virulence factors (PL and SAP activity) in catheterized patients may explain that defect in neutrophil function may predispose to infections with more aggressive *candida* species. Koziol-Montewka *et al.* (2006) demonstrated that patients with neutropenia and significant low levels of MPO were more susceptible to infections with different *Candida* species. In conclusion, there is a strong relationship between host risk factors (old age, antibiotic use, catheterization, female sex, ICU stay, diabetes mellitus, hospitalization) and the expression of various virulence factors of *Candida* species causing candiduria and their resistance to antifungals. This may be exaggerated by decreased MPO concentrations which should be considered and improved by effective treatment.

Recommendations

Risk factors and serious co-morbid conditions should be corrected to avoid the development of candiduria. There is an urgent need to develop new treatment modalities such as human recombinant MPO to face the condition of resistance by candida and the simultaneous expression of their virulence factors. Surveillance and management of candiduria should be multi-disciplinary considering all those factors.

Table.1 *In vitro* antifungal susceptibility tests among *Candida* species of patients with candiduria

Antifungal agents	*C. albicans* (No= 35)			*C. glabrata* (No = 26)			*C. krusei* (No = 1)		
	S	DD	R	S	DD	R	S	DD	R
	No %	No %	No %	No %	No %	No %	No %	No %	No %
5Fc	6 17.1	13 37.1	10 28.7	2 7.7	13 50	11 42.3	- -	- -	1 100
Flu	17 48.6	- -	3 8.6	6 3.1	13 50	7 26.9	- -	- -	1 100
Itr	- -	2 5.7	25 71.4	- -	- -	26 100	- -	- -	1 100
Mcz	12 34.3	22 62.8	1 2.8	15 42.8	11 31.4	- -	- -	1 100	- -
Vor	14 40	- -	3 8.6	22 62.8	- -	4 15.4	1 100	- -	- -
AmB	35 100	- -	- -	26 100	- -	- -	1 100	- -	- -
Eco	28 80	7 20	- -	26 100	- -	- -	- -	1 100	- -
Ktc	34 97.5	- -	- -	23 88.2	3 11.8	- -	1 100	-	- -

S = Sensitive; DD = Dose dependent; R = Resistant; 5Fc = Flucytosine; Flu = Fluconazole; Itr = Itraconazole; Mcz = Miconazole; Vor = oriconazole; AmB = Amphotericin; B Eco = Econazole; Ktc = Ketoconazole

Table.2 Frequency of secretory aspartic proteinase production among
Candida species of patient groups

Candida species No = 62	Positive SAP production		Catheterized patients (group I) No = 39				Non catheterized patients (group II) No = 22*				P value
			+		++		+		++		
	No	%	No	%	No	%	No	%	No	%	
C. albicans No = 35	32/35	91.4	2/19	10.5	17/19	89.5	2/13	15.4	11/13	84.6	<0.01
C. glabrata No = 26	-	-	-	-	-	-	-	-	-	-	-
C. krusei No = 1	-	-	-	-	-	-	-	-	-	-	-
Total +ve	32/62	51.6	19/20		95		13/15		86.7		<0.05

*One patient in group II was infected with *C. albicans* and *C. glabrata*
 The zone of clarification around the growth (mm): (+) = Mild activity (1-2), (++) = Strong activity (3-5)

Table.3 Frequency of phospholipase production among *Candida* species of patient groups

Candida species No = 62	Positive Phospholipase production		Catheterized patients (group I) No = 39								Non catheterized patients (group II) No = 22*								P value
			+		++		+++		++++		+		++		+++		++++		
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	
C. albicans No = 35	24/35	68.6	8	53.3	6	40	-	-	1	6.7	4	44.5	3	33.3	1	11.1	1	11.1	<0.01
C. glabrata No = 26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. krusei No = 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total +ve	24/62	38.7	15/20				75				9/15						60		<0.05

*One patient in group II was infected with *C. albicans* and *C. glabrata*.
Precipitation zone (mm): (+) = 1.26-1.50, (++) = 1.51-1.75, (+++) = 1.76-2.00, (++++) = 2.01-2.25

Table.4 Frequency of biofilm formation among *Candida* species of patient groups

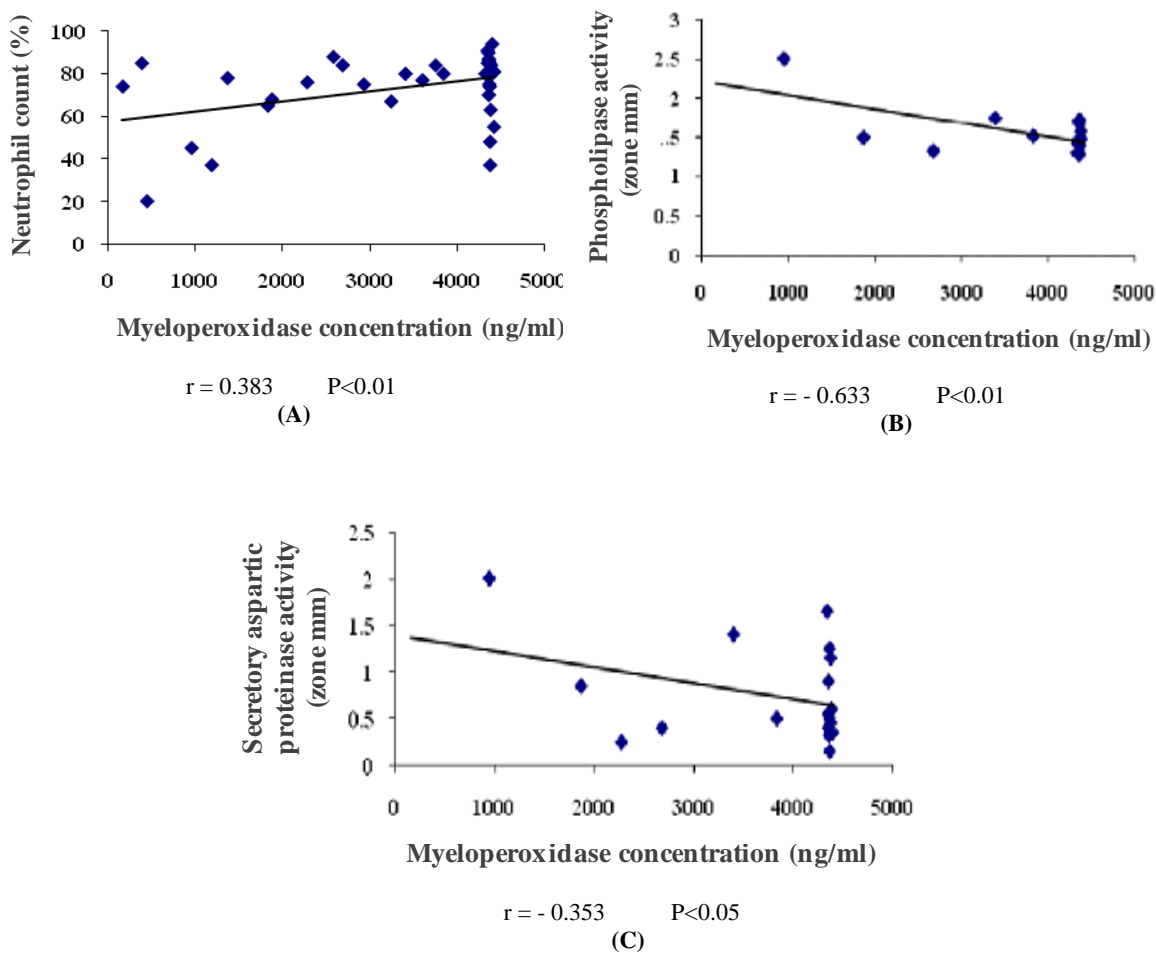| *Candida* species No = 62	Positive biofilm formation		Catheterized patients (group I) No = 39		Non catheterized patients (group II) No = 22*		P value
	No	%	No	%	No	%	
C. albicans No = 35	9/35	25.7	4/20	20	5/15	33.3	< 0.05
C. glabrata No = 26	-	-	-	-	-	-	-
C. krusei No = 1	1/1	100	1/1	100	-	-	< 0.01
Total +ve	10/62	16.1	5	50	5	50	< 0.05

*One patient in group II was infected with *C. albicans* and *C. glabrata*

Table.5 Neutrophil counts and mean myeloperoxidase concentrations in sera of the patient groups and healthy controls

Parameter	Catheterized patients (Group I) No=39	Non catheterized patients (Group II) No=22	Healthy controls (Group III) No=19	P value
Neutrophil count (%) Mean±SD	73.51±16.46	63.00±13.95	52.00±13.21	P <0.01 P_1<0.01 P_2<0.01
Myeloperoxidase concentration (ng/ml) Mean±SD	3401.5±1375.2	3598.6±1214.8	1851.6±1109.0	P<0.01 P_1<0.01 P_2<0.01

P = group I vs. group II P_1 = group I vs. group III P_2 = group II vs. group III

Fig.1 The correlation coefficient between myeloperoxidase concentration and neutrophil count (A), phospholipase activity (B), and secretory aspartic proteinase activity (C) in catheterized patients

r = 0.383   P<0.01

(A)

r = - 0.633   P<0.01

(B)

r = - 0.353   P<0.05

(C)

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