Brief Report

Phospholipase Activity of *Candida* Species Isolated from Diabetic Patients

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Abstract

Background: Candidiasis is one of the prevalent fungal infections caused by the genus *Candida*. The clinical manifestation ranges from mucocutaneous colonization to disseminated and fatal infections such as candidemia. Diabetes mellitus is one of the significant predisposing factors for this fungal infection. *Candida* spp. may release many exoenzymes such as phospholipase to debilitate the immune system and facilitate adherence and invasion of the fungus to the host cells. The aim of the present study is evaluation of phospholipase activity of *Candida* species isolated from candidemia and gastroesophageal candidiasis (GEC) among diabetic patients.

Materials and Methods: Eighty-three *Candida* isolates were evaluated for enzyme activity by phenotypic (the precipitation zone around the colonies) and molecular methods (detection of phospholipase genes using duplex polymerase chain reaction with specific primers).

Results: Eight out of eighty-three clinical isolates (9.6%) were negative for phospholipase production. All phospholipase producers among candidemia and GEC isolates were categorized in high production group.

Conclusions: Our findings revealed no differences in phospholipase activity among isolates obtained from different body sites (blood, oesophagus and stomach); however, non-*albicans Candida* species had less phospholipase activity.

Keywords: *Candida* species, candidemia, diabetes mellitus, gastroesophageal candidiasis, phospholipase activity

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Introduction

Candida species are opportunistic yeasts that cause a wide spectrum of infections in immunocompromised patients, which range from superficial to disseminated infections.[1] Diabetes mellitus is one of the main risk factors for this fungal infection. Elevated serum glucose level can impair neutrophil and monocyte adhesion and movement, phagocytosis and killing of pathogens. The increased glucose level in involved tissues increments *Candida* colonization and invasion.[2] *Candida* species may release many exoenzymes such as phospholipase to impair of cellular membranes, which facilitate adherence and invasion of the fungus to the host cells.[3] In the present study, we examined the *in vitro* phospholipase activity of *Candida* species isolated from candidemia and gastroesophageal candidiasis.

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Materials and Methods

Clinical strains

The protocol of the present study was approved by the Ethics Committee of Isfahan University of Medical Science (no. IR.MUI.MED.REC.1399.316). Eighty-three *Candida* isolates were tested among which, 37 and 46 strains were related to candidemia and GEC, respectively.

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Thirty-four and 12 isolates were obtained from the stomach and oesophagus, respectively. With *Hpa*II restriction enzyme, clinical isolates were previously identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as follows: *Candida albicans* ($n = 67$; 80.7%), *Candida parapsilosis* ($n = 8$; 9.6%), *Candida glabrata* ($n = 6$; 7.2%) and *Candida krusei* ($n = 2$; 0.25%).

Detection of phospholipase activity

This test was performed according to the method of Price *et al*.[4] Briefly, Sabouraud Dextrose Agar (SDA) was supplemented with 0.005 mol/L $CaCl_2$, 1 mol/L NaCl, and 8% sterile egg yolk emulsion and was poured (about 23 mL) in 90 mm Petri dishes. Ten microliter of the yeasts suspension was inoculated on the test medium, and plates were incubated at 37°C for 5 days. Phospholipase activity (Pz) was measured according to the precipitation zone around the colonies. The value of Pz was determined as the growth diameter ratio to the colony's total diameter plus the precipitation zone. It was scored as follows: negative (–), low production (0.75–0.9), moderate production (0.51–0.74) and high production (0.35–0.5).[5]

Detection of Candida phospholipase genes

Detection of phospholipase genes (*PLB1* and *PLB2*) were performed by duplex PCR (dPCR) and specific primers containing: *PLB1* (forward: 5′-CCT ATT GCC AAA CAA GCA TTG TC-3′ and reverse: 5′-CCA AGC TAC TGA TTT CAC CTG CTC C-3′) and *PLB2* (forward: 5′-GTG GGA TCT TGC AGA GTT CAA GC-3′ and reverse: 5′-CTC AAA GCT CTC CCA TAG ACA TCT G-3′).[6] The size of amplicons for *PLB1* and *PLB2* were 179 bp and 270 bp, respectively.

Statistical analysis

SPSS Statistics 25 (IBM, Chicago, USA) was used for data analysis. Kolmogorov–Smirnov test was applied for normal data distribution, the independent *t*-test and Chi-square test were used to compare quantitative and qualitative data between the two groups, respectively. A P value of < 0.05 was considered significant.

Results

Among blood isolates, 4 out of 37 (10.8%) *Candida* spp. (*C. glabrata* = 2 and *C. parapsilosis* = 2) were negative for phospholipase production. In GEC group, 4 out of 46 (8.7%) isolates (*C. glabrata* = 1 and *C. albicans* = 3) didn't secrete phospholipase. All phospholipase producers among candidemia and GEC isolates were categorized in high production group (0.35–0.5) [Figure 1]. The *PLB1* and *PLB2* gene expression percentage in the candidemia and GEC groups were 76.5% and 64.7%, and 86.4% and 77.3%, respectively [Figure 2]. There was no statistically significant difference in the expression of both phospholipase genes between the two groups (p-value = 0.15).

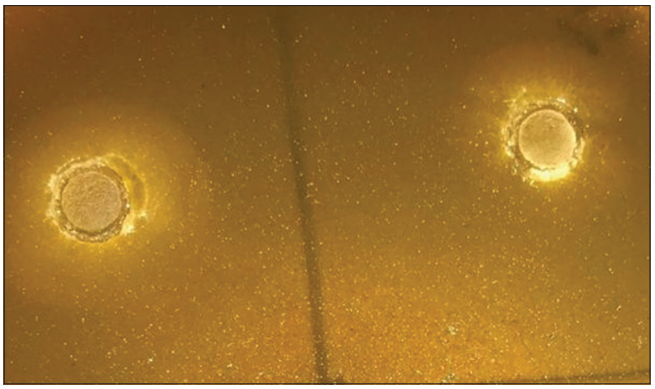

Figure 1: Enzymatic activity of *Candida* spp. on SDA supplemented with $CaCl_2$, NaCl and egg yolk emulsion

Discussion

Virulence factors in microorganisms may vary depending on the type of infection, different species, the site of infection, geographical origin and host response. Pathogenesis of *Candida* spp. closely related to these virulence factors such as phospholipase, proteinase and haemolysin. Detection of pathogenicity factors may help new drug discovery to improve therapeutic regimens.[7] Pakshir *et al*.[8] reported that among 84 clinical *Candida* spp., *C. parapsilosis* strains had less phospholipase activity. In agreement, *C. parapsilosis* and *C. glabrata* isolates had the lowest phospholipase activity in the present study. In line with our findings, Kantarcioglu and Yucel did not show any discrepancy in enzyme production by *C. albicans* from various anatomical sites.[9] Although no remarkable differences of phospholipase activity among the *Candida* spp. from different sites have been found in the current study, we declared that catheter isolates presented lower phospholipase activity than other isolates obtained from candidemia. The phospholipase family contains four distinct classes (A, B, C and D); however, only the products of the *PLB1* and *PLB2* genes have been detected extracellularly,[6] and this is the reason to select these two genes in the present investigation. Bassyouni *et al*.[2] indicated that the *PLB1* and *PLB2* genes were positive in 87.5% -and 45% of *Candida* isolates of diabetic patients, but these percentages were 82.1% and 71.8% in our study.

Conclusion

The results of the present investigation showed no differences in phospholipase activity in different sites of infection (blood, oesophagus and stomach); however, non-*albicans Candida* species had less phospholipase activity. This study suggests that the pathogenicity of genus *Candida* can be related to the fungal species.

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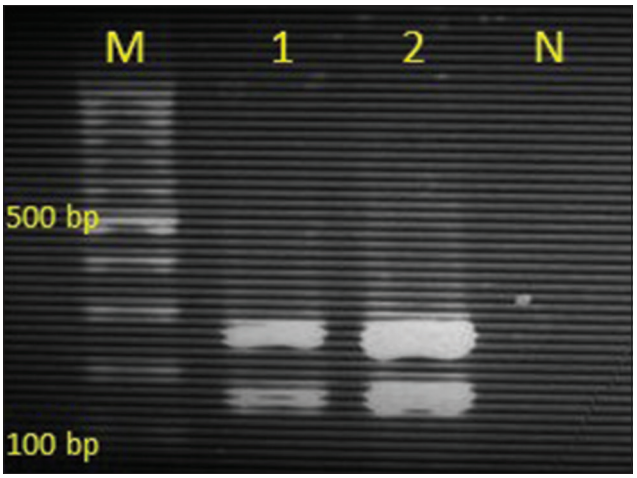

Figure 2: Amplification of *PLB1*and *PLB2* genes by dPCR, lane M is 100 bp DNA size marker, lanes 1, 2 are *C. albicans* isolates that show both PLB1 (179 bp) and PLB2 (270 bp) amplicons, and N is negative control

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Conflicts of interest

There are no conflicts of interest.

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