Short Communication

# Comparison of proteinase activity, hemolysin production, and adherence ability of *Candida albicans* isolates obtained from gastroesophageal lesions and urinary tract infections

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**Background:** Adhesion of *Candida* to host cell receptors, hemolysin production, and proteinase activity are assumed as principal virulence factors and infection establishment. These virulence factors are essential for colonization, biofilm formation, and attack on the host cells. **Materials and Methods:** A total of 97 *Candida albicans* isolates obtained from gastroesophageal lesions and urinary tract infections were included in the study. Adhesion assay, proteinase activity, and hemolysin production were measured. Statistical analysis was performed using the independent *t*-test and Chi-square test to compare quantitative and qualitative data between the two groups. **Results:** The adherence ability to the buccal epithelial cells was the same in the two groups. Proteinase activity was seen in all clinical isolates. Hemolytic activities were not statistically significant in the two groups. **Conclusion:** Our results recommend that the pathogenicity of *C. albicans* in the mucous membranes cannot be connected to the infected site.

**Key words:** Adhesion ability, *candida albicans*, gastroesophageal candidiasis, hemolysin production, proteinase activity, urinary tract infections

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## INTRODUCTION

Adhesins, proteases, and hydrolytic enzymes are pivotal virulence factors in colonization, biofilm formation, and the establishment of infection, especially in mucocutaneous membranes.[1,2] *Candida albicans* has multiple virulence factors and is the most prevalent *Candida* species in the gastroesophageal candidiasis (GEC) and urinary tract infections (UTIs). These infections are major sources of disseminated candidiasis because *C. albicans* can enter the bloodstream by translocating through the mucosal surfaces. It is important to understand the pathogenesis of *Candida* and its role in systemic infections to develop more effective antifungal agents. The aim of this study was to investigate the activity of *C. albicans* virulence factors isolated from GEC and UTIs.

## MATERIALS AND METHODS

The protocol of the present study was approved by the Ethics Committee of Isfahan University of Medical Science (no.IR.MUI.MED.REC.1399.325). A total of 97 *C. albicans* strains obtained from GEC and UTIs were included in the study.

### Adhesion assay

The buccal epithelial cells (BEC) were washed with phosphate-buffered saline (PBS) and adjusted to a final concentration of 2–3 × $10^5$ cells/mL. A yeast stock of

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$1 \times 10^7$ yeast/mL was provided. Yeast suspension (600 µL) was mixed with 600 µL of the BEC and incubated at 37°C with agitation for 1 h. Membrane filter (20 µm) was used to filter the mixture. The filter was washed with PBS and then transferred to a slide by pushing the filter paper against it to remove nonadhered yeasts. Methanol and Gram-Nicolle technique were used for fixation and staining, respectively, and finally the number of adhered cells to 100 BEC was counted.[3]

### Proteinase activity

Ten µL of *C. albicans* suspension was inoculated into Bovine Serum Albumin agar. The plates were incubated at 37°C for 6 days. Proteolytic activity (Pz) was determined by the ratio of the diameter of colony and total diameter of colony plus precipitation zone.[4,5]

### Hemolysin production

Hemolytic activity was evaluated on Sabouraud Dextrose Agar + 7% sheep blood + 3% glucose. Suspended yeasts were inoculated onto the medium and incubated at 37°C for 48 h. Hemolytic index (colony diameter/semitransparent zone in mm) was applied to indicate the amount of hemolysin activity.[6]

### Statistical 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 $P < 0.05$ was considered significant.

## RESULTS

### Adhesion assay

The adherence ability to BEC was 34.46 ± 10.14 and 37.27 ± 14.96 in the UTIs and GEC groups, respectively, which were not statistically significant ($P = 0.285$). In the GEC group, the percentage of adhesion in gastric juice with an average of 41.12 ± 17.35 was higher than esophagus (37.62 ± 15.35) and stomach (33.95 ± 12.36); however, this difference was not statistically significant ($P = 0.349$) [Figure 1a].

### Proteinase activity

Proteinase activity was seen in all clinical isolates. The mean proteinase activity was 0.25 ± 0.11 and 0.28 ± 0.08 in the UTIs and GEC groups, respectively, which were not statistically significant ($P = 0.14$) [Figure 1b].

### Hemolysin production

Hemolytic activities were 0.39 ± 0.10 and 0.41 ± 0.13 in the UTIs and GEC groups, respectively, which were not statistically significant ($P = 0.569$). In the GEC group, hemolytic activity among stomach clinical strains with an average of 0.42 ± 0.13 was more than isolates obtained from the esophagus (0.38 ± 0.08) and gastric juice (0.41 ± 0.16); however, this difference was not statistically significant ($P = 0.569$) [Figure 1c].

## DISCUSSION

Exoenzymes are produced at high rates in *C. albicans* in comparison to non-*albicans Candida* species, and this was the

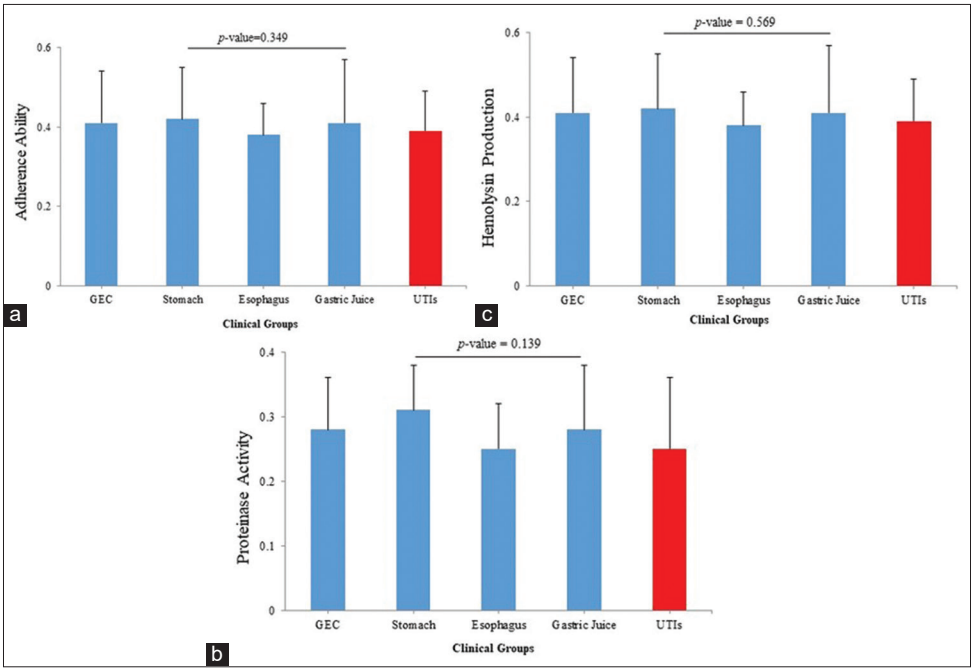


**Figure 1:** The adherence ability (a), proteinase activity (b), and hemolysin production (c) of *Candida albicans* isolates collected from UTIs and GEC

reason for the focus of this study on *C. albicans* isolates.[7-9] We verified that gastric and catheter-related isolates presented higher proteinase activity than isolates obtained from gastric juice and esophagus. Although surveys on the hemolysin activity are limited in *C. albicans*, Manns *et al*. described the conditions and factors under which *C. albicans* can show hemolytic activity.[10] They discovered that hemolysis does not occur when no glucose is accessible in the culture media. Luo *et al*.[6] have examined 80 clinical *Candida* strains in different geographical locations and found only alpha, and not any beta, hemolysis in examinations with glucose-free sheep blood agar. These data are in accordance with our findings because all secreted hemolysins were alpha-hemolysin in the present study. Most of the research has shown that *C. albicans* from the catheter and oral cavity is more adherent to BEC.[11,12] Nevertheless, we did not observe a significant difference for this pathogenesis factor in patients using catheters. The adherence to host receptors is thought an event possibly linked to invasion of that cell or tissue. High adhesion ability was detected among *C. albicans* strains isolated from gastric juice (41.12 ± 17.35). We were not able to find any connection for this issue and further research are needed to illuminate this matter.

## CONCLUSION

The results of the present investigation revealed no discrepancies in the adherence ability, proteinase activity, and hemolysin production of clinical isolates which are not related to the sites where the samples were collected.

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### Conflicts of interest

There are no conflicts of interest.

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