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Adherence of Candida Albicans to Uroepithelial Cells

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Abstract

Background Urinary tract infection (UTI) is usually associated with multiplication of microorganisms in urinary tract followed by adhesion of these organisms to the uroepithelial cells which is considered as the first step for colonization and infection. Adhesion of Candida on the epithelium of the urinary tract stands as the first step in the pathogenesis of Candidiasis. The adhesion process is under the impact of many factors.

Objective This study was designed to determine the susceptibility of uroepithelial cells obtained from patients with different urinary tract diseases, for adherence with Candida albicans in vitro.

Methods Forty eight urine samples collected from women attending Medical City Hospital /Baghdad suffering from urinary tract diseases and proved to have Candida growth. Ten urine samples were collected from apparently healthy age matched females and used as a control. The uroepithelial cells of each sample have been collected and incubated with Candida albicans cells and the percentage of uroepithelial cells with an adhered Candida cells (% EC). The mean number of Candida/epithelial cells (C/E) was estimated before and after adherence assay.

Results Klebsiella infection showed the highest percentage of uroepithelial cells with Candida (%EC), next in frequency was samples with high number of uroepithelial cells, then, Streptococcus pyogenes, Escherichia coli, pus cells, and finally samples with high number of RBCs. This study indicates that receptivity of Candida adhesion to the uroepithelial cells of urinary tract have different affinity depending on the type of modulating factor surrounding these uroepithelial cells.

Conclusion From the obtained data it can be concluded that urine findings including bacterial growth might modulate epithelial cell surface and increase their receptivity for Candida adherence.

Key word Adherence, Candida albicans, uroepithelial cells.

Introduction

Urinary tract infections (UTIs) which are not properly treated from their onset can become a real threat in time, finally leading to renal failure. This is partly due to the fact that urinary signs and symptoms are often not reliable in distinguishing upper and lower urinary tract diseases [(1)].

UTI is usually associated with the demonstration of pathogenic organisms in urine; bacteria, fungi or parasite. It is more common among females due to the shortness of urethra [(2)]. Candida play an important role in UTI since Candida albicans and related species are the leading cause of disseminated fungal infection in immuno- compromised diabetics, postoperative patients and in patients with chronic mucocutaneous candidiasis. Candida causes an erosive dermatitis and severe inflammation of vaginal tract [(3)].

An understanding of the mechanism of attachment and a delineation of the adhesive

molecules on the surface of Candida (adhesion) as well as those on cell membranes (receptors) has suggested new approaches to the prevention of serious candidiasis infection. Such adherence enables the organisms to avoid elimination by the cleansing action of mucosal secretions, allowing the yeast to colonize [4].

Adhesion of Candida species on epithelium of the gastrointestinal or urinary tract stands as a critical first step in the pathogenesis of candidal infection [3].

A work on the adherence of some Gram positive bacteria such as Staphylococcus aureus to human epithelial cells was carried out and it was found that fibronectin-binding proteins are the major staphylococcal adhesin responsible to this adherence [5].

The objective of this study is to determine the effect of different urine findings including bacteria on susceptibility of uroepithelial cells obtained from patients with different urinary tract infections, for adherence with Candida species in vitro. It is an attempt to assess the role of urine deposit findings and bacterial growth on modulation of uroepithelial surface receptivity for Candida albicans adhesion.

Methods

Epithelial cells

Uroepithelial cells were collected from freshly voided urine of females with urinary tract disease with or without bacteria. The suspended cells in urine were centrifuged 3000 rpm for 15 minutes. The pellet was washed 2-3 times with phosphate buffer saline (PBS). Using Neubauer chamber, uroepithelial cells were examined and counted for attached bacteria and yeast. Control uroepithelial cells had no evidence of yeast contamination or attachment [6].

Candida growth and isolation

Candida cells were obtained from females urine samples with urinary tract diseases associated with Candida albicans infection by using Sabouraud dextrose agar. Candida isolates were diagnosed by applying appropriate diagnostic tests [7]. The yeast cells were incubated for 48h at 37^{o}C and the cells were suspended in 5 ml PBS with pH 7.2, washed twice with PBS by centrifugation at 3000 rpm for 5minutes. The pellet was collected and resuspended in 5 ml PBS. Yeast cells were diluted with PBS and adjusted to a concentration of 10^8 yeast cells/ ml and kept in deep freeze until used [6].

Urine samples

Urine samples were grouped into; urine from patients with positive bacterial growth, urine from patients with negative bacterial growth and urine samples from controls. The following tests were applied to the above samples:

1. General urine examination: This examination was carried out according to Fischach & Dunning (2004) [8].

It included the following:

-Macroscopic examination e.g. color, reaction, appearance.

-Biochemical tests e.g. sugar, albumin, bile pigment.

-Microscopic examination: urine samples were centrifuged at 3000 rpm for 15 minutes, deposits were examined and the results estimated according to the following scores:

a) Pus cells > 5/HPF considered positive.
b) RBC >3/ HPF considered positive.
c) Casts> 2/HPF considered positive (other than hyaline cast).

Urine culture

Both Gram positive and Gram negative bacteria were isolated on different media. Klebsiella pneumoniae, Escherchia coli and Streptococcus pyogenes were identified in urine samples grouped as samples with bacterial growth. Uroepithelial cells were obtained from urine samples of all groups and incubated with candida albicans.

Adhesion assay

This assay was performed as described by Centeno et al [6]. Uroepithelial cells (0.5 ml from a PBS suspension of 5x10^6 cells per ml) and yeast organisms (0.5 ml from a PBS suspension of 10^8 yeast cells per one ml) were placed in screw top vials (ratio of cells/ yeast 1:20) and incubated at 37^{o}C for 60

minutes in water bath with gentle shaking. After incubation, the suspension was filtered through Nuclepore schisto kit (12 μm pore size) instead of polycarbonate filter Nuclepore Corp., Pleasanton, Calif. unattached yeast cells were removed by rinsing the filter two times with 3-5 ml of pipetted PBS. The filters were labeled and allowed to dry for 24 hours at room temperature. The dried filters were floated, uroepithelial cells down side, on the dye solution (Methylene blue 0.5% in PBS) for 10 to 15 seconds and then removed. Excess dye was removed from filters with three successive rinses by floating filters in PBS – filled Petri plate. After the final rinse, filters were placed, epithelial cells side up, on large glass slides and viewed as wet mounts by light microscopy at 400X. Uroepithelial cells viewed were counted for yeast attachment by preparing 4-6 slides for each sample. Percentage of epithelial cells with attached candida (% EC) and mean number of candida per epithelial cells (C/E) were counted by using direct microscopic examination.

Adherence assay was applied to study Candida adherence to the epithelial cells isolated from patients with urinary tract disease as well as apparently healthy individuals (control).

Statistical analysis

t -Test was used with P value < or = 0.05 considered significant [9].

Results

A- Urine from patients with bacterial growth:

The percentage of uroepithelial cells with adhered Candida (%E C) was counted before and after Candida adherence assay. Table (1) showed that %EC after adherence assay were significantly higher (P<0.01) compared to that before assay. The highest differences in % EC before and after assay was noticed with uroepithelial cells obtained from urine infected with Klebsiella (35.3%) followed by that with Streptococcus (32%) and then with E.coli (29.2%).

Table 1. Percentage of EC (%EC) obtained from urine with different bacterial infections before and after Candida adherence assay.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
Klebsiella pneumonia	25.3±6.4	60.6±4.5*	35.3
Escherichia coli	35.9±14.8	65.1±13.7*	29.2
Streptococcus pyogenes	34±7.8	66±1.8*	32

EC= Epithelial cells with Candida, *P< 0.01

The mean number of candida per uroepithelial cell (C/E), (Table 2), was found to be higher with uroepithelial cells obtained from urine sample with different bacterial infections after assay compared to that before assay (P<0.01) and the difference between C/E after assay and before assay was the highest with epithelial cells obtained from urine with Klebsiella (49.6%) followed by that with Streptococcus (47%) and then with E.coli (32.2%).

Table 2. Mean number of Candida per epithelial cell (C/E) obtained from urine with different bacterial infections before and after adherence assay.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
Klebsiella pneumonia	37±18.5	86.6±20.8*	49.6
Escherichia coli	55.8±17.9	89±10.5*	33.2

Streptococcus pyogenes	47±15.1	94±18.2*	47

EC= Epithelial cells with Candida, *P< 0.01

B-Urine from patients with negative bacterial growth but with positive urine deposits:

Table (3) showed that %EC was significantly increased (P<0.01) after Candida assay compared to that before assay. %EC difference was found to be the highest with uroepithelial cells having high number of uroepithelial cells (32.7%) compared to the epithelial cells with pus cells (24.5%)and then epithelial cells with red blood corpuscles (RBCs) (21%).

Table 3. Percentage of EC in epithelial cells obtained from urine deposits with different microscopic findings and no bacterial growth.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
>10 epithelial cells/ HPF	37.5±9.1	70.2±8.1*	32.7
> 5 pus cells/ HPF	36.5±20.3	61±13.2*	24.5
> 3 RBCs/ HPF	48.6±11.2	69.6±11.2*	21

* P < 0.01

Table (4) revealed that C/E in the presence of different urine deposit findings were significantly higher (P<0.01) after adherence assay than before. The positive epithelial findings showed the highest difference followed by RBCs and pus cells (31.5, 26.6, and 25.3 respectively).

Table 4. Mean number of C/E obtained from urine with different abnormal urine findings and no bacterial infection.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
>10 epithelial cells/ HPF	55.7±21.1	87.2±13.8*	31.5
> 5 pus cells/ HPF	53.7±26.1	79±14.8*	25.3
> 3 RBCs/ HPF	68±13.6	94.6±8.7*	26.6

* P < 0.01

It was noticed that urinary tract disease with and without bacterial growth has an enhancing effect on adherence activity compared to control (Table 5). Percentage of EC in uroepithelial cells obtained from urine with bacterial growth was found to be higher than that without bacterial growth. The same is true for mean number C/E.

Table 5. %EC and C/E mean difference between results before and after Candida adherence assay for patients compared to control.

	Mean of difference		
	Uroepithelial cells from urine with (-) growth	Uroepithelial cells from urine with infection	Control
% EC	32.2	26.1	3.5
Mean C/E	43.3	27.9	5

Discussion

C. albicans are commonly found on mucous membranes (e.g. vagina and gastrointestinal tract) and it is an opportunistic pathogen. Vaginal colonization is increased by Diabetes mellitus, pregnancy and the use of oral contraceptive agents [10]. Previously, colonization with bacteria was thought to inhibit fungal infections in situation where the bacterial flora does not adhere to yeast [11]. Later studies suggested that certain pilliated bacterial strains do significantly enhance yeast adherence to host epithelial cells. It was shown by Ahearn et al [12] that C. albicans is frequently found concomitantly with bacterial urinary tract infections. Working on adherence of three species of Candida it was indicated that C. albicans adhered to vaginal and buccal epithelial cells to a significantly greater degree than other species tested [13]. Date extract inhibit germ tube formation of C. albicans which might contribute to the effects on reducing adhesion (14). It can be concluded from the above data that Candida adherence could be under the effect of many factors.

Adherence of Candida species to mucosa and particularly C. albicans is probably an important initial step in the pathogenesis of infection caused by these yeasts [15]. This adhesion occurs as a result of the interaction between yeast and epithelial cells receptors [13]. It was found that the cell wall of C. albicans contains floccular materials which are probably involved in yeast attachment [6]. Candidal adherence to the epithelium and the production of carboxylic acids of short chain as a product of sugar metabolism, produce an acid environment that could affect the pathological process in several ways such as cleavage of secreted IgA, an important factor to prevent Candida adherence [16].

As Candida adheres, their ability to grow and survive contributes to the hydrolytic enzymes, which are extracellularly secreted by the fungus. They may play a central role in the pathogenesis of candidiasis [17].

It is well known that C. albicans adhesion to mucosal cell is enhanced by several factors such as germ tube production, phospholipase, protease, other extracellular enzymatic activities, carbohydrates, pH and temperature [18]. It was suggested that cell wall mannoprotein is an essential component of the C. albicans adhesins [19].

Adherence of K. pneumoniae to mammalian epithelial cells is mediated by the adhesins, Fim H and MrKD which are associated with type 1 and type 3 fimbriae respectively [20]. All the RD6 isolates of K. pneumoniae were found to adhere strongly to mammalian cells in vitro [21]. Regarding E. coli adhesion, it was stated that E. coli has Fim H gene responsible for adherence to epithelial cells [22].

Many studies have shown that bacterial attachment to host cells can be augmented by pilli [6]. The capacity of K. pneumoniae RD6 isolate (genotype) to cause UTI may be mediated by its striking adherence to mammalian cells [19]. The increase in yeast attachment to epithelial cells preincubated with pilliated bacteria is due to an attachment of yeast to mannose-sensitive pilli on bacteria already attached to epithelial cells. This attachment may be an important factor in C. albicans pathogenicity.

In the present study the possible involvement and capabilities of Candida adherence to epithelial cells isolated from urinary tract of patients in the presence or absence of bacterial growth compared to normal was evaluated in vitro. This study revealed that the percentage of epithelial cells with candida (% E C) was higher in the presence of bacterial growth, especially K. pneumoniae after adherence assay where the difference was 35.3 which is similar to findings reported previously by King et al [13]. Candida adhesion to urogenital epithelial cells in the presence of Streptococcus pyogenese was found to be increased. An in vitro study on oral streptococci showed that these bacteria suppressed C. albicans infection which could be due to competition between Streptococcus and Candida for cell receptors

and alteration of the environment by bacterial infection [23]. On the other hand it was found few years later that, group A streptococci is responsible for the attachment of these organisms to mucosal cells [24], which is in agreement with our study. The same result was detected in the presence of E.coli.

Also the mean number of Candida per epithelial (C/ E)after conducting adherence assay was found to be higher in the presence of bacterial growth in particular K. pneumoniae where the difference was (49.6%), whereas for E. coli (33.2%) and then for S. pyogenes it was (47%) with P value < 0.01

Suman et al [25] concluded that increased receptivity of vaginal epithelial cells to pathogens associated with lower local immunity may play role in the pathogenesis of recurrent UTI in females.

In the present study, urine samples without bacterial growth and high number of epithelial cells showed the highest difference in EC% before and after applying adherence assay (Table 3). From these findings it can be concluded that epithelial cells from urine with high number of sloughed epithelial cells are more susceptible to adhesion than others. This may be explained on the same basis of Williams et al [26] findings which demonstrated that increased adherence was associated with increased keratinization of epithelial cells among smokers. It was stated that Candida showed more adherences to superficial keratinize epithelial cells than intermediate cells [27].

Increased number of RBCs in urine which is seen in 40-60% of patients with acute cystitis. Haematuria may be caused by, non-infective pathological conditions or renal mycobacterial infection with or without pyuria. High umber of RBCs or pus cells indicates certain pathological conditions that are not associated with bacterial growth. Such situation was met with this study, which showed an increase in Candida adhesion (EC %) but to a lower extent compared to those with bacterial growth (Tables 1 & 4) at P value<0.01.

In table (4), C/E in the presence of positive epithelial cells showed a significant increase (31.5) compared to positive pus cells (25.6) and positive RBC (26.6) with P value<0.01.

Finally, table (5) revealed that mean% EC and mean C/E in urine obtained from apparently healthy controls were the lowest in comparison to those with bacterial and without bacterial infections before and after applying adherence assay. Also it was noticed that the mean difference in %EC and C/E in a group with bacterial growth is higher than that with only urine findings group and control. Recently an attempt to prepare an anti-candidal IgA in egg yolk (IgY) showed to decrease Candida adhesion which can be used as an adjunct to antifungal drugs [28].

In conclusion, the increase in adhesion of Candida to uroepithelial cells was associated variably with urinary tract diseases with different urine deposits and bacterial growth factors. These changes in Candida adhesion may be due to modulation effect of these factors on uroepithelial surface.

References

1. Wojnicz D. Virulence factors of uropathogenic Escherichia coli strains isolated from children with chronic pyelonephritis. *Adv Clin Exp Med*, 2007; 16(5): 651-657.
2. Davison AM, Cumming AD, Swainson CP, Turner N. Diseases of the kidney and urinary system. In: Davidson' s principles and practice of medicine. London: Churchill Livingstone. 1995.
3. Hostetter MK. Adhesins and ligands involved in the interaction of Candida species with epithelial and surfaces. *Clin Microbiol Rev*, 1995; 7(1): 29-42.
4. Jabra-Rizk MA, Falkler WA, Merz WG, Baqui MA, Kelley JI, Meiller TF. Cell surface hydrophobicity-associated adherence of Candida du bliniensis to human buccal epithelial cells. *Rev Iberoam Micol*, 2001; 18: 17-22.
5. Mongodin E, Bajolet O, Cutrona J, Bonnet N, Dupuit F, Puchelle E, Bentzmann S. Fibronectin-binding proteins of Staphylococcus aureus are involved in adherence of human airway epithelium. *Infec Immun*, 2002; 70(2): 620-630.
6. Centeno A, Davis CP, Cohen MS, Warren MM. Modulation of Candida albicans attachment to human epithelial cells by bacteria and carbohydrates. *Infec Immun*, 1983; 39(3): 1354-1360.

7. Bello MD, Gondalez A, Barnabe C, Larrouy G. First characterization of Candida albicans by random amplified polymorphic DNA method and comparison of the diagnosis methods for vaginal candidiasis in Nicaraguan women. *Mem Inst Oswaldo Cruz*, 2002; 97(7): 985-989.
8. Fischbach FT, Dunning MB. Urine studies. In: A manual of laboratory diagnostic tests (7th eds.). Chapter 3, London, Lippincot William & Wilkins. 2004; p. 263.
9. Petrie A, Sabin C. Medical statistics at a glance. (2nd ed.) Massachusetts, Blackwell Publishing Ltd. 2005; p.156
10. Parslow TG, Stites DP, Terr AL, Imboden JB. Medical Immunlogy. 10th ed. New York: Mc Graw-Hill; 2001; pp. 814.
11. Paine TF. The inhibitory actions of bacteria on Candida growth. *Antibio Chemoth*, 1958; 8: 273-281.
12. Ahearn DG, Jannach JR, Roth FJ. Specification and densities of human urine specimens. *Sabouraudia*, 1966; 5: 110-119.
13. King RD, Lee JC, Morris AL. Adherence of Candida albicans and other Candida species to mucosal epithelial cells. *Infec Immun*, 1980; 27(2): 667-674.
14. Abo-Elteen KH. Effects of date extract on adhesion of Candida species to human buccal epithelial cells in vitro. *J Oral Pathol Med*, 2000; 29(5): 200-205.
15. Sturtevant J, Calderone R. Candida albicans adhesions: Biochemical aspects and virulence. *Rev Iberoam Micol*, 1997; 14: 90-97.
16. Coutinho HDM. Factors influencing the virulence of Candida spp. *West Indian Med J*, 2009; 58 (2): 153-158.
17. Sandovsky-Losica H, Segal E. Infection of HEp2 epithelial cells with Candida albicans: adherence and postadherence events. *Immunol Med Microbiol*, 2006; 46 (3): 470-475.
18. Vidotto V, Mantoon B, Pugliese A, Pontòn J, Quindôs G, Aoki S, Ito-Ku WA. Adherence of Candida albicans and Candida dubliniensis to buccal and vaginal cells. *Rev Iberoam Micol*, 2003; 20: 52-54.
19. Lee JC, King D. Characterization of Candida albicans adherence to human vaginal epithelial cells in vitro. *Infec Immun*, 1983; 41(3): 1024-1030.
20. Krogfelt KA, Bergmans H, Klemm P. Direct evidence that the FimH protein is the mannose-specific adhesion of Escherichia coli type 1 fimbriae. *Infec Immun*, 1990; 58: 1995-1998.
21. Kil KS, Darouiche RO, Hull RA, Mansouri MD, Musher DM. Identification of a Klebsiella pneumoniae strain associated with nosocomial urinary tract infection. *J Clin Microbiol*, 1997; 35 (9): 2370-2374
22. Sokurenko EV, Courtney HS, Maslow J, Siitonen A, Hasty DL. Quantitative differences in adhesiveness of type 1 fimbriated Eschericha coli due to structural differences in FimH genes. *J Bacteriol*, 1995; 177: 3680-3686.
23. Lijemark WF, Gibbons RJ. Suppression of Candida albicans by human oral streptococci in gnotobiotic mice. *Infec Immun*, 1973; 8: 846-849.
24. Ofek I, Mirelman D, Sharon N. Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. *Nature (London)*, 1977; 265: 623-625.
25. Suman E, Gopalkrishna-Bhat K, Hegde BM. Bacterial adherence and immune response in recurrent urinary tract infection. *Int J Gynaecol Obstet*, 2001; 75(3): 263-268
26. Williams DW, Walker R, Lewis MA, Allison RT, Potts AJ. Adherence of Candida albicans to oral epithelial cells differentiated by Papanicolaou staining. *J Clin Pathol*, 1999; 52: 529-531.
27. Kumar GA, Kumari GR, Shivananda PG, Arun kumar G, Girija RK. Adhesion of Candida albicans to human buccal and vaginal epithelial cells. *Indian J Med Microbiol*, 1997; 15(2): 61-63.
28. Fujibayashi T, Nakamura M, Tominaga A, Satoh N, Kawarai T. et al. Effect of IgY against Candida albicans and Candida spp. adherence and biofilm formation. *Jpn J Infect Dis*, 2009; 62: 337-342.

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