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ORIGINAL ARTICLE

Candiduria in Renal Transplant Recipients and Identification of isolated *Candida* species by Morphological and Molecular methods

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ABSTRACT
Candiduria in renal transplant recipients may represents colonization, lower or upper urinary tract infection but, there is no established diagnostic test that distinguishes infection from colonization.The aimof this study was to determine candiduria in renal transplant patients and identify Candida isolates by mycological and molecular methods. Urine samples of 70renal transplant patients were collected during a period of 3 months for diagnosis of candiduria. Primary identification of Candida isolates was doneby morphological method and confirmed by using restriction fragment length polymorphism (PCR-RFLP) technique. Candiduria was diagnosed in 11(15.71%) patients and isolated Candida specieswere identified asCandida albicans (n: 6),C. glabrata (n: 3), C. krusei (n: 1), and C. tropicalis (n: 1), respectively. Candiduria in renal transplant patients could be related to Candida urinary tract infection. Therefore, a high index of suspicion is necessary to diagnose and treatment of Candida infection in renal transplant recipients. In this study Candida albicans was predominant etiologic agent of candiduria.
***Keywords**: Renal Transplantation, Candiduria, Candida albicans, Candida spp., PCR-RFLP*

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INTRODUCTION

Candiduria is rarely seen as a community acquired infection in healthy people with a structurally normal urinary tract [1] it may represent cystitis, pyelonephritis or fungus ball in the urinary tract system. On the other hand, candidemia and upper urinary tract involvement are some complications of candiduria. . It is increasingly becoming an important subgroup of nosocomial urinary tract infections in organ transplant recipients [2-4]. Prolonged hospitalization, treatment with broad spectrum antibiotics and corticosteroids, urinary tract abnormality, prophylaxis by antifungal agents and immunosuppressive regimens are important risk factors for candiduria in renal transplant recipients [7].The incidence of candiduria in these patients is unknown and the indication for antifungal therapy is not well established. Some antifungal drugs may select drug resistant *Candida* species, or interact with immunosuppressive agents and renal transplant recipients usually present vague clinical symptoms of infection [5].Although C. albicans (52%) is the most common etiologic agent of candiduria, non-albicans Candida (NAC) species can also be related to UTIs and in 10% of cases, different Candida spp. may be isolated from a urine sample. The resistance of Candida spp. especially NAC spp. to antifungal drugs has increased in recent years.Thus to improve the prognosis, a high index of suspicion is necessary in renal transplant recipients and identification of etiological agents for early treatment, and preventing the invasion is highly recommended [5-8].The aim of this study was to determine the frequency of candiduria in renal transplant patients and identified isolated *Candida* species.

MATERIALS AND METHODS

This cross-sectional study was carried out on renal transplant patients from December 2014 to December 2015 in a teaching hospital in North of Tehran, Iran. The aim of research was clearly explained to patients and all patients agreed to participate in this study, which was approved by the local Ethics Committee. The reference strains *Candida albicans* (ATCC 10261) were used as positive control. For each patient, the following data were recorded: demographic characteristics and variables as patient symptoms, information pertaining to transplantation, previous treatment with antibiotics and antifungals, use of steroids, duration of hospital stay and outcome. Urine samples were obtained insterile urine bottles and immediately transferred to medical mycology laboratory.

Yeasts identification

10µl of each urine sample was cultured on CHROM agar candida medium (CHROM agar Candida®, France) before and after centrifugation and incubated at 35°C for 48 hours , evaluated based on color and number of growth colonies. If no growth was observed, the media were incubated for several additional days. isolated colonies were cultured on corn-meal agar (DIFCO laboratories, Detroit, Mich., USA) with 1% tween80. *Candida* species were identified by phenotypic methods. In this study, urine wet-mount examination was performed to detect fungal elements in the urine sediment. One colony on each identification strains was stored for molecular identification.

DNA Extraction

PCR-RFLP method was performed for definite identification of species. All isolated strains subcultured on Sabouraud dextrose agar medium (Sigma, USA) Genomic DNA was extracted, using the method of phenol-chloroform disruption (13). Briefly, 300 µL of lysis buffer (Tris-HCL 1 M , EDTA 0.25 M (pH 8)), 1% SDS, 300 µLof phenol- chloroform-isoamyl alcohol (25:24:1) solution and equal to 300 µL of 0.5 mm diameter glass beads, were added to yeast. After 5 min of vigorous shaking which followed by 5 min centrifugation at 10000 x g, the supernatant was isolated and transferred to a new tube and equal volume of chloroform - isoamyl alcohol (24:1) was added, mixed gently, centrifuged and its supernatant was transferred to a new tube. For alcohol precipitation, 0.01mL volume sodium acetate (pH 5.2) and 0.5 mL volume of cold absolute ethanol were added and the mixture was gently shaken and centrifuged at 10000 x g for 10 min at 4°C. After washing with 70% ethanol, the pellets resuspended in 100 µL TE buffer (10 mMTris, 1 mM EDTA) and were stored at -20°C prior to use.

RCR- RFLP analysis

The PCR-RFLP method was performed as previously described. Briefly, PCR amplification of ITS1-5.8S-ITS2 rDNA regions was achieved using the universal primers ITS1 (5 -TCC GTA GGT GAA CCT GCG G-3 and ITS4 (5 -TCC TCC GCT TAT TGA TAT GC-3). PCR amplification was performed in a final volume of 50 µl. Each reaction contained of 2µl (100-150ng) of template DNA, each forwardand reverse primer at 0.2µM, each deoxynucleoside triphosphate (dNTP) at 0.2 mM,,5U Taq DNA polymerase and 5 µl 10× PCR buffer. The amplification parameters consist of 35 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 45 sec, extension at 72°C for 1 min. In the first cycle, the denaturation step was 94°C for 5 min and in the final cycle the final extension step was 72°C for 7min. Subsequently, PCR products were digested by *MspI* restriction enzyme. Amplified and digested products were visualized by 1.5% agarose gel electrophoresis in TAE buffer (0.09 M Tris, Glycial Acetic acid and EDTA 0.5 M , pH 8.3) respectively, and stained with ethidium bromide (0.5 µg/ml) and photographed. The size of DNA fragments determined directly with comparison of molecular size marker and distinct banding patterns.After the digestion with *MspI* enzyme the results were evaluated based on the sizes of PCR products for *Candida* species.

RESULTS

In the present study seventy patients who included 45 (64.28%) males and 25 (35.71%) females with the age range from 17 to 71 years (median age 44±1.3 years) were enrolled. None of the patients received antifungal prophylaxis or antifungal treatment previous. Maximum cases of candiduria were seen in 50-60 yr age group. One hundred two urine samples were evaluated from renal transplant patients. 15.7 % of the patient urines were yielded *Candida*species. by morphological method, the Candida isolates were identified as *C. albicans* (n: 6) (Fig 1) *C.glabrata* (n: 3) (Fig 2), *C.tropicalis* (n:1)(Fig 3) , *C.krusei* (n:1) (Fig4).Fig 6 demonstrates the patterns of ITS region for *Candida* strains after digestion with *Msp I* enzyme. *Candida albicans* was confirmed in 6 (54.54%) cases as the most common *Candida*, followed by *C. glabrata* 3 (27.27%), *C. krusei*1 (9.9%) and *C. tropicalis* 1(9.9%).

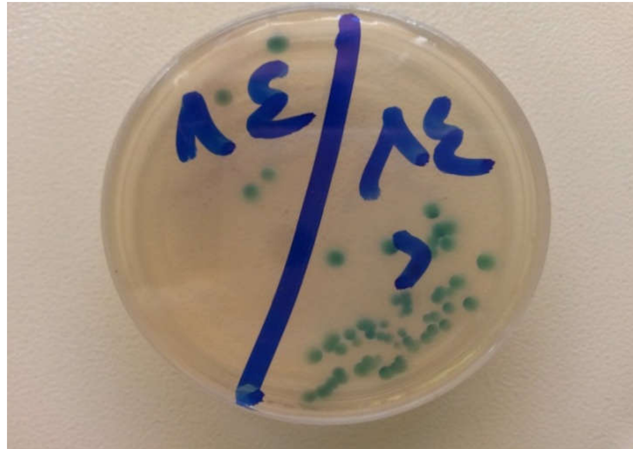

Fig 1: *C. albicans* isolated from urine culture (CHROMagar Candida medium) incubation at 35°C for 48 hours

Fig 2 : *C . glabrata* isolated from urine culture (CHROM agar Candida medium) incubation at 35°C for 48 hours

Fig 3 : *C . tropicalis* isolated from urine culture (CHROMagar Candida medium) incubation at 35°C for 48 hours

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Fig 4 : *C . krusei* isolated from urine culture (CHROMagar Candida medium) incubation at 35°C for 48 hours

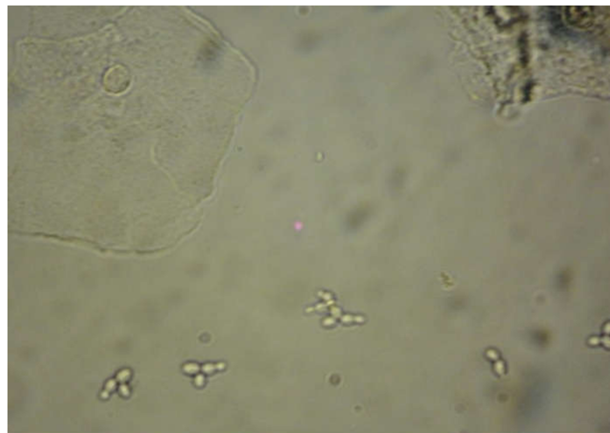

Fig 5 :Budding yeast cells in direct examination of urine sediment (×400)

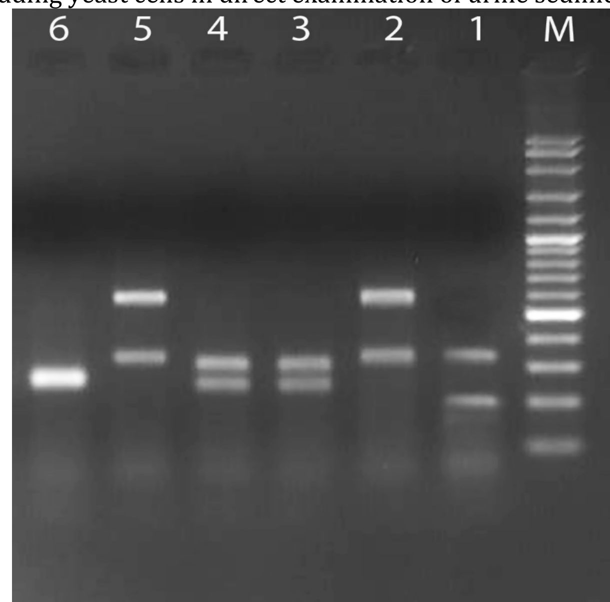

Fig 6: Patterns of PCR-RFLP products of *Candida* spp. Isolated from candiduria after digestion by the restriction enzyme *MspI*. Lanes of 1 represent *C. tropicalis (184 ,340bp)*; 3, 4*C. albicans (238, 297 bp)*; 2,5 *C. glabrata(557 , 314 bp)*; and 6*C. krusei (261 , 249bp)*. Lane M is 100 bp ladder molecular size marker.

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DISCUSSION

Due to the use of immunosuppressive regimens and environmental exposure, infections are a significant problem in transplant patients worldwide and remains the major cause of death in those individuals [8].The urinary tract is the most common site of infections that occur after renal transplantation (35% to 79%) [15-17]. Appropriate management of candiduria in renal transplant recipients is important for patient and graft survivals [18].The incidence of candiduria among recipients of renal transplants is not clear, however identify agents and antifungal susceptibility for the treatment are important [18, 19].Treatment of candiduria depends on the clinical status of patients and individuals with symptomatic UTI and underlying diseases should be treated with appropriate antifungal drugs. This study demonstrates the frequency candiduria of 15.7 % of patients. In our study, candiduria were seen in women (54.54%) more than men (45.45%).This may be due to shorter urethra in women or anti-Candida properties of prostate fluid in men. None of our patients suffered from vulvovaginal candidiasis. In direct examination of urine samples, budding yeast cells were seen in only two (2.85%) cases of candiduria. This finding suggests that, negative direct examination does not rule out candiduria and both direct examination and culture should be done. It was also shown that the relatively large lipid contents in cell wall of some Candida species caused yeast cells to float in urine. Therefore, in our study urine samples were cultured before and after centrifugation. But, the cultures of urine sediments yielded much greater numbers of yeast colonies in comparison with whole urine samples. A urine sediment culture in this study also yielded whole urine cultures colonies the same. Some researchers believe that; 10^3cfu/ml is valuable for diagnosis of UTIs in patients without urinary catheter. In other researches, 10^4cfu/ml in patients with an indwelling catheter was considered as UTIs. However, urinary colonization has been reported as 10^4 to $\geq 10^5$cfu/ml. Therefore, unlike bacteria there is no standard colony counting for differentiation of UTI from urine contamination and usually, isolated colonies are interpreted depending on the patient's underlying factors. Although, in our study we could not certainly confirm infection based on colony counting, but the underlying diseases of the patients, including hematologic malignancy, renal failure and renal transplantation, emphasizes follow-up on them.

Consistent with the results of previous studies, the overall candiduria rate was high in our study group[16, 20]. This result is in agreement with other studies conductedthat female gender increased the risk to develop a candiduria among renal transplant recipients [15]. We investigated to know the prevalence of *Candida* species in renal transplantation recipients using mycological and PCR-RFLP method. A striking result of this study is that *C. albicans* 54% as predominant species followed by *C. glabrata* 27%, *C. krusei* 9% and *C. tropicalis* 9%.This result is in agreement with the study conducted by Febre *et al*. who isolated yeasts in 18.6% of patients with urinary catheters, and *C. albicans* (46.15%) the most frequently, followed by *Candida glabrata* (30.77%) and *Candida krusei* (7.7%), were isolated from urine specimens (21).Also, Ghahri *et al* [22] showed that candiduria prevalence in patients with urinary catheter 16.2%. *Candida albicans* (27%) wasthe most frequently, followed by *Candida tropicalis* (27.8 %), *Candida glabrata* (22.2 %), *Candida parapsilosis* (16.7 %) in patients with urinary catheter use were identified by PCR-RFLP.In contrast the study by Fakour *et al* [23] and Delgado *et al* [15] showed *C glabrata* was the most frequently, followed by *C albicans*, *C krusei,* and *C tropicalis* in renal transplantation. This could be related to different populations of patients, variation in hospital setting and different geographic regions. Molecular diagnostic provide a rapid and frequently highly discriminatory means of identifying infectious organisms. In the present study, we apply mycological and molecular (PCR-RFLP) methods to identify of the medically important *candida* species and in both, the same results were obtained.

CONCLUSION

Candiduria in renal transplant patients may represent urinary tract infection and requires early diagnosis and treatment. It is difficult to differentiate urinary infection from colonization and candiduria caused by drug resistant non-albicans Candida species should also be considered

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