Original Article

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Study of Colonisation Pattern and Antifungal Sensitivity Profile of *Candida* Species in Diabetic Patients

Internal Medicine Section

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ABSTRACT

Introduction: In recent times, various studies have pointed out the rise in prevalence of *Candida* species causing Urinary Tract Infections (UTIs). Candiduria appears to be underdiagnosed entity and thus has been the source of morbidity and mortality. Emergence of drug resistant *Candida* species has further complicated the patient management.

Aim: To understand the colonisation pattern and antifungal sensitivity profile of *Candida* species in diabetic patients.

Materials and Methods: This cross-sectional study was conducted for a period of six months (July 2019 to December 2019) at a tertiary care teaching centre of southern Haryana, India. A total of 360 urine samples were collected from patients admitted both out-patient and in-patient. Glycated Haemoglobin (HbA1c) ≥6.5% was taken as having diabetes. The urine samples were collected as per standard guidelines. Urine wet mount examination was performed. Confirmation of identification and antifungal susceptibility testing was done for all the *Candida* isolates. Data was analysed using statistical software.

Results: The prevalence of candiduria was 65 (18.1%) among study subjects. Out of significant candiduria patients 19 (29.23%) were having *Candida albicans*, 30 (46.16%) were *Candida tropicalis*, 10 (15.38%) were *Candida krusei*, 6 (9.23%) *Candida glabrata*. *Candida albicans* was found to be sensitive to amphotericin-B in 100% of cases. Sensitivity to flucytosine, voriconazole and fluconazole was found to be 89.47%, 89.47% and 84.21% respectively in case of *Candida albicans*.

Conclusion: The prevalence of candiduria is definitely high in this region. Non albicans *Candida* species are more resistant to antifungal drugs compared to *Candida albicans*.

Keywords: Colony, Diabetes mellitus, Susceptibility, Urinary tract infection

INTRODUCTION

Diabetes mellitus is a common and frequently encountered health problem among patients, many of them present with UTIs. In past 2-3 decades, various studies have pointed out the rise in prevalence of *Candida* species causing UTIs. *Candida* species is found to be responsible for 10-15% of nosocomial UTIs in Indian scenario [1]. Not all cases of candiduria are diagnosed and reported hence it remains underdiagnosed entity and thus causes morbidity and mortality [2]. All *Candida* species has potential of causing UTIs but as per the literature non *albicans Candida* species have replaced *Candida albicans* as the predominant pathogen [3].

Candida species are commensal under normal conditions but turns to pathogenic microorganism as defence system and mechanisms becomes weaker and may cause various kinds of infections in the body of host [4]. Environmental conditions in the urinary tract of humans usually favour the growth of *Candida* species as observed in the literature [5]. As per the available literature, higher susceptibility of fungal infections (including *Candida* species) to the the urinary tract of the host, is attributed to higher levels of urine glucose concentration in the host having diabetes [6]. Pathogenic bacteria and fungi grow easily in any environment with raised levels of glucose concentrations.

Over a period of time irrational (prolonged and inappropriate) use of empirical therapy resulted in emergence of drug resistant *Candida* species that has further complicated the patient management. With this background, the present study was carried out to understand the colonisation pattern and antifungal sensitivity profile of *Candida* species in diabetic patients.

MATERIALS AND METHODS

The present cross-sectional study was planned and rolled out under the aegis of Department of General Medicine in close collaboration of Department of Microbiology at a tertiary care teaching medical college situated at a lone aspiration district Nuh of southern Haryana, India. Study was conducted for a period of six months July 2019 to December. 2019. The study was instituted only after obtaining necessary clearance from Institutional Ethics Committee (IEC) of the medical college vide letter number SHKM/CM/2016/901 Dated 23.02.2016.

A total of 360 urine samples were collected from patients admitted both outdoor and indoor. Written informed consent was obtained from all the study subjects.

Inclusion criteria: Diabetic patients of both sexes and all age groups were considered for this study. HbA1c ≥6.5% was taken as having diabetes. Both OPD and IPD patients having urinary tract infections were included. Microbiologically, samples showing pure growth of yeast isolates with significant colony count were also included.

Exclusion criteria: Those urine samples in which *Candida* species was isolated but was not there, were excluded from this investigation. Similarly, those urine samples in which *Candida* with colony count was ≤1000 Colony Forming Unit (CFU)/mL and showing mixed growth (polymicrobial growth) were also excluded.

Sample size calculation: The calculation of sample size was done (n=360) considering the prevalence of Candida species causing vaginitis as 64.5% [7], with confidence level of 95% and 5% absolute error by applying the following formula: $n=(Z^{1-a/2})^2 \times p(1-p)/d^2$; where Z=Standard normal variate, a=Level of significance (0.05), p=Prevalence, d=Absolute allowable error (5%), n=sample size.

Study Procedure

The urine samples were collected as per standard guidelines [8]. All samples were collected in containers that were sterile, leak proof and had screw capped lids. Samples were shifted to microbiology laboratory with no delay. Urine wet mount examination was performed. After that, urine samples were used for culture. Urine culture was done as per standard protocol [9]. Samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) and Mac Conkey agar. Incubation of culture plates was done at 37°C for

24-48 hours. *Candida* species isolated on culture plates with colony count >10000 CFU/mL were taken as significant [10].

Candida species were identified by inoculating isolates on Chromogenic (CHROM) agar. This was incubated for approximately 48 hours at 30°C. Observations were made as per manufacturers instruction [11]. The identification of colonies was done on the basis of chromogenic reaction. *Candida albicans* was identified with Light green colonies on the CHROM Agar. Similarly *Candida tropicalis* was identified with blue colonies with pink halo, *Candida glabrata* was identified with cream to white colonies and *Candida krusei* was identified with purple fussy colonies on the CHROM Agar [12].

After that, confirmation of identification and antifungal susceptibility testing was done for all the *Candida* isolates using Vitek-2 compact system of biomerieux. The antifungal susceptibility was looked for amphotericin B, flucystosine, voriconazole, and fluconazole.

STATISTICAL ANALYSIS

Any possible personal identifiers were delinked with the data before the analysis. Data collected was entered in Microsoft excel 7, and then data was analysed using Statistical Package for the Social Sciences (SPSS) 22.0 statistical software. Descriptive values were expressed as mean and Standard Deviation (SD). Categorical variables were written as the numbers of cases and percentage value.

RESULTS

A total of 65 *Candida* species were isolated from 360 urine samples thus the prevalence of candiduria was 18.1% among study subjects. Out of significant candiduria patients, different species showed different pattern [Table/Fig-1].

Candida species	Number	Percentage
Candida albicans	19	29.23
Candida tropicalis	30	46.16
Candida krusei	10	15.38
Candida glabrata	6	9.23

[Table/Fig-1]: Pattern of *Candida* species observed in study subjects (N=65).

Considering the age and sex distribution maximum percentage of significant candiduria was seen in age group 31-60 yrs of age (50.76%), with male being 33.84% and females being 16.92%. Followed by age group of greater than 60 years (30.77%), Male were 18.46% and females were 12.30%. In age group 1-30 years total of 18.46% were having significant Candiduria, Male 9.23% and Females 9.23% [Table/Fig-2].

Age group (years)	Male n (%)	Female n (%)	Total n (%)
1-30	6 (9.23)	6 (9.23)	12 (18.46)
31-60	22 (33.85)	11 (16.92)	33 (50.77)
>60	12 (18.46)	8 (12.31)	20 (30.77)
Total number of isolates	40 (61.54)	25 (38.46)	65 (100.00)

[Table/Fig-2]: Distribution of *Candida* species as per age and gender of study subjects (N=65).

Candida albicans was found to be sensitive to amphotericin B in 100% of cases. Sensitivity to flucytosine, voriconazole and fluconazole is shown in [Table/Fig-3].

In clinical isolates of *Candida tropicalis* sensitivity to amphotericin B, flucytosine, voriconazole and fluconazole is shown in [Table/Fig-3] respectively. Antifungal sensitivity pattern in case of *Candida krusei* with amphotericin B, flucytosine, voriconazole and fluconazole is shown in [Table/Fig-3] respectively. Antifungal sensitivity pattern of *Candida glabrata* with amphotericin B, flucytosine, voriconazole and fluconazole is shown in [Table/Fig-3] respectively.

DISCUSSION

More than 150 varieties of *Candida* species are currently existing around us as saprophytes. Majority of them are harmless, only a few are pathogenic for human beings. *Candida* infections are frequently encountered in the patients having diabetes mellitus. Present study observed a total of 65 *Candida* species isolated from 360 urine samples thus the prevalence of candiduria was 18.1% among study subjects. This was in contrast to the study from Madhya Pradesh that which observed the prevalence of candiduria as 65% among diabetic patients [7]. On the other hand, another study from Bihar reported the prevalence of candiduria as 11.25% [13].

Authors observed higher prevalence of candiduria in this investigation. It is really important to note the rising burden of non *albicans Candida* species. This is a matter of concern that needs to be addressed [14]. Certain factors are considered responsible for such rising trend of candiduria, a few of them are prolonged antibiotic therapy, prolonged hospital stay, immunocompromised status of patient, immunosuppressive therapy, catherisation, etc [15]. Process of catheterisation involves migration of microbes from outer surface into the urinary bladder thus increase the chances of episodes of UTIs. Emergence of resistance for *Candida* indiscriminate is attributed to irrationale use of antifungal drugs, especially azole group [16]. Immunocompromised state and critically ill patients are at high risk of developing candidemia [17].

Present study observed the isolation rate of non *albicans Candida* was 70.77% i.e. 46.16% were *Candida tropicalis*, 15.38% were *Candida krusei*, 9.23 % *Candida glabrata*. Various scientific reports have proved the rise in prevalence of non *albicans Candida* species among candiduria. Another study by Kumari KS et al., was also in concordance with present study observations [18]. They observed that, of 500 urine specimens, 66 (13.2%) yielded *Candida* isolates whereas among the 66 *Candida* species isolated from urine, 43 were non-*albicans Candida* species and 23 were *Candida albicans*.

Regarding antifungal sensitivity profile and pattern of *Candida* species among study subjects, this study noted that *Candida albicans* was found to be sensitive to amphotericin B in 100% of cases. Sensitivity to flucytosine, voriconazole and fluconazole was found to be 89.47%, 89.47% and 84.21% respectively in case of *Candida albicans*. Identification of *Candida* species is important as *non albicans Candida* are more resistant to azoles compared to that of *Candida albicans*.

Candida krusei is intrinsically resistant to fluconazole. Present study also observed that antifungal sensitivity pattern in case of *Candida krusei*, antifungal sensitivity pattern with amphotericin B, flucytosine, voriconazole and fluconazole was 70%, 70%, 50% and 40% respectively. Antifungal sensitivity pattern of *Candida glabrata* with amphotericin B, flucytosine, voriconazole and fluconazole

	Amphotericin B			Flucytosine			Azoles					
							Voriconazole			Fluconazole		
Candida species	S (%)	I	R	S (%)	I	R	S (%)	I	R	S (%)	I	R
Candida albicans	19 (100)	-	-	17 (89.47)	1 (5.26)	1 (5.26)	17 (89.47)	2 (10.53)	-	16 (84.21)	1 (5.26)	2 (10.53)
Candida tropicalis	26 (86.66)	2 (6.67)	2 (6.67)	20 (66.67)	6 (20)	4 (13.33)	22 (73.33)	5 (16.67)	3 (10)	21 (70.00)	3 (10)	6 (20)
Candida krusei	7 (70.00)	2 (20)	1 (10)	7 (70.00)	1 (10)	2 (20)	5 (50.00)	4 (40)	1 (10)	4 (40.00)	3 (30)	3 (30)
Candida glabrata	5 (83.33)	1 (16.67)	-	3 (50.00)	2 (33.33)	1 (16.67)	3 (50.00)	2 (33.33)	1 (16.67)	4 (66.67)	2 (33.33)	-

[Table/Fig-3]: Antifungal sensitivity profile and pattern of *Candida* species among study subjects.
S=susceptible, I=intermediate, R=resistant

was 83.33%, 50%, 50% and 66.67% respectively. Antifungal susceptibility shows that *Candida* isolates were more susceptible to amphotericin B and flucytosine as compared to azole group. Clinicians commonly use azole group to treat candiduria thus increasing to fluconazole is noteworthy and challenging to us. Present study observed that *Candida albicans* were found to be more susceptibility to azole group compared to non *albicans Candida*. The result of this study was in agreement with previous study by Pramodhini S et al., [19]. Understanding the colonisation pattern and antifungal sensitivity profile of *Candida* species are important for planning the management of such subjects. This adds to the strength of study investigation.

[Table/Fig-4] summarises the pattern of colonisation and sensitivity pattern as observed by various authors from different parts of India [13,20-23].

Name of author, place of study [reference no]	Year of publication	Sample size	Highest colonisation	Highest sensitivity pattern
Prakash A et al., Muzaffarpur [13]	2020	400	*C. glabrata, C. albicans*	-
Prakash V et al., Bareilly [20]	2018	4192	*Candida tropicalis*	Fluconazole, Voriconazole
Ponnambath DK et al., Coimbatore [21]	2017	101	*C. albicans*	Amphotericin B, Caspofungin, Flucytosine
Gupta S and Goyal R Bareilly [22]	2017	89	*C. tropicalis, C. albicans*	Fluconazole, Voriconzole, Caspofungin
Shaik N et al., Guntur [23]	2016	150	*C. tropicalis*	Amphotericin B, Nystatin, Voriconazole,
Present study	2022	360	*Candida tropicalis*	Amphotericin B

[Table/Fig-4]: Summary of colonisation and sensitivity pattern of *Candida* species [13,20-23].

Limitation(s)

Due to financial and logistics constraints, authors could not perform comprehensive analysis of urine as samples of polymicrobial growth and pyuria were excluded.

CONCLUSION(S)

The findings of this study shows that the prevalence of candiduria is definitely high in this region. Various species of *Candida* are responsible for causing UTI. Information on antifungal susceptibility pattern will help clinicians for better management of patients. Non *albicans Candida* species are more resistant to antifungal drugs compared to *Candida albicans*. Better understanding of colonisation pattern and antifungal sensitivity profile shall definitely help for better management of candiduria.

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