

# Antifungal Drug Susceptibility of Oral Candida Species Isolates in Chronic Renal Failure Patients with Type 2 Diabetes Mellitus

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## Abstract

**Background:** Chronic renal failure (CRF) patients with superseded diabetes are immune-compromised and are prone for oral candidal infection. **Aim:** To study the Antifungal drug susceptibility of oral candida species isolates in chronic renal failure patients with type 2 diabetes. **Material And Methods:** A total of 98 individuals including 73 cases of chronic renal failure with type 2 diabetes mellitus and 25 healthy individuals as controls. The diabetic patients were divided into 3 groups according to their glycemic index; 22 controlled diabetes (HbA1c  $\leq$ 5%), 27 moderately controlled (HbA1c 5-7%) and 24 Uncontrolled diabetes (HbA1c  $\geq$ 7%). Salivary samples were collected in as sterile container with phosphate buffered saline and then immediately transported to various mycological investigations and antifungal susceptibility tests. **Results:** There was significant difference in incidence of candida species in uncontrolled diabetes when compared to moderately controlled, controlled and normal patients ( $P < 0.05$ ). The higher number of colony count was seen among uncontrolled and moderately controlled diabetes than controlled and healthy subjects. *Candida albicans* is the most prominent species among the groups. *C. albicans* showed increased resistance to Amphotericin B and fluconazole in diabetic patients in comparison to control group. Other species showed variable sensitivity patterns. **Conclusion:** An accurate identification of oral candida species and their drug susceptibility, in chronic renal failure with type 2 diabetes patients mandates proper treatment to avoid recurrence and drug resistance.

**Keywords:** Antifungal susceptibility, candida species, Type 2 Diabetes, Fluconazole, Amphotericin B.

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## INTRODUCTION

The prevalence of chronic renal failure with diabetes mellitus is becoming a major public health problem with high number of cases in India. Chronic renal failure patients with superseded diabetes are prone for oral candidal infection [1]. These patients are immuno-compromised and their salivary flow tends to slow. When the immune system is suppressed, microbial agents of normal oral flora have an opportunity to become pathogenic, which causes infection and destruction of the oral cavity. A depressed immune system with diabetes predisposes opportunistic infection by candida species. The transition of candida from commensal to pathogen is often associated with predisposing factors like prolonged antibiotic use, viral infections, nutrition, high salivary glucose aid the overgrowth of candida in the oral cavity [2].

*Candida albicans* is the most common pathogen, however there is an increase in non *albicans* in immunocompromised patients [3]. It is important to

accurately identify the candida species because of their specific resistance to antifungal drugs. Antifungal drugs mainly azole groups such as itraconazole, ketoconazole are used as initial drugs in the treatment of fungal infections. However there is difficulties in complete eradication of fungus owing to their resistance due to genetic differences among fungal species or overuse of azole drugs [4]. Drugs like amphotericin B and fluconazole are used in treatment of critically ill and immunocompromised patients. An attempt was made to study the Antifungal drug susceptibility of oral candida species isolates in chronic renal failure patients with type 2 diabetes.

## MATERIAL AND METHODS

### Study Design

This study was conducted at Department of Oral Pathology, Vydehi Institute of Dental sciences and Research Centre, Bangalore. Patients who were clinically diagnosed cases of chronic renal failure with diabetes reported to the department of Nephrology at Vydehi medical college hospital, Bangalore were

considered for the study. Ethical clearance was obtained from the institutional ethical committee for human experimentation as per standard guidelines and informed consent was obtained from all the individuals.

It is a hospital based case control study consisting of 98 individuals including 73 cases of Chronic renal failure with type 2 diabetes mellitus and 25 healthy individuals, age and sex matched individuals were randomly selected as controlled group. Detailed Oral examination of all the individuals were carried out using diagnostic instruments. Demographic data as well as details such as fasting blood sugar level, post prandial blood sugar, glycated hemoglobin level (HbA1c level), duration of the disease and medication taken were recorded. The patients of diabetes were divided into 3 groups according to their glyceamic index; 22 controlled diabetes (HbA1c  $\leq$ 5%), 27 moderately controlled (HbA1c 5-7%) and 24 Uncontrolled diabetes (HbA1c  $\geq$ 7%). Patients subjected to radiotherapy / chemotherapy, Patients on any long term medications like systemic corticosteroids, antibiotics, and immunomodulants. Patients wearing dentures were excluded from the study.

### Microbial Sampling

#### Collection of Unstimulated whole saliva- Navazesh's method [5-7]

The subjects were asked to refrain from intake of any food, beverage, smoking or chewing gum for one hour before the sample collection. The subjects were seated on a dental chair, asked to rinse their mouth with distilled water and then to relax for five minutes. They were instructed to minimize movements and asked to lean the forehead over a funnel and a test tube kept below it. The subjects were asked to keep their mouth slightly open and allow saliva to drain into the tube for five minutes.

### Culturing for Candida

The collected salivary sample was centrifuged at 2000 rpm for 15 minutes and the sediment was obtained for fungal culture. A loop of sample was inoculated on SDA plates and incubated at 37<sup>0</sup> C. The colonies were periodically checked for the growth at 0, 12, 24, 48 and 72 hours. Gram stain and Germ tube testing. The colonies grown on the plates were subjected to Grams stain. The species which showed positivity to candida were again subjected to germ tube test. Germ tube test: Germ tube test is the rapid screening procedure for observing germ tubes formation, identifies and differentiates *C. albicans* from other candida species. The culture of candida species was treated with 1 ml of sterile mammalian (fetal- bovine, sheep or normal human) serum & incubated at 37<sup>c</sup> for 2-4 hours. After incubation, a drop of suspension was examined on the glass slide under the microscope for the presence of germ tube.

1ml of human serum taken in a test tube + 1 loop of candida colony were mixed and incubated for 2 hours at 37<sup>0</sup> Celsius. A drop of it is taken on sterile slide and coverslip is placed and observed under microscope for presence of germ tube [10-14].

### Candida species isolation and Identification

The fungal colonies that showed positivity for germ tube formation were streaked on CHROM agar plates by using sterile plastic inoculating loops for candida species differentiation based on color and morphology was done. The colonies were periodically checked for the growth at 0, 12, 24 and 48 hours. Identification of colonies: *Candida albicans* -Green colonies, *Candida kruzei* - Pink colonies, *Candida Tropicalis* - Purple or blue colonies.

### Counting of Candida Colonies

The number of colonies thus obtained were counted and quantitatively expressed as number of colonies obtained per ml of saliva. i.e. Colony Forming Units/ml (CFU) [1].

### Biochemical Tests

#### Glycated hemoglobin HbA1c

The HbA1c levels were determined by the borate affinity assay (Nycocard, Axis –shield PoC AS, Norway) Briefly 2 ml of blood was collected in an ethylenediamine tetracetic acid tube and HbA1 c levels were assayed as per instructions supplied with the kit [8].

### Antifungal susceptibility

The clinical samples of saliva and obtained from the chronic renal failure patients with type 2 diabetes were subjected to antifungal sensitivity. The susceptibility of the candida isolates to antifungal agents was determined by the disk diffusion method with azoles and polyenes drugs [9].

### Statistical Analysis

Data obtained were computed on Microsoft excel sheet. Statistical analysis was carried by using Statistical package for social sciences (SPSS version 17.0, Chicago, USA) using Chi-square has been used to find the significance of study parameters on categorical scale between the groups. P value: <0.05 was considered statistically significant.

## RESULTS

The study population of consisted 98 patients with 76 males and 22 females. Their age varied from 43 years to 64 years with mean age of 53.11 $\pm$ 8.02 for uncontrolled diabetes, 60.48 $\pm$ 7.02 for moderately controlled diabetes, 61.90 $\pm$ 6.91 for controlled diabetes and 64.08 $\pm$ 6.37 years for healthy controls. The glycated hemoglobin values varied from 5.5% to 14.1% (mean values=7.82  $\pm$ 1.65 %). There was significant difference (P<0.05) in frequency of candida in uncontrolled

diabetes (75%) when compared to moderately controlled (59.2%), controlled (40.9%) and normal patients (44%) (Table 1).

The higher number of colony count was seen among uncontrolled and moderately controlled diabetes than controlled and healthy subjects (Table 2). *Candida albicans* is the most prominent species among the groups (Figure 1).

The total number of case studied 98, but we could perform antifungal sensitivity with amphotericin

B and fluconazole testing to 49 cases only and rest of the cases we could not perform due non suitability of the tests. *C. albicans* showed increased resistance to Amphotericin B (72.5%) and Fluconazole (90%) in CRF with diabetic patients in comparison to healthy controls. *C. Krusei* showed increased sensitivity to Amphotericin B (66.6%) and fluconazole (83.3%) in CRF with diabetic patients and *C. Tropicalis* showed increased sensitivity for fluconazole (100%) and resistance to Amphotericin B (66.6%) (Table 3).

**Table 1: Correlation between the groups and presence and absence of candida**

Candidal growth	CRF with Diabetes			Healthy Controls (%)	p Value
	Uncontrolled diabetes (%)	Moderately controlled diabetes (%)	Controlled diabetes (%)		
Present	18 (75)	16(59.2)	9 (40.9)	11(44)	0.0042*
Absent	6 (25)	11(40.7)	13(59.01)	14 (56)	
Total	24 (100)	27 (100)	22(100)	25(100)	

P<0.05\* Significant

**Table 2: Correlation between the groups and colony forming units**

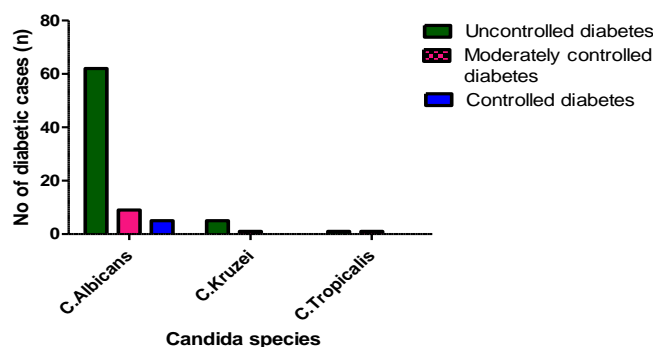
CFU/ml counts	CRF with Diabetes			Healthy controls n (%)	p value
	Uncontrolled diabetes n (%)	Moderately controlled diabetes n (%)	Controlled diabetes n (%)		
	(n=14)	(n=16)	(n=13)	(n=11)	
1-1000	0	0	1(7.6)	2 ( 18.1 )	0.0175*
1001-5000	2 (14.2)	6(37.5)	3(23.0)	4 (36.3)	0.0335*
5001-10,000	3(21.4)	4(25)	4(30.7)	5 (45.4)	0.0374*
>10,000	9(64.2)	6(37.5)	5 (38.4)	0	0.010*

\*Significant (p<0.05)

**Table 3: Comparison of antifungal drug susceptibility of candida species in CRF cases with type 2 diabetes mellitus and Normal healthy controls**

Antifungal agent	CRF with Diabetic (n=49)		Healthy Controls (n= 16)		P value
	S (%)	R (%)	S (%)	R (%)	
Amphotericin B					
<i>C. Albicans</i>	11(27.5)	29(72.5)	4(25)	12(75)	0.0024*
<i>C. Krusei</i>	4(66.6)	2(33.3)	0	0	-
<i>C. Tropicalis</i>	1(33.3)	2(66.6)	0	0	-
Fluconazole					
<i>C. Albicans</i>	4(10)	36(90)	16(100)	0	0.001*
<i>C. Krusei</i>	5(83.3)	1(16.6)	0	0	-
<i>C.Tropicalis</i>	3(100)	0	0	0	-

p<0.05 Significant\*



**Fig 1: Distribution of candida species in CRF with diabetic patients**

## DISCUSSION

*Candida* species account for 8–15% of all hospital acquired infections of which patients with diabetes are more prone to fungal infections. When the immune system is suppressed, microbial agents of normal oral flora have an opportunity to become pathogenic, which causes infection and destruction of the oral cavity [13-15]. The various species diversity poses a challenge in management of such cases owing to its variable susceptibility of the species to the antifungals. An efficient identification is of *Candida* species of *Candida* is a paramount important to successful treatment and complete eradication.

Diabetes mellitus is common in developed countries with higher occurrence in ages between 45 and 64 [16]. Our study showed the age ranges from 43 years to 64 years with mean age of 53.11±8.02 for uncontrolled diabetes, 60.48±7.02 for moderately controlled diabetes, 61.90±6.91 for controlled diabetes and 64.08±6.37 years for healthy controls. Higher prevalence of *Candida* carriage with diabetes was 64% as reported by Belazi *et al.*, [17]. And 87% was reported by Prem Kumar *et al.*, [18]. Our study revealed the glycated hemoglobin values varied from 5.5% to 14.1% (mean values=7.82 ±1.65 %). There was significant difference ( $P<0.05$ ) in frequency of *Candida* in uncontrolled diabetes (75%) when compared to moderately controlled (59.2%), controlled (40.9%) and normal patients (44%).

The present study showed the higher number of colony count was seen among uncontrolled and moderately controlled diabetes than controlled and healthy subjects. *Candida albicans* is the most prominent species among the groups. Similar findings were reported by Epstein *et al* who performed colony count of *Candida albicans* in saliva in which statistical analysis showed significant difference between colony count in saliva from patients with chronic candidiasis with diabetes ( $p<0.002$ ) which indicates individuals <400 CFU/ml carriers & those with >400CFU/ml were having acute or chronic candidiasis [19]. Rate of candidal growth significantly correlated with glycemic control was reported by Darwazeh *et al.*, [20].

Our study revealed *C. albicans* showed increased resistance to Amphotericin B (72.5%) and Fluconazole (90%) in CRF with diabetic patients in comparison to healthy controls. *C. Kruzei* showed increased sensitivity to Amphotericin B (66.6%) and fluconazole (83.3%) in CRF with diabetic patients and *C. Tropicalis* showed increased sensitivity for fluconazole (100%) and resistance to Amphotericin B (66.6%). Our findings were in agreement with study of Lyon *et al.*, [21]. In vitro antifungal susceptibility to azoles like fluconazole and polyene Amphotericin B

were performed on all positive isolates showed resistance to Amphotericin B and *C. albicans* was seen in disagreement to Yar Ahmadi *et al.*, [22] and Zomorodian *et al.*, [23]. This may be due to building up of sterol intermediates and mutation of ERG3 in resistant strains [18].

## CONCLUSION

An accurate identification of oral *Candida* species and their drug susceptibility, in chronic renal failure with type 2 diabetes patients mandates proper treatment to avoid recurrence and drug resistance. *C. albicans* species isolated and their susceptibility to antifungals in CRF with diabetes indicate special diagnostic and therapeutic management. Dental health practitioners should make patients aware of possible risk factors associated with poor oral health and should provide guidance for effective orodental care and oral hygiene.

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