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# Oral *Candida* colonization in renal disease patients between diabetes and non-diabetes; a comparative study

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## Abstract

**Introduction:** Oral colonization with fungi requires attainment from the oral atmosphere, attachment and growth replication but host defense acts to remove or kill invading fungi. This function is hampered in renal failure patients. This is the reason why in case of immune system defects, a rise in Candida colonization is seen. Oral candidiasis is a common opportunistic infection in immune-suppressed and immune-compromised patients.

**Objectives:** This study is planned to see the existence of Candida in different renal conditions with diabetic and non-diabetic etiology.

**Patients and Methods:** The study comprises a total of 45 patients, which includes 15 chronic kidney disease (CKD) patients, 15 end-stage renal disease (ESRD) and 15 renal transplanted patients. Each group is further divided as diabetic (group 1) and non-diabetic (group 2). Whole saliva samples were cultured on Candida chrome agar.

**Results:** Each group showed positivity for *Candida* species with the highest positivity of a total of 67% in CKD group. The diabetic group (group 1) showed 64% positivity and non-diabetic group (group 2) showed 55% positivity respectively.

**Conclusion:** Immunosuppression states like CKD, ESRD and renal transplant recipients are associated with increased risk of oral *Candida* colonization and diabetic further worsens this occurrence.

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## Introduction

Oral cavity is a reflection of the systemic health of the person (1). Systemic diseases like diabetes are associated with insulin deregulation, which has shown to have direct as well as indirect effect on oral cavity with increased rate of infections (2). Oral cavity may also give a clue to underlying disease like renal failure with altered salivary composition and periodontal related diseases even though it is not in proximity to oral cavity (3). The presence of both these diseases increases the likelihood of oral manifestations (4). Nowadays, the incidence of the renal diseases like chronic kidney disease (CKD) and end-stage renal disease (ESRD) is rising probably due to increased health awareness and availability of simple laboratory tests to diagnose renal failure (5). Diabetes is expanding like a pandemic and diabetes itself is a major reason for CKD (6). Consequently the incidence of CKD and ESRD is rising at a faster pace (7). CKD and ESRD diminish immune system and thus it is a great risk factor for infections. Alterations in immune system in CKD explained as, sequential mechanisms like defects in effector T-cell, antibody production, antigen recognition and presentation (8,9). Diabetes decreases cellular immune response which explains the dampening of immune system. Diabetes state increases adhesion to mucosal surfaces, decreased cellular immunity which can lead to spread of infection by Candida

**Key point**

This study showed the prevalence of *Candida* in 3 groups. As the prevalence is more in transplant group, clinicians should have a low threshold to diagnose in transplant patient that ultimately leads to early initiation of treatment and thus morbidity and mortality can be improved in long run.

some time masking the signs and symptoms (10,11). Additionally CKD due to diabetic etiology further increases the risk of infections (12). Though in renal transplantation immunity regained to some extent, this effect is offset by administration of immunosuppression medications to salvage graft (13,14). Fungal infections are rare in healthy persons and mostly they occur as opportunistic infections (15). CKD, ESRD, transplantation and diabetes provide a perfect environment for these opportunistic infections (16). The Candida organisms are normal commensals in more than 30- 50% of healthy population resides in oral cavity without clinical evidence of infection (17). The normal host system has particular defense mechanisms which will prevent growth by inhibiting colonizing in the oral cavity further preventing infection. The immune compromised states like CKD and diabetes can convert this organism as commensal to pathogenic state (18).

## Objectives

To compare the abundance of Candida species in diabetic and non-diabetic individuals with underlying renal disease state.

## Patients and Methods

### *Study population*

The present study is an observer open labeled study conducted in a period of five months from November 2015 to March 2016 with the approval of college ethical committee. The study was designed to compare the three groups by associating the Candida growth. The study comprises of a total of 45 samples divided in to three groups. Each group contained 15 samples. Group 1 consisted of patients who are diagnosed as CKD. Group 2 consisted of ESRD patients who were on hemodialysis for at least 4 months. Group 3 consisted of individuals who had successfully undergone kidney transplantation (renal transplanted recipients) with restored normal kidney function. Each group is further divided in to two subgroups as diabetic and non-diabetic patients. Each sample comprises of whole saliva collected in sterile containers, and cultured to check the positivity of Candida using Candida chrome agar. Demographic data was also collected as a part of the study with exclusion criteria as the patient receiving anti- fungal medication. All patients were prospectively recruited from outpatient clinics, from department of nephrology in a tertiary care hospital in south India.

### *Methods*

Whole saliva sample was collected in sterile bottles for culturing. Streaking was done with 0.5 mm loop on CHROMagar candida. These cultures were incubated for 48 hours to 72 hours at 37°C for isolation of Candida species. After 72 hours colonies were identified as *Candida albicans* (green), *Candida tropicalis* (metallic blue) and *Candida krusei* (pink), by observing color and morphology of the colony. Cultures showing 1-5 colonies were considered as negative and above five colonies were considered positive. The cultures positivity were confirmed by doing grams staining for *Candida* species. The cultures which have not shown positivity within 72 hours were considered as negative.

### *Ethical issues*

The study protocol was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of PMVIDS (PMVIDS & RC/IEC/OMFP/PR/0056-15). All participants gave their informed written consent to enter the study.

### *Statistical analysis*

Relative risk or risk ratio (RR) was used to calculate the ratio of the probability of an event occurring in diabetic group to the probability of the event occurring in a comparison, non-diabetic group. Quantitative variables were expressed as percentages, frequencies and odds ratio. The odds ratio represents the odds that an outcome will occur given a particular exposure (diabetes with underlying renal condition), compared to the odds of the outcome occurring in the absence of that exposure (non-diabetic with underlying renal condition).

## Results

We monitored 45 patients with renal diseases to test for association between *Candida* and diabetes. Renal patients were monitored mainly CKD, ESRD, renal transplant recipients (Table 1). Based on culture growth, cases were classified as *Candida* positive and *Candida* negative. We further analyzed by classifying the groups as group I (diabetes positive) and group II (diabetes negative) with the presence of *Candida* or absence of *Candida* presented in Table 2.

On statistical analysis of relative risk ratio towards diabetes positive group was 1.1723 in comparison with diabetes negative group. The statistical output of2 x2 contingency with risk ratios was presented in Table 3. To find the relative risk of candidiasis depending on the presence of diabetes with CKD, we found the risk ratio of *Candida* positive is 1.774 between diabetes and non-diabetic group. This high risk ratio is an indication that the presence of *Candida* is probably higher in diabetes patient.

## Discussion

The oral cavity in healthy individuals contains number of different bacterial, viral, and fungal species most of these live in an environment called the "oral ecosystem". These micro-flora live as commensal species, which are essential for survival of human beings (19). During immunosuppressive state, oral ecosystem is disturbed and the microflora become pathogenic in responses to changes in the environment or other triggers in oral cavity, including an individual's personal hygiene (3). The mode of microbial contribution to health and disease is an interesting phenomenon. During immunosuppressive

**Table 1.** Sample distribution with Candida positive cases

| | Diabetic | Candida positive | Non-diabetic | Candida positive |
|---|---|---|---|---|
| Transplanted kidney | 3 | 2 | 12 | 10 |
| CKD | 7 | 6 | 8 | 4 |
| ESRD | 4 | 1 | 11 | 3 |
| Total | 14 | 9 (64%) | 31 | 17 (**55%**) |

**Table 2.** Positive percentages in groups 1 and 2

| | Diabetic group 1 | | | Non-diabetes group 2 | | | Group 1 + group 2 |
|---|---|---|---|---|---|---|---|
| | Candida negative | Candida positive | Percent | Candida negative | Candida positive | Percent | Candida positive percent |
| Transplant | 1 | 2 | 67% | 2 | 10 | 83% | 80% |
| CKD | 1 | 6 | 86% | 4 | 4 | 50% | 67% |
| ESRD | 3 | 1 | 25% | 8 | 3 | 27% | 27% |
| Total | 5 | 9 | 64% | 14 | 17 | 55% | 58% |

**Table 3.** Comparison of absent and present in two groups in a 2x2 contingency table with risk ratios

| | Absent | Present | Total | Rate | Risk ratio |
|---|---|---|---|---|---|
| Group 1 | 5 | 9 | 14 | 0.6429 | 1.1723 |
| Group 2 | 14 | 17 | 35 | 0.5484 | |
| Total | 19 | 26 | 45 | | |

conditions, fungal organisms switch from harmless commensal to a pathogenic organism (17). This change is intricately related to variation in host environment that lead to expression of a range of virulence factors. Among the fungi, Candida albicans is the most commonly found species. Various factors are associated with promoting the infection (15), consisting adhesion of Candida to oral surfaces and disturbances in cellular and adaptive immunity (20). Conditions such as renal failure and diabetes act as perfect nidus for these organisms because of the imbalance created in specific immune mechanisms. Imbalances are seen as alterations in the activation of macrophages by neutrophils (18). The normal host immune system fights against Candida by two primary mechanisms which are dehydration of cells as they become mature and continuous shedding of superficial layers which prevent the colonization of these organisms (21). The ability of Candida to resist host immune defense mechanism and release of hydrolytic enzymes that can induce damage to host cells are not clearly known. However, it is suggested that uremic environment, altered biochemistry of blood and change in the salivary composition may impact the virulence. The key danger recognition pathways in innate immune cells are toll like receptors expressed on neutrophils, monocytes, and antigen presenting cells which bind to pathogen-associated molecular patterns (PAMPs) and recognize fungi (18). This recognition triggers the signaling pathways to induce cytokines that lead to differentiation of Th1 and Th17 CD4+ T cells responsible for anti-fungal immunity. These cells produce pro-inflammatory cytokines such as INF-gamma and IL-17 which recruit and activate phagocytes to kill fungi (15,18). These pathways were also found to fail in diabetes. It is a common knowledge that immunosuppression leads to opportunistic infection. Dongari-Bagtzoglou et al (22) conducted on the prevalence of Candida in kidney and heart transplant subjects. They concluded that prevalence (75%) of oral candidiasis is higher in renal transplanted patients compared to normal population. They highlighted the underlying role of immunosuppression in renal transplanted patients in Candida growth. López-Pintor et al (23) conducted a study to analyze the prevalence and risk factors of oral candidiasis in a group of renal transplant recipients. Their study showed a lower prevalence of oral candidiasis in renal transplant patients than previous reports, appeared as 79% of oral cavity of cases. However, they stressed the significance of periodic pre-transplant and post-transplant oral health and denture maintenance to prevent infection. Our results are in accordance with the above two studies with positivity showing 80% in the transplant group. The similarity could be contributed to the immunosuppressive medication given to sustain graft tissue.

The importance and role of various systemic and local factors involved in promoting Candida infection were brought into spotlight by Kumar et al (24), who conducted a study on the influence of diabetic type I and type 2 on oral candidiasis. Their study concluded that Candida in the oral cavity was higher in diabetic subjects than in non-diabetic subjects with an estimated prevalence rate of 69% in type II. Our study results are slightly similar with 64% in diabetic individuals in the Hada et al (25), conducted a study on the influence of diabetes on renal damage with focus on ESRD. The study showed 75% people showed

positive for the existence of candida. They found, renal failure brings certain oral changes which may be due to underlying diabetic uremia. Our study is in contrast with this study with least positivity of 25%in ESRD group. The reasons for difference can be explained as ESRD group on our sample is under strict medication with controlled diabetic individuals.

As the aforementioned studies indicate the role of host in promoting Candida infection with underlying various systemic illnesses we intend to verify the variation in prevalence of Candida among patient suffering from various stages of renal failure confounded by diabetes. In our study, we found the higher prevalence of Candida in patients suffering diabetic group (64%) compared diabetic free individuals (55%) with a risk ratio of 1.173. Among the renal failure groups, the highest risk was associated with CKD followed by transplant group though immunosuppression was pounced among transplant patients. This is in contrast to the argument that higher immunosuppression leads to severe opportunistic infections. This higher prevalence could indicate that increased association of diabetes with CKD promotes Candida growth.

Other possible reasons for variation in prevalence could be difference in geographical location, administration of immunosuppressive drugs in transplant recipients and difference in prevalence in diabetic as compared to our study. In our study diabetic group has shown increased colonization in culture growth. In fact, few studies which are resembling our study with few dissimilarities in sample size, grouping, and the method of investigating and different geographical groups. There are few differences in information on Candida carriage in diabetic patients, while are often conflicting too. This condition possibly because of the various techniques that have been employed and also to the dissimilarities in patients and control populations chosen by various investigators.

## Conclusion

Immunosuppression states like CKD, ESRD, and transplant recipients are associated with increased risk of oral Candida colonization and diabetic condition further exacerbates this phenomenon. Using non- invasive techniques like cultures are beneficial to the patients and clinicians for periodic evaluation. These results should be confirmed by studies involving larger sample size and longitudinal observations.

## Limitations of the study

The low proportion of patients is a limitation of our study. We suggest larger investigation on this subject of renal failure patients.

### Conflicts of interest

There is no conflict of interest to be declared regarding the manuscript.

### Authors' contribution

All authors equally contributed to design the research and contributed significantly to the scientific revision of the manuscript. All authors read and approved the final manuscript.

### Conflicts of interest

The authors declared no competing interests.

### Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by authors.

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