

A Study to Evaluate *Candida albicans* at Pre and Post Insertion Stages of Removable Dentures in Diabetics and Non Diabetics – An in Vivo Study

Running Title: A study to evaluate *Candida albicans* at pre and post insertion stages of removable dentures in diabetics and non diabetics

Dr. Keerthi R¹, Dr. Anil Kumar Gujjari², Dr. Sushant A Pai³, Dr. Sowmya S⁴, Dr. Smitha V Shetty⁵, Dr. Sukanya Abigail⁶

¹Reader, Department of Prosthodontics, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Bangalore

²Professor, Department of Prosthodontics, JSS Dental College and Hospital, Mysore

³Professor & Head, Department of Prosthodontics, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Bangalore

⁴Senior Lecturer, Department of Prosthodontics, JSS Dental College and Hospital, Mysore

⁵Reader, Department of Orthodontics, M R Ambedkar Dental College and Hospital, Bangalore

⁶PG Student, Department of Prosthodontics, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Bangalore

Abstract: Introduction: A considerable proportion of the population carries detectable numbers of yeast in the mouth; very few of those people suffer from oral *Candida* infection. The adhesion of microorganisms (yeasts) to a denture surface is a prerequisite for colonization. Aims: The Present study was done to evaluate the presence of *Candida albicans* in diabetic and non-diabetic first time denture wearers at pre and post insertion stages of complete dentures and transitional acrylic removable partial dentures at varying time periods of 0, on the 15th day and on the 30th day of insertion of removable dentures. Materials and Methods: Twenty completely edentulous and twenty partially edentulous patients aged between 50 to 70 years were selected. Each group constituted of 10 diabetic and 10 non diabetic patients. The following parameters were recorded at the appropriate time: fasting blood glucose and post prandial blood glucose. The culture swabs were taken and were inoculated on to Sabouraud's dextrose agar medium, incubated and then subjected to evaluation of *Candida albicans*. Results: There was no evident growth of *Candida albicans* species in both complete denture and partial denture wearers irrespective of their diabetic status for the study period of 30 days. Conclusion: The results of this study showed that elderly diabetic patients who were first time transitional acrylic removable partial denture wearers were more susceptible to Candidal infections than the elderly non diabetic partial denture wearers.

Keywords: *Candida albicans*, Colonization, Diabetic, and Non-diabetic.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia, associated with irregularities in the metabolism of carbohydrates, lipids, and proteins. This type of diabetes generally occurs after the age of 40, and its prevalence increases with age, with a maximum peak between 65 and 74 years.^[1]

The presence of complete denture prosthesis in edentulous patients creates another environment with its own micro flora.^[2] *Candida* is present in the oral cavity of almost half of the population. Studies have shown a higher prevalence of *Candida* in diabetic versus non diabetic individuals. *Candida* infection is also found commonly in denture wearers and can occur as a side effect of medications.^[3]

Hence the present study was done to compare the number of *Candida albicans* in the palatal mucosa of completely edentulous (diabetic, non diabetic) and partially edentulous (diabetic, non diabetic) patients at varying time periods.

2. Aims and Objectives

The Present study was done to evaluate the presence of *Candida albicans* in diabetic and non-diabetic first time denture wearers at pre and post insertion stages of complete dentures and transitional acrylic removable partial dentures at varying time periods of 0, on the 15th day and on the 30th day of insertion of removable dentures

3. Materials & Methods

Selection of subjects:

Twenty completely edentulous and twenty partially edentulous patients aged between 50 to 70 years will be selected for the study from the Out Patient Department of Prosthodontics, JSS Dental College and Hospital, Shivarathreshwaranagar, Mysore, Karnataka, India, for replacement of lost teeth with removable prosthesis.

The patients were grouped as follows:

Group I: Patients for transitional acrylic removable partial denture with controlled diabetes mellitus.

Group II: Patients for transitional acrylic removable partial denture not associated with diabetes mellitus.

Group III: Patients for complete denture associated with controlled diabetes mellitus.

Group IV: Patients for complete denture not associated with diabetes mellitus.

All these individuals will be subjected to evaluation of *Candida albicans* as follows:

- a) Prior to impression making.
- b) On the fifteenth day of insertion.
- c) On the thirtieth day of insertion.

Inclusion criteria:

- a) Subjects were first time denture wearers.
- b) Study group: Subjects were controlled diabetics.
- c) Control group: Normal subjects not associated with any conditions of immunosuppression.

Numbering of study subjects:

- a) Completely edentulous patient.
- b) Partially edentulous patients.
- c) Patients belonging to control group (non diabetic).
- d) Patients belonging to study group (diabetic).

Clinical study design:

A questionnaire was prepared to know the patient's diet and oral hygiene habits,

Questionnaire:

Serial no:

Case for R.P.D/C.D.

Diabetic Non diabetic Unaware of diabetes

To be answered by the patient

Name: _____ Age/Sex: _____

Address: _____ Occupation: _____

1. Are you already wearing a denture? Yes / No

If no when was the last tooth to be extracted?

For how long have you been without teeth? _____

2. Are you suffering from any other disease or had you been hospitalized in the past? Yes/ No

If yes please specify-

If you are a diabetic please specify since how long?

3. Are you taking any other medications for control of diabetes?

Yes No.

If yes do you take-

- a) Oral :
- b) Insulin:
- c) If others please specify:

4. How frequently do you visit your physician?

5. Have you got the latest reports of your blood sugar level?

6. Oral hygiene habits.

- a) How do you clean your mouth?(in cases of completely edentulous patients)
- b) How do you clean your teeth?(in cases of partially edentulous patients)
- c) What do you use?
- d) What is the duration?

e) What is the frequency?

7. What is the type of diet you take routinely?

8. Do you have the habit of smoking? Yes No.

If yes since how long?

Patient's signature

Observations made by the operator:

- 1) General examination
- 2) Built
- 3) Nourishment
- 4) Neuromuscular evaluation
- 5) Intra oral examination
 - a) Labial mucosa
 - b) Buccal mucosa:
 - c) Palatal mucosa:
 - d) Tongue:
 - e) Teeth present:
 - In case of presence of teeth:
 - f) Gingival status: i. Gingival recession-
 - ii. Furcation involvement-

Testing of blood sugar level was done using an electronic Glucometer* (fig 1) in J.S.S. Dental college, prior to the collection of culture swabs and the values were noted down as follows:

- 6) Fasting blood sugar level : _____(normal value- 80 to 110 mg/dl).
- 7) Post prandial blood sugar level : _____(normal value- 80 to 140 mg/dl).

Remarks: case selected case not suited for study

(Patients with controlled diabetes and patients with no diabetes were selected for the study.)

- 1) Date of collection of first swab: _____Result..... (prior to impression making)
- 2) Date of collection of the second swab: _____Result..... (on the 15th day of insertion)
- 3) Date of collection of the third swab: _____Result..... (on the 30th day of insertion)

After knowing the diabetic status of the patients, 20 controlled diabetic and 20 non diabetic patients for removable dentures were selected for the study.

The first culture swab was collected from the palatal mucosa using a sterile cotton swab (fig 1, 2) prior to impression making. Then the insertion of the dentures was done for the patient. Patients were instructed to wear dentures only during the day and instructed to remove their dentures before going to bed. They also will be strictly instructed to clean the prosthesis by brushing with soap and water only.



Figure 1: Collection of swabs for complete denture patients



Figure 2: Collection of swabs for Removable partial denture patients

After the collection, it was directly inoculated onto a plate containing Sabouraud's dextrose agar medium (fig 3).



Figure 3: Inoculation of swabs

The medium was incubated at 37 degree centigrade for 24 hours.

Gram staining was done for the culture isolates and will be observed under the microscope for any yeast isolate. In order to identify an unknown yeast isolate, Germ tube test was performed.



Figure 4: Macroscopic view of Candida colonies

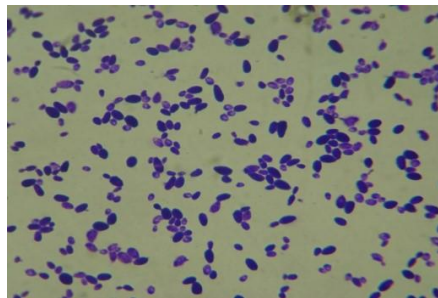


Figure 5: Microscopic view of Candida colonies

Procedure for Germ tube test:

A small portion of an isolated colony of the yeast was suspended in a test tube containing 0.5 ml of human pooled serum. The test tube was incubated at 37 degree Centigrade for two hours. A drop of yeast- serum suspension was placed on a microscopic slide, overlaid with a cover slip and was examined under the microscope.

This confirmatory test was done to see the presence or absence of *Candida albicans* for the respective culture swabs.

The collection of the second and third culture swabs was done on the 15th day and the 30th day of insertion of removable dentures and the same procedure mentioned above for the first culture swab was carried out for the second and third swabs respectively.

Then the data was subjected for statistical analysis.

Statistical analysis

The data were subjected to Pearson's chi square test. All the statistical calculations were done using SPSS (Statistical package for the social sciences) 11.5 package [Table 5].

4. Results

On comparing the two major groups that is,
I] Transitional acrylic removable partial denture wearers,
II] Complete denture wearers.

There was evident growth of *Candida* in the palatal mucosa of diabetic patients on the 30th day of insertion of transitional acrylic removable partial dentures and all of them were chronic smokers. There was evident growth in 40% of the patients who wore transitional acrylic removable dentures with the p value of 0.094, which was not statistically significant. There was no evident growth of *Candida albicans* which was of prime concern in this study.

There was neither evident growth of genera *Candida* nor *Candida albicans* species in the palatal mucosa of both diabetic and non diabetic complete denture patients, prior to impression making, on the 15th day and on the 30th day of insertion. All twenty complete denture wearers in the study had no history of smoking.

Interpretation of tables:

[Table 1]: shows the presence of candidal growth and absence of *Candida albicans* in 40% of partially edentulous diabetic patients on the 30th day of insertion, and it also

shows the absence of candida in 60% of partially edentulous diabetic patients.

Sr. No.	Group	Prior to impression making	15 days after insertion	30 days after insertion
1.	Bd1	No growth	No growth	Growth present <i>Candida albicans - ve</i>
2.	Bd2	No growth	No growth	Growth present <i>Candida albicans - ve</i>
3.	Bd3	No growth	No growth	No growth
4.	Bd4	No growth	No growth	Growth present <i>Candida albicans - ve</i>
5.	Bd5	No growth	No growth	No growth
6.	Bd6	No growth	No growth	No growth
7.	Bd7	No growth	No growth	No growth
8.	Bd8	No growth	No growth	Growth present <i>Candida albicans - ve</i>
9.	Bd9	No growth	No growth	No growth
10.	Bd10	No growth	No growth	No growth

[Table 2]: shows absence of candidal growth in all 10 non diabetic partially edentulous patients prior to impression making, on the 15th day of insertion and also on the 30th day of insertion.

Sr. No.	Groups	Prior to impression making	15 days after insertion	30 days after insertion
1.	Bc1	No growth	No growth	No growth
2.	Bc2	No growth	No growth	No growth
3.	Bc3	No growth	No growth	No growth
4.	Bc4	No growth	No growth	No growth
5.	Bc5	No growth	No growth	No growth
6.	Bc6	No growth	No growth	No growth
7.	Bc7	No growth	No growth	No growth
8.	Bc8	No growth	No growth	No growth
9.	Bc9	No growth	No growth	No growth
10.	Bc10	No growth	No growth	No growth

[Table 3]: shows the absence of candidal growth in all 10 completely edentulous diabetic patients prior to impression making, on the 15th day of insertion and also on the 30th day of insertion.

No.	Groups	Prior to impression making	15 days after insertion	30 days after Insertion
1.	Ac1	No growth	No growth	No growth
2.	Ac2	No growth	No growth	No growth
3.	Ac3	No growth	No growth	No growth
4.	Ac4	No growth	No growth	No growth
5.	Ac5	No growth	No growth	No growth
6.	Ac6	No growth	No growth	No growth
7.	Ac7	No growth	No growth	No growth
8.	Ac8	No growth	No growth	No growth
9.	Ac9	No growth	No growth	No growth
10.	Ac10	No growth	No growth	No growth

[Table 4]: shows absence of candidal growth in all 10 completely edentulous non diabetic patients prior to impression making, on the 15th day of insertion and also on the 30th day of insertion.

Sr. No.	Groups	Prior to impression making	15 days after insertion	30 days after Insertion
1.	Ad1	No growth	No growth	No growth
2.	Ad2	No growth	No growth	No growth
3.	Ad3	No growth	No growth	No growth
4.	Ad4	No growth	No growth	No growth
5.	Ad5	No growth	No growth	No growth
6.	Ad6	No growth	No growth	No growth
7.	Ad7	No growth	No growth	No growth
8.	Ad8	No growth	No growth	No growth
9.	Ad9	No growth	No growth	No growth
10.	Ad10	No growth	No growth	No growth

[Table 5]: shows isolation of candida in among partially edentulous diabetic patients with the p value of 0.094 which is not statistically significant.

		CANDIDA		
DIABETES		Present	Absent	Total
	Present	4	6	10
	Absent	0	10	10
	Total	4	16	20

Chi Square Value (Yates Corrected): 2.813, .d.f. 1; P=0.094

[Table 6]: shows the absence of isolation of candida among complete denture wearers. Hence CHI SQUARE TEST was not applicable for this group.

		CANDIDA		
DIABETES		Present	Absent	Total
	Present	0	10	10
	Absent	0	10	10
	Total	0	20	20

Chi Square Test Not Applicable

5. Discussion

Candida albicans, was isolated in greater quantities from saliva of patients with diagnosed diabetes mellitus than from patients without diagnosis of diabetes. [4] Patients with a diagnosis of diabetes mellitus present a higher susceptibility to infections due to a deficiency in polymorphonuclear leukocytes, as a result of vascular alterations and neuropathies. An increased risk of infections has been observed with reduced salivary flow, leading to low salivary buffering capacity and inadequate hygiene of removable dentures by the patients. Hence it may be concluded that the maintenance of oral health and prevention of oral diseases are prerequisites for the maintenance of good systemic health. [3]

Various other studies done previously showed the increase in the number of *Candida albicans* colonies in the patients who were subjected to prolonged use of dentures. There is no study done on diabetic and non diabetic first time denture wearers. Hence the present study was done to evaluate the candida albicans colonies in first time denture wearers for the maximum duration of 30 days after insertion of the removable prosthesis.

In the present study the data collected was based on the observation of Candidal growth, before insertion and after insertion of removable denture prosthesis in the palatal mucosa both in diabetic and non diabetic patients at varying

time periods viz. prior to impression making, 15 days after insertion and 30 days after insertion.

Candida albicans species which was of prime concern in this study were not found in any of the transitional acrylic removable partial denture wearers irrespective of their diabetic status for a study period of 30 days whereas the controlled diabetic patients who wore transitional acrylic removable partial dentures showed genera Candidal growth on the 30th day of insertion.

Table 1 and 2 shows the comparison between diabetic and non diabetic transitional acrylic removable partial denture wearers, where table 1 shows the evidence of genera Candidal growth (fig 4, 5) on the 30th day of insertion of dentures in patients with controlled diabetes aged between 61 to 70 years (P value of 0.094). The confirmatory GERM TUBE TEST showed no evident growth of *Candida albicans* colonies which were of prime concern in the present study.

There was no evidence of candidal growth prior to impression making and on the 15th day of insertion of transitional acrylic removable partial dentures in controlled diabetic patients. This is in accordance with the study done by Neppelenbroek KH which states that the rate of diffusion of the monomer content in the resin will take approximately to about 14 days.^[5] This might be attributed to the failure of isolation of candidal growth for the 15 day period as observed in this study.

Table 3 and 4 shows the comparison between diabetic and non diabetic complete denture wearers, where table 3 shows no evidence of genera Candidal growth prior to impression making, on the 15th day of insertion and on the 30th day of insertion of complete dentures in patients with controlled diabetes. There was no need for GERM TUBE TEST in this particular group as there was no growth of *Candida*.

6. Conclusion

From this study, an inference can be drawn that the elderly patients who are first time denture wearers and have history of smoking are more susceptible to Candidal infections than the first time denture wearers who had no history of smoking. The results are in concurrence with the previous study done by Jones LMT.^[11] The limitation of this study is that, the patients were followed up only for duration of 30 days post insertion, whereas studies have shown that *Candida albicans* colonies increased with the increase in the duration of removable prosthesis wear but at the same time there are no studies stating when the candidal colonies can be isolated at the earliest. The following conclusions were drawn from the findings.

a) Among complete denture wearers:

- *Candida albicans* species which was of prime concern in this study was not found in any of the complete denture wearers irrespective of their diabetic status for a study period of 30 days.

b) Among transitional acrylic removable partial denture wearers:

- *Candida albicans* species which was of prime concern in this study were not found in any of the transitional acrylic

removable partial denture wearers irrespective of their diabetic status for a study period of 30 days.

- The controlled diabetic patients who wore transitional acrylic removable partial dentures showed Candidal growth on the 30th day of insertion.
- Transitional acrylic removable partial denture wearers who showed Candidal growth on the 30th day of insertion did not show any Candidal growth prior to the impression making nor on the 15th day of insertion.

It is our duty as prosthodontists to create awareness among the masses regarding the ill effects and correlation between smoking, diabetes and susceptibility towards developing candidal infections.

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