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Assessment Of Denture Adhesive On Candida Albicans Growth Between Smokers And Non-Smokers – An Invivo Study

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Abstract:

Background*:Candida albicans is a common oral fungal pathogen that frequently colonizes denture surfaces, leading to denture-related stomatitis. Denture adhesive usage is widespread, yet the impact of smoking on the efficacy of denture adhesives in preventing Candida albicans growth remains unclear.This in vivo study aims to assess the influence of smoking on the effectiveness of denture adhesive in Candida albicans growth on denture surfaces. The study also investigates whether there are differences in this outcome between smokers and non-smokers.*

Materials and Methods:*. A group of 16 completely edentulous healthy male individuals were selected, ranging from 40 – 70 years of age group. The selected 16 subjects were divided into 2 groups (Group A – 8 non-smoker, Group B – 8 smoker). The selected subjects had no significant medical history, no clinical signs of candidiasis, & had not received any recent medication for previous 6 weeks.*

A standardized acrylic denture was provided to each participant. Denture adhesive application followed manufacturer guidelines. Candida albicans growth was measured using colony-forming units (CFUs) per unit area of denture surface, sampled at baseline, Two samples i.e. saliva sample and swab from palate and intaglio surface of maxillary denture were collected at the time of denture insertion and 40 days after denture insertion. Patients were instructed to use the denture adhesive (FIXON) twice a day for 40 days. All the subjects were told to clean their denture using a soft tooth brush under running water. The samples were kept in closed sterile container and stored under refrigeration until they were processed.

To ensure standardization, dentures were fabricated identically, and adhesive application was supervised by a single examiner. Intra-examiner reliability was assessed prior to the study. Sample size calculation indicated a power of 80% at a significance level of 0.05.

Results: *The mean baseline CFUs were comparable between smokers and non-smokers in both adhesive and control groups ($p > 0.05$). Based on the results a positive correlation was found between duration and frequency of smoking and C.albicans level. There was no correlation seen between mode and frequency of cleaning denture and mouth and C.albicans level Also there is a positive correlation between saliva and swab with groups of non msokers and smokers using adhesive and a significant difference was found in the Candida Albicans colony growth when compared to a group of smokers and non smokers with use of denture adhesive.There is marked increase in Candida Albicans with use of denture adhesive in smoker.*

Conclusions:

Thus it was concluded from results of above study that smoking causes increase in C.albicans levels in complete denture patient using denture adhesive. Though patients are always advised to quit smoking habit but if the use of denture adhesive is inevitable it should be used cautiously in smoker patients. Adhesive containing anti-fungal agent should be prescribed. Regular recall and follow up should be done for such patients. Along with this

meticulous attention should be given to denture care and oral hygiene instruction and its importance should be explained to patient.

***Keyword**: Candida albicans , Denture Adhesive , Denture Wearer, Oral Fungus, Smoking*

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I. Introduction

Aging is a universal process and a normal inexorable biological phenomenon. Throughout the world, a demographic upheaval is underway as the total number of older people is growing faster than any other age group of people.

Complete denture is one of the most important treatment possibilities in prosthodontics, more so because of the increase in average life expectancy of the individual. Newly fabricated dentures can be lacking in retention or stability and could cause anxiety or psychological stress to the patient. Henceforth pose a problem to the dentist as well as patient.

The use of denture adhesive in such patients will overcome patients' anxieties upon their introduction to new dentures, particularly during the immediate post insertion adjustment stage. It improves the confidence and they become more willing to adjust to eating and socializing in public with their new dentures.

Most of the researchers suggest that denture adhesive do not alter the oral micro biota but none of the studies so far have compared the effect of smoking and use of denture adhesive on oral microflora. This present study compared the effect of denture adhesive on oral microflora between the smokers and non- smokers.

Candida albicans is a commensal of the oral cavity of45–65% of healthy individuals. In denture wearers, Candida occurrence increases up to 60-100%and the organism can be opportunistic,this can be elucidated by the fact that dentures decrease the flow of oxygen and saliva to the underlying tissue producing a local acidic and anaerobic microenvironment that further favors the overgrowth of yeast. Furthermore, Candida has attraction towards the acrylic surface of dentures.

Cigarette smoking (CS) has stronger influence on oral microflora which may encourage the growth of pathogenic microorganisms. Cigarette Smoke has been shown to intensify the biofilm formation. Several studies have proposed an association between oral Candida level and Cigarette Smoke, as a major percentage of smokers carry Candida as compared to non-smokers.

II. Material & Methods

Subject selection

This study was conducted after approval from The Institutional Ethics Committee, Peoples University (established under MP Act 17 of 2007). All the subjects signed an informed consent and were explained regarding the experimental procedure & a brief questionnaire was filled by them. A questionnaire was prepared both in english and hindi, regarding frequency & duration of smoking and frequency & mode of cleaning mouth & denture (fig 2).

A group of 16 completely edentulous healthy male individuals were selected, ranging from 40 – 70 years of age group. The selected 16 subjects were divided into 2 groups (Group A – 8 non-smoker, Group B – 8 smoker). The selected subjects had no significant medical history, no clinical signs of candidiasis, & had not received any recent medication for previous 6 weeks.

For all the individuals well adapted new conventional complete maxillary and mandibular complete denture were fabricated with appropriate retention and stability.

Two samples i.e. saliva sample and swab from palate and intaglio surface of maxillary denture were collected at the time of denture insertion and 40 days after denture insertion (fig1). Patients were instructed to use the denture adhesive (FIXON) twice a day for 40 days. All the subjects were told to clean their denture using a soft tooth brush under running water. The samples were kept in closed sterile container and stored under refrigeration until they were processed.

Microbiological Evaluation

The overall collected samples were processed for microbiological evaluation, for isolation of yeast selective medium for fungi- Sabouraud dextrose agar (HiMedia™ Laboratories Pvt Ltd) with chloramphenicol was used and for bacterial count brain heart infusion agar (HiMedia™ Laboratories Pvt Ltd) was used. The Petri dish containing SDA culture medium was taken and 0.1 ml of saliva sample was directly seeded onto the culture media and swab samples from palate and intaglio surface of denture were streaked onto the other petri dish containing SDA medium. The samples were incubated in an oven at 37 degree Celsius for 48 hrs. The above microbiological procedure was followed at the time of denture insertion and 40 days after usage of denture adhesive.

Similar procedure was carried out for bacterial count except from culture medium which was brain heart infusion agar (BHI).

The incubated plates were examined and isolated colonies were evaluated according to the morphology and pigment formation

After the incubation of inoculated culture plates the cell forming units (CFU) of bacteria and fungi were counted separately with the help of the Digital Colony Counter

III. Results

Table 1: Distribution of colony count diameters amongst study population based on smoking status and adhesive usage in salivary sample - Post

Groups	N	Mean	S.D	Minimum	Maximum
Smokers + Adhesives (Group A)	8	115.2413	.37115	114.80	115.83
Non - Smokers + Adhesives (Group B)	8	91.3063	4.68671	83.64	94.01
Smokers without adhesive (Group C)	8	69.7600	3.41044	63.67	73.35
Non-smokers without adhesive (Group D)	8	61.3688	1.32283	59.07	62.86
F statistic	524.149				
Df	3				
P value	0.000*				

*=Significant; NS=Not Significant

Table 2: Post hoc / Pairwise comparison of colony count diameters amongst study population based on smoking status and adhesive usage in salivary sample - Post

Pairs	Mean difference	Std. Error	Significance
Group A versus Group B	23.93500*	1.48921	.000*
Group A versus Group C	45.48125*	1.48921	.000*
Group A versus Group D	53.87250*	1.48921	.000*
Group B versus Group C	21.54625*	1.48921	.000*
Group B versus Group D	29.93750*	1.48921	.000*
Group C versus Group D	8.39125*	1.48921	.000*

*=Significant; NS=Not Significant

Table 3: Distribution of colony count diameters amongst study population based on smoking status and adhesive usage in salivary sample - Pre

Groups	N	Mean	S.D	Minimum	Maximum
Smokers + Adhesives (Group A)	8	90.4758	2.75402	84.90	93.86
Non - Smokers + Adhesives (Group B)	8	74.5750	4.79151	64.97	78.79
Smokers without adhesive (Group C)	8	59.5959	4.05583	53.97	64.67
Non-smokers without adhesive (Group D)	8	55.9050	3.74201	51.97	61.96
F statistic	130.637				
Df	3				
P value	0.000*				

*=Significant; NS=Not Significant

Table 4: Distribution of colony count diameters amongst study population based on smoking status and adhesive usage in swab - Post

Groups	N	Mean	S.D	Minimum	Maximum
Smokers + Adhesives (Group A)	8	76.2275	2.14397	72.57	78.76
Non - Smokers + Adhesives (Group B)	8	67.1225	1.22861	65.76	68.85
Smokers without adhesive (Group C)	8	63.0938	2.45441	59.85	66.86
Non-smokers without adhesive (Group D)	8	59.2350	1.63809	57.38	61.96
F statistic	114.755				
Df	3				
P value	0.000*				

*=Significant; NS=Not Significant

Table 5: Post hoc / Pairwise comparison of colony count diameters amongst study population based on smoking status and adhesive usage in swab

Pairs	Mean difference	Std. Error	Significance
Group A versus Group B	9.10500*	.96221	.000*
Group A versus Group C	13.13375*	.96221	.000*
Group A versus Group D	16.99250*	.96221	.000*
Group B versus Group C	4.02875*	.96221	.001*
Group B versus Group D	7.88750*	.96221	.000*
Group C versus Group D	3.85875*	.96221	.002*

*=Significant; NS=Not Significant

Table 6: Distribution of colony count diameters amongst study population based on smoking status and adhesive usage in swab - Pre

Groups	N	Mean	S.D	Minimum	Maximum
Smokers + Adhesives (Group A)	8	63.8200	2.69975	60.07	67.97
Non - Smokers + Adhesives (Group B)	8	56.3362	.95797	54.97	57.97
Smokers without adhesive (Group C)	8	47.0113	17.06324	5.00	54.97
Non-smokers without adhesive (Group D)	8	47.0113	17.06324	5.00	54.97
F statistic	3.590				
Df	3				
P value	0.026*				

*=Significant; NS=Not Significant

Table 5: Groupwise comparison of CFU/ml between salivary and swab samples – Post intervention

Groups	Specimen	Mean	S.D	't' test statistic	df	P value
Group A	Saliva	115.2413	.37115	50.714	14	.000*
	Swab	76.2275	2.14397			
Group B	Saliva	91.3063	4.68671	14.118	14	.000*
	Swab	67.1225	1.22861			
Group C	Saliva	69.7600	3.41044	4.487	14	.001*
	Swab	63.0938	2.45441			
Group D	Saliva	61.3688	1.32283	2.866	14	.012*
	Swab	59.2350	1.63809			

*=Significant; NS=Not Significant

Graph 1: Groupwise comparison of CFU/ml between salivary and swab samples

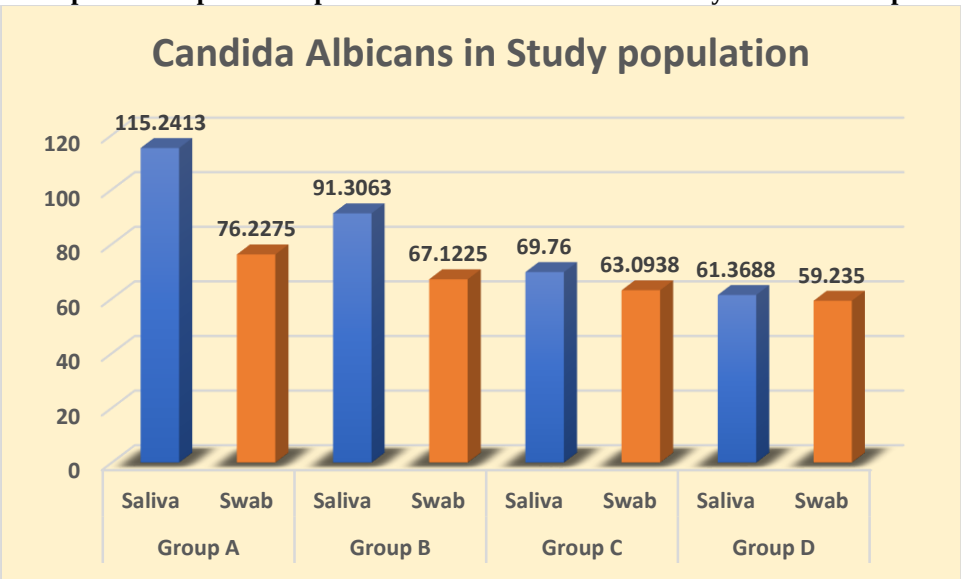


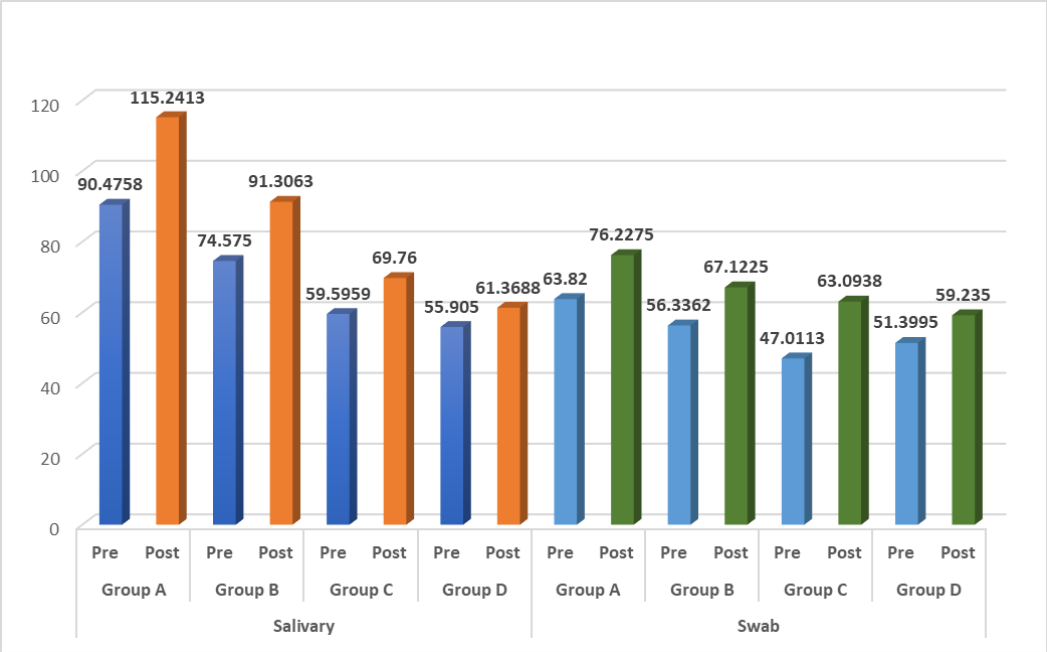
Table 6: Comparison of groups between pre and post intervals in salivary and swab cultures

Groups	Specimen	Mean	S.D	't' test statistic	df	P value
Saliva						
Group A	Pre	90.4758	2.75402	-25.100	7	.000*
	Post	115.2413	.37115			
Group B	Pre	74.5750	4.79151	-9.565	7	.000*
	Post	91.3063	4.68671			
Group C	Pre	59.5959	4.05583	-6.200	7	.000*
	Post	69.7600	3.41044			
Group D	Pre	55.9050	3.74201	-3.318	7	.013*
	Post	61.3688	1.32283			
Swab						
Group A	Pre	63.8200	2.69975	-9.891	7	.000*
	Post	76.2275	2.14397			
Group B	Pre	56.3362	.95797	-18.054	7	.000*

	Post	67.1225	1.22861			
Group C	Pre	47.0113	17.06324	-2.739	7	.029*
	Post	63.0938	2.45441			
Group D	Pre	51.3995	3.50649	-9.384	7	.000*
	Post	59.2350	1.63809			

*=Significant; NS=Not Significant

Graph 2: Comparison of groups between pre and post intervals in salivary and swab cultures



Data analysis:

The data obtained was entered into spread sheets and analysed using SPSS software 23.0 version ((IBM Corporation, Armonk, NY, USA).). Microbial count were entered as mean and standard deviation. Analysis of Variance (ANOVA) test was applied to find significant differences between saliva and swab culture in both pre and post interval period. Independent t test was run to estimate difference in saliva and swab cultures in each group in the post intervention period. Paired t test was run to evaluate difference between pre and post intervention in each group of salivary and swab cultures. P value lesser than 0.05 was considered statistically significant.

Results

Based on the results a positive correlation was found between duration and frequency of smoking and C.albicans level. There was no correlation seen between mode and frequency of cleaning denture and mouth and C.albicans level.

Also there is a positive correlation between saliva and swab with groups of non msokers and smokers using adhesive and a significant difference was found in the Candida Albicans colony growth when compared to a group of smokers and non smokers with use of denture adhesive.

There is marked increase in Candida Albicans with use of denture adhesive in smoker.

IV. Discussion

The increased stability and retention due to denture adhesive allow their use in patients with extremely resorbed residual alveolar ridge, loss of neuromuscular control, xerostomia where retention and stability are compromised. It provides a cushioning effect, reduces the amount of food particles collecting under the denture, and assists in the distribution of forces of occlusion over the denture bearing regions, thereby minimizing local pressure points. It alleviates the discomfort of tissue irritation, and lessens the need for frequent adjustments.

Adverse effect of smoking in the oral cavity shows increased candida albicans level in oral cavity which in turn may cause denture stomatitis. Smoker's have significantly greater loss of bone height than nonsmokers, long-term smoking compromises the function of the salivary glands which is reflected in the reduced amount(xerostomia) and poorer quality of saliva(thick saliva) which in turn affects retention of denture. It also causes loss of taste sensation and poorer oral hygiene is found among smokers.

Several studies have been done on effect of denture adhesive on oral micro flora such as Kim et al[1] in 2003 in his study suggested that denture adhesive tested did not alter denture micro biota during 14 day trial

period. Similarly Oliveira et al[3] (2010) on the effect of adhesive on complete denture on colonization of candida species reported that the denture adhesive tested did not significantly alter the oral microbiota during 14 days trial period. Another study by Ozkan et al[2] (2012) effect of denture adhesive on microorganisms in vivo concluded prolonged use of denture adhesive tested up to 2 months did not increase the microorganisms of oral flora.

The literature suggests increase in candida level in smokers. The precise mechanism by which candida presence may be altered by cigarette smoke is not yet established. Arendorf and walker [4](1980) told that smoking may lead to localized epithelial alteration which facilitates candidal colonization. Cigarette smoke can contain nutritional factors for candida albicans. Smoking also depresses the activity of oral leukocytes[5](leukocyte are important in inhibiting colonization of candida in oral cavity).There was a marginally significant positive correlation between the number of cigarettes smoked per day and the density of Candida growth in oral rinse cultures. So, cigarette smoke can be labeled as an infection promoting agent.[8,9]

All patients were given oral and denture hygiene instructions which all of them followed so no corelation was found between mode and frequency of cleaning mouth & denture and C.albicans level.

In denture wearers, the prevalence of Candida increases to 60-100% and the organism can be opportunistic, which can be explained by the fact that dentures decrease the flow of oxygen and saliva to the underlying tissue producing a local acidic and anaerobic microenvironment that favors yeast overgrowth. Additionally, Candida has affinity for the acrylic surface of denture[7.]

Surface characteristics of denture base acrylic resins, such as hydrophobicity, have generally been acknowledged to be one of the factors contributing to the adhesion, which is a crucial step in biofilm formation

V. Conclusion

Thus it was concluded from results of above study that smoking causes increase in C.albicans levels in complete denture patient using denture adhesive. Though patients are always advised to quit smoking habit but if the use of denture adhesive is inevitable it should be used cautiously in smoker patients. Adhesive containing anti-fungal agent should be prescribed. Regular recall and follow up should be done for such patients. Along with this meticulous attention should be given to denture care and oral hygiene instruction and its importance should be explained to patient.

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