

ANTIFUNGAL ACTIVITY OF DENTURE CLEANSERS: A COMPARATIVE STUDY

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ABSTRACT

The objective of current study was to compare the efficacy of denture cleansers and to find out the most efficacious denture cleanser against Candida. This randomized control trial study with sample size eighty was conducted at Prosthodontic Department de, Montmorency College of Dentistry, Lahore, Microbiological test were performed at PGMI Lahore from Febuary 2003 to October 2003. Patients who were regularly wearing their dentures without any occlusal disharmony for more than six months were included. Group A, B, C, and D of dentures were used to determine the effect of "Sno Wite" (4% sodium hypochlorite), "Klinzar" (effervescent tablet of sodium metaborate), "Lacer Chlorhexidine" (0.12% Chlorhexidine), and plain water (control) respectively on Candida. Sabouraud's dextrose agar (Laboratorios Britania S.A. Los Patos, Argentina) was used as a culture medium. Germ tube test was performed to identify Candida albicans. Where sodium hypochlorite was added Candida CFUs were found in non of the cases, no gram negative rods or gram positive streptococci were seen. With sodium metaborate only in one case of candida CFUs (5%) were detected, with chlorhexidine also in one case of CFUs (5%) were detected. While with distilled water as a control group 13 cases with candida (65%) has been detected. It was concluded that denture cleansers are more effective than the plain water (control) and sodium hypochloride is more effective denture cleaners as compared to sodium metaborate and 0.12% chlorhexidine.

INTRODUCTION

The oral cavity is colonized by various pathogens that may affect the course of a number of systemic diseases including aspiration pneumonia, gastrointestinal infection and chronic obstructive pulmonary disease. It is known that complete and removable dentures accumulate microorganisms, particularly *Candida albicans*, on the porous surface of the acrylic resin. This microbial colonization is one of the main etiological factors associated with denture induced stomatitis.¹ Correct prosthetic use and daily hygiene are important factors for good oral health, greater longevity of the prosthesis, and health of supporting tissues.² In the latest guideline for care and maintenance of complete dentures, the American College of Prosthodontists states that regular brushing of the denture with toothpaste has no impact on *Candida* species colonization. Which enhances the need of chemical denture cleaners.³ Commonly used denture cleansers are Chlorhexidine, Sodium hypochlorite⁴, Sodium perborate, Cetylpyridinium chloride⁵, 5% and 10% white vinegar.⁶ Sodium hypochlorite causes oxidation of metal and causing spot corrosion so it should not be used with cast partial denture or cast

complete denture.⁷ The highest *Candida* biofilm growth was shown to occur on polyamide resin when compared with Polymethyl- methacrylate resin.⁸ Pasricha N⁹ conducted study about awareness and knowledge of denture cleaners in dental professionals and observed highest number of respondents (55.5%) used tablet form of denture cleanser. While 47.5% reported that they had little knowledge about adverse effects of denture cleansers. A significant percentage (36%) reported that no knowledge is imparted about denture cleansers in their curriculum which shows lack of awareness about denture cleaners in dental professionals. So the aim of the study was to increase awareness among dental professional about denture cleansers, assess the efficacy of different denture cleaners and compare current study with international studies. Local published studies about denture cleansers are rare.

METHODOLOGY

This randomized control trial study with sample size of eighty was conducted at Prosthodontic Department de, Montmorency College of Dentistry, Lahore and Microbiology Department, PGMI, Lahore. Patients who were regularly wearing their dentures without any occlusal disharmony for more than six months were included. The patient's age ranged from 40-65 years. Subjects using denture cleansers or antiseptic mouth washes, history of radiotherapy, and history of medication known to predispose to oral candidiasis

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(i.e. antibiotics, steroids, cytotoxic drugs, immunosuppressive drugs) were excluded. Selected subjects were randomized into four equal groups namely A, B, C and D (each group of 20 subjects). Information about each subject's denture age, method of denture cleansing, and denture wearing habit was recorded. Group A, B, C, and D dentures were used to determine the effect of "Sno Wite" (4% sodium hypochlorite), "Klinzar" (effervescent tablet of sodium metaborate), "Lacer Chlorhexidine" (0.12% Chlorhexidine), and plain water (control) respectively on *Candida*. Fitting surface of upper complete denture was divided into five zones as follows.

- 1) Incline area between midline of denture and depth of alveolar sulcus from canine region anteriorly to post-dam area posteriorly on right side.
- 2) Incline area between midline of denture and depth of alveolar sulcus from canine region anteriorly to post-dam area posteriorly on left side.
- 3) Right side flange area from canine region to Tuberosity region.
- 4) Left side flange area from canine region to Tuberosity region.
- 5) Anterior flange area from canine to canine region.

Each area was subjected to four unilateral brushing strokes. The operator brushed the fitting surface of denture with sterile tooth brush, while assistant was pouring saline solution (10 ml) over the respective areas of fitting surface. After washing and brushing, the resultant solution was divided into two equal parts (part-I and part-II). Part-I solution (5 ml) was diluted 10 times by adding 50 ml saline solution. Part-II solution (5ml) was diluted five times by adding 25ml of saline solution because in future five milliliter denture cleanser has to be added to it, thus getting total dilution of 10 times. After dilution, five milliliter was secured from both solutions (solution I and II) in sterile syringes. The needles of the syringes were capped carefully. The cap was sealed with sticky tape. The denture was returned to patient. Syringes were labeled by putting the patient's name and OPD number and stored in refrigerator. The sample was transported to microbiology laboratory within one hour.

Sabouraud's dextrose agar (Laboratorios Britania S.A. Los Patos, Argentina) was used as a culture medium. It contains 10 gms/litre mycological peptone, 40 gms/litre dextrose, 0.05 gms/litre chloramphenicol, and 15 gms/litre agar No. 1. It is a general purpose medium for the cultivation of moulds and yeasts. To rehydrate the medium, 6.5 gms powder was mixed and suspended in 100 ml distilled water. The mixture was heated to boiling point to ensure dissolution. It is

then sterilized in an autoclave for 15 minutes at 15 lb pressure at 121°C. The solution was allowed to cool and then dispensed in sterile Petri dishes before gellation. After cooling to room temperature, they were stored in refrigerator in sterile plastic bags. From refrigerator, the prepared culture plates were transferred to incubator and allowed for 30 minutes at 37°C to eliminate moisture. After 30 minutes, culture plates were taken out of incubator. A line was drawn on outer surface of base of culture plate to divide it into two equal halves. One portion was marked as 'plate I' (for solution I) and the other as 'plate II' (for solution II). Solution I (5ml) was left unmixed. Equal amount of denture cleanser (5ml) was added to solution II as follows (according to assigned groups).

- 1) Sodium hypochlorite (Sno Wite) was added to solution II of group A subjects' dentures.
- 2) Solution of sodium metaborate (Klinzar) was added to solution II of group B subjects' dentures.
- 3) Chlorhexidine (Lacer chlorhexidine) was added to solution II of group C subjects' dentures.
- 4) Distilled water (control) was added to solution II of group D subjects' dentures.

The mixture (solution II and denture cleanser) was kept for 20 minutes for antimicrobial action. Solution I and II were inoculated on respective areas of culture medium (relative to 'plate I' and 'plate II' of culture plate) for this purpose, Pasteur spreader was red heated over a flame to ensure sterilization. It was then air cooled. The spreader was dipped in solution I. The specimen was inoculated on the portion of culture plate marked as 'plate I'. The specimen was spread as primary, secondary, tertiary inoculums and its tail. Solution II was inoculated on 'plate II' following the same procedure. For the sake of standardization, same size of Pasteur spreader was used for all cases. After inoculation, culture plates were covered with sterile covers. The plates were transferred to incubator and left there for 48 hours at 37°C in aerobic condition. This allowed growth of *Candida* if present. After 48 hours, culture plates were observed for colony forming units (CFUs). The CFUs of *Candida* were identified by their morphologic characteristics and distinct odor. The CFUs on both halves of plate were counted and charted down in the data collection form. To confirm the presence of *Candida*, Gram staining and germ tube test were performed.

Gram staining:

- 1) With sterile Pasteur spreader, a drop of saline was put on a slide.
- 2) The spreader was sterilized again, and a small portion of CFU was mixed with saline on the slide.

- 3) The slide was air dried and fixed by passing over the flame.
- 4) Crystal violet was put over the smear for two minutes. The slide was then washed with water.
- 5) Gram iodine was applied for 1-2 minutes and then washed with water.
- 6) Acetone alcohol was used to decolorize the smear for few seconds. It was thoroughly washed with water.
- 7) Safranin was applied for 2-3 minutes. The slide was washed with water and air dried.

A drop of cedar wood oil was put on the slide for better visibility. The slide was examined under high magnification light microscope for the presence of Candida. Germ tube test was performed to identify Candida albicans.

Germ tube test:

- 1) Five hundred micro liter (0.5 ml) of human serum was pipetted into a small test tube.
- 2) Using a sterile Pasteur spreader, the serum was inoculated with a portion of CFU.
- 3) The tube was placed in the incubator at 35-37°C for 2-3 hours.
- 4) Using a wire loop. A drop of serum yeast culture was transferred to a glass slide and covered with a cover slip.
- 5) The preparation was examined using 10x and 40x objectives with the condenser iris diaphragm closed sufficiently to give good contrast.
- 6) When tube like out growths from the cell (germ tubes) were examined and the culture was reported as 'Candida albicans isolated'.
- 7) In case where yeast cells did not show sprouting (germ tubes), the culture was reported as 'yeast other than Candida albicans'.

Occasionally, gram negative bacteria (rods) and gram positive streptococci were seen in the smear. They were identified by their morphologic characteristics. The

data base of all observations was developed in SPSS 8.0 for windows. The mean, standard deviation, and range for quantitative variables were computed with the help of SPSS analysis. Significance in difference was determined in the CFUs of fitting surface of upper complete denture. For this purpose Fisher Exact test was employed at 95% confidence interval to determine the P value. P value greater than 0.05 will be considered non significant difference and vice versa.

RESULTS

The age of 80 selected patients ranged from 40-65 years with mean age of 57.56 years (SD \pm 6.78). There were 45 males and 35 females (56% and 44% respectively). Group wise patient distribution is given in Table 1. Thirty-three patients (41.25%) were in the age range of 61-65 years followed by 19 (23.75%) and 12 (15%) patients falling in age group 56-60 and 46-50 years respectively.

Group A:

On 'plate-I' (without sodium hypochlorite), Candida species were detected in 12 cases (60%) out of 20. Their CFUs ranged from 10 to 4500. Out of Candida species, Candida albicans was isolated in 10 cases which are 83.3% of cases where candida species were isolated. Gram negative rods were seen in two cases (10%). Gram positive streptococci were also found in two cases (10%).

On 'plate-II' (where sodium hypochlorite was added), Candida CFUs were found in none of the cases. The efficacy of sodium hypochlorite against Candida species was highly significant (P value 0.000). On 'plate-II', no gram negative rods or gram positive streptococci were seen (Table 2). The culture of five cases (20%) did not show any growth on either part of culture plate (plate-I or plate-II). (Table 2)

Group B:

On 'plate-I' (without sodium metaborate) Candida species was found in 12 cases (60%). Their CFUs ranged from 1 to 3000 (Table 3). Candida albicans was

TABLE 1: DISTRIBUTION OF GROUPS ACCORDING TO GENDER AND MEAN AGE

Groups	No. of male patients	No. of female patients	Mean age (years) (males & females)	Standard Deviation (\pm SD)
A n=20	14	06	59.05	\pm 6.54
B n=20	10	10	55.90	\pm 7.05
C n=20	12	08	57.85	\pm 6.91
D n=20	09	11	57.45	\pm 6.68
Total n = 80	45	35	57.56	\pm 6.78
	Mean age (years) 59.66 \pm 6.25	Mean age (years) 54.85 \pm 6.52		

TABLE 2: EFFECT OF SODIUM HYPOCHLORITE ON CFUS OF DIFFERENT MICROORGANISMS (GROUP A SUBJECTS)

No.	Microorganisms	CFUs		No.	Microorganisms	CFUs	
		Without cleanser	With cleanser			Without cleanser	With cleanser
01	Candida alb.	10	00	11	00	00	00
02	00	00	00	12	Candida alb.	1360	00
03	Candida alb.	20	00	13	00	00	00
04	Candida alb.	1700	00	14	00	00	00
05	Candida alb.	3950	00	15	Candida alb.	4500	00
06	Candida non alb.	3200	00	16	Candida alb.	10	00
07	00	00	00	17	Candida alb.	3370	00
08	Candida alb.	3500	00	18	Gram +ve streptococci	3000	00
	Gram -ve rods	600					
09	Candida non alb.	130	00	19	Candida alb.	2900	00
10	Gram -ve rods	10	00	20	Gram +ve streptococci	3500	00

TABLE 3: EFFECT OF SODIUM METABORATE ON CFUS OF DIFFERENT MICROORGANISMS (GROUP B SUBJECTS)

No.	Microorganisms	CFUs		No.	Microorganisms	CFUs	
		Without cleanser	With cleanser			Without cleanser	With cleanser
01	00	00	00	11	00	00	00
02	Candida alb.	01	00	12	Candida non alb.	500	00
03	Candida alb.	350	17	13	Candida alb.	2500	00
04	Candida non alb.	80	00	14	Candida alb.	1000	00
05	Gram (-) rods	350	00	15	Candida alb.	4500	00
06	Candida non alb.	30	00	16	Candida alb.	10	00
07	00	00	00	17	Candida alb.	3370	00
08	00	00	00	18	Gram +ve streptococci	3000	00
09	Candida non alb.	3000	00	19	Candida alb.	2900	00
10	Candida alb.	01	00	20	Gram +ve streptococci	3500	00

TABLE 4: EFFECT OF CHLORHEXIDINE ON CFUS OF DIFFERENT MICROORGANISMS (GROUP C SUBJECTS)

No.	Microorganisms	CFUs		No.	Microorganisms	CFUs	
		Without cleanser	With cleanser			Without cleanser	With cleanser
01	Candida alb.	6500	00	11	Candida alb.	40	00
02	00	00	00	12	00	00	00
03	Candida alb.	20	00	13	00	00	00
04	00	00	00	14	00	00	00
05	Gram (-) rods	150	00	15	Gram (-) rods	100	00
	Candida alb.	01	10	16	Candida non alb.	30	00
06	00	00	00	17	Candida alb.	3500	00
07	Candida alb.	30	00	18	Candida alb.	200	00
08	00	00			Candida alb.	3100	
09	Candida alb.	450	00	19	Candida non alb.	4500	00
10	Candida alb.	170	00	20	Candida non alb.	400	00

TABLE 5: EFFECT OF DISTILLED WATER ON CFUS OF DIFFERENT MICROORGANISMS (CONTROL GROUP)

No.	Microorganisms	CFUs		No.	Microorganisms	CFUs	
		Without cleanser	With cleanser			Without cleanser	With cleanser
01	00	00	00	11	Candida non alb.	200	150
02	Gram (-) rods	12	01	12	Candida alb.	320	210
03	Candida alb.	6000	4500	13	Candida alb.	450	385
04	Gram (-) rods	1050	950	14	Candida alb.	03	13
05	00	00	00	15	Candida non alb.	650	250
06	Gram (-) rods	300	250	16	Candida non alb.	90	55
07	Candida alb.	30	19	17	Candida alb.	10	05
	Gram (-) rods	00	150		Gram (-) rods	12	25
08	Candida alb.	2500	2000	18	00	00	00
09	Candida non alb.	500	350	19	Candida alb.	510	380
10	Candida alb.	01	00	20	Candida non alb.	00	15

isolated in eight cases (40%) which are 66.6% of cases having Candida CFUs. Candida CFUs ranged from 1 to 4500 (Table 3). Gram negative rods were observed in three cases (15%). The efficacy of sodium metaborate against all Candida species was highly significant (P value 0.00). In case of gram negative rods the efficacy of this cleanser was statistically non significant (P value 0.231). No microorganism was isolated in four cases.

On plate-II (with sodium metaborate) Candida species was found in 1 case (5%). no gram negative rods or gram positive streptococci were seen. (Table 3)

On 'plate-I' (without chlorhexidine), Candida species were isolated in 13 cases (65%). Candida albicans was isolated in 10 cases (76.9% of total Candida species). Their CFUs ranged from 1 to 6500. Gram negative rods were isolated in two cases. In both cases they were found along with Candida species. There was significant correlation between Candidal CFUs inhibition and chlorhexidine activity against Candida species (P value 0.001). Antimicrobial activity of chlorhexidine against gram negative rods was found non significant (P value 0.487). In this group, the culture of seven cases (35%) did not show growth of any microorganisms.

On plate-II (with chlorhexidine) Candida species was found in 2 cases (10%). No gram negative rods or gram positive streptococci were seen in any case. (Table 4)

Group D (Control):

In control group (where distilled water was used) Candida species were isolated in 14 cases (70%) (Table).

Their CFUs ranged from 01 to 6000 (Table 5). Candida albicans was present in nine cases (69% of all Candida species) (Table 5). Gram negative rods were isolated in five cases (25%). No microorganism was isolated in one case. Distilled water was found ineffective in CFUs inhibition of all types of isolated microorganisms (P value 1.00). (Table 5)

COMPARISON AMONG DIFFERENT GROUPS

In group A the difference was statistically significant when compared to control group (P-Value 0.00) but was non significant by comparing to B & C. Group B (sodium metaborate group) and group C (chlorhexidine group) have also significant anti fungal activity when compared to control group (P value 0.00).

All the three cleansers had some antimicrobial activity against Gram negative rods but the results were non significant (P Value > 0.05). A gram positive streptococcus was isolated in group A only. The effect of sodium hypochlorite was found to be statistically non significant against gram positive Streptococci. (P Value 0.487).

DISCUSSION

Prosthesis is provided to replace the lost part and also to restore the lost or impaired function due to missing part or organ of the body. To make it more meaning the care and maintenance of the prosthesis is of paramount importance including maintaining hygienic conditions. Inadequate home care can seriously compromise the clinical results of the dental prosthesis utilizing the excellent material and techniques.

In current study as compared to the other denture cleansers sodium hypochloride is compared to be the best denture cleanser as *Candida* CFUs were found in non of the cases and no gram negative rods or gram positive streptococci were seen. Same results has also been shown by Cem Sahin et al 10 who compared sodium hypochloride with glutaraldehyde. Study done by Chethan et al 11 also showed that sodium hypochloride is more effective denture cleanser as compared to Trisodium phosphate, Sodium perborate and Chlorhexidine gluconate in assessing the colony forming unit of yeast and *Streptococcus* species. De silva PM et al observed that both sodium hypochloride and chlorhexidine are effective against *Candida albicans* but sodium hypochloride is better because it removes the dead *Candida* species from heat polymerized acrylic resin.¹² Dhamande et al¹³ observed that sodium laural sulfate has higher *Candida* removing activity as compared to sodium hypochloride but in current study sodium laural sulfate was not used as denture cleaner.

In current study Cleansing agents were found to be effective in the following order, Sodium hypochlorite solution, Sodium metaborate and Chlorhexidine gluconate. Same sequence is also found in Chethan et al¹¹ study but the sequence was slightly different in study of De silva FC¹⁴ which showed that 1% sodium hypochlorite, 2% glutaraldehyde, and 2% chlorhexidine digluconate were most effective against the analyzed microorganisms, followed by 100% vinegar, 3.8% sodium perborate.

In current study it was observed that denture cleansers were not only effective against *Candida* but also against other gram positive and negative bacteria. Same result was observed by Ida Suraya et al.¹⁵

All the three cleansers completely eradicated gram negative rods. Gram positive streptococci were found in group A and B. They were also completely eradicated but the result shows to be statistically non significant. This is because of small sample size. Clinical significance of these cleaners against gram negative rods and gram positive streptococci is evident from this study. In group D distilled water was used as a control. The CFUs count was reduced. This may be because of dilution factor by adding equal amount of water to solution II instead of denture cleaners.

CONCLUSION

From current study it was concluded that all denture cleaners are effective against *Candida* species, gram negative rods and gram positive streptococci but sodium hypochloride is more effective than the two.

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