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# COMPARATIVE EFFICACY OF COMBINED VS. SINGLE-METHOD DENTURE CLEANING PROTOCOLS FOR HALITOSIS AND BIOFILM REDUCTION. A RANDOMIZED CONTROL TRIAL IN COMPLETE DENTURE-WEARING POPULATION OF INDIA

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## ABSTRACT

*One among the common complications faced by complete denture wearing population is halitosis and biofilm associated complications. Even though the recommendation of mechanical or chemical means of cleaning denture is quite a common practice, the combined/integrated or individual efficacy of these methods in reducing the microbial load and halitosis especially in complete denture wearing population remains underexplored. This randomized control trial aimed to compare the efficacy of mechanical, chemical and the combination of both mechanical and chemical methods of denture cleaning in reducing halitosis and microbial load among complete denture wearers. Seventy-five participants using complete dentures (PMMA) with the age of 60-75 were randomly allotted to one of the 3 groups; mechanical brushing (group 1) using denture brush and toothpaste, chemical intervention of soaking denture overnight with sodium perborate tablet (group 2) and the combined protocol employing both the mechanical and chemical methods (group 3). Halitosis levels were interpreted using halimeter, detecting levels of VSCs (volatile sulphur compounds) and the microbial load (CFU) was assessed by a digital colony counter upon culturing the samples taken as swabs from denture surface at baseline and post four weeks. The collected data were analyzed using one-way ANOVA for mean difference comparison, Tukey post-hoc test to identify the level of significance among the groups and paired t-tests for comparison within groups at baseline and post intervention ( $\alpha=0.05$ ). The combined protocol (group 3) showed the highest level of reduction of VSCs and microbial load (CFU) at 35% and 39% respectively by outperforming chemical method (group 2) with 22% (VSC) and 25% (CFU) reduction. The mechanical group (group 1) revealed the least reduction levels with 11% (VSC) and 6% (CFU) despite showing significance difference ( $P=0.03$ ). A combined approach of integrating both chemical and mechanical cleaning methods with affordable and locally available products can have a significant impact on the denture hygiene by reducing the microbial load that is responsible for production of VSCs which in turn reflects in controlling the halitosis levels in complete denture wearers in Indian population.*

## CLINICAL IMPLICATIONS

Abiding to the combined protocol of using denture brush and toothpaste followed by immersion of denture overnight in sodium perborate tablet solution can be an effective choice in controlling halitosis by maintaining complete denture hygiene in Indian population where advanced aids such as ultrasonic cleansers cannot be an affordable choice to a significant sum of population.

Halitosis, the term derived from Latin ("halitus" meaning breath) and Greek ("osis" meaning a pathological process), is the condition defined as unpleasant odor from the mouth.<sup>1</sup> Commonly denoted as bad breath is one among the most common issues faced by the denture-wearing population.<sup>2</sup> The prevalence rate of halitosis especially in older population was 54.16%, out of which 84.61% were removable denture users.<sup>3</sup> It impacts the self-esteem and social life of an individual to a greater extent, leading to isolation and depression.<sup>4</sup> The etiology behind halitosis is linked to the production of volatile sulfur compounds (VSCs), comprising hydrogen sulfide, dimethyl sulfide, and methyl mercaptan<sup>5</sup>, by colony-forming microorganisms such as *Candida albicans* and a few other majorly prominent organisms in the biofilm.<sup>6</sup> In removable denture wearers, particularly in complete dentures, the biofilm formation occurs majorly on denture surfaces. The irregular and porous surface of PMMA dentures makes them more susceptible to biofilm formation.<sup>7</sup> It is further accelerated due to the deposition of food debris and prolonged contact of the denture with saliva, leading to the accumulation of plaque that harbors pathogens that are responsible for biofilm formation and the production of VSCs.<sup>8,9</sup>

Maintaining good oral hygiene is crucial in preventing the buildup of plaque on the denture surface. Along with it, a proper regimen for denture cleaning is also necessary to remove the biofilm from the denture surface. Conventional denture cleansing involves mechanical (using a denture brush and paste) and chemical interventions that aid in the disruption of the biofilm. Mechanical cleaning protocol using a denture brush can be effective in only removing food debris and is less effective in eliminating plaque as the bristles of the brush cannot reach the intricate areas of the porous surface of a denture.<sup>10-13</sup> Chemical cleansing agents are often categorized as bleach-based (sodium hypochlorite), enzyme-based, and finally peroxide effervescent tablets<sup>6,14,15</sup> and are well known for their capabilities in reducing the microbial load and release of volatile sulfur compounds (VSCs) as they are more potent in

disrupting the biofilm and aid in the reduction of pathogens causing halitosis in comparison to the mechanical means of denture cleaning, thus adding to improved hygiene of the oral cavity.<sup>8,16-19</sup> However, the *Candida* colonies do not exhibit a significant reduction in counts when exposed to chemical agents such as enzymatic peroxide-based cleanser.<sup>20</sup> This shows that following protocols that only employ either of the two methods might not be as effective as an integrated approach when it comes to denture cleaning.<sup>21-24</sup> Implementing an optimal protocol is quite a challenge, as the general denture-wearing population in India is not aware of such measures and resorts to suboptimal methods such as the usage of a toothbrush with only tap water.<sup>17,22,25</sup> It was revealed in a study by Pothala et al that about 48% of denture wearers have poor knowledge and awareness of denture cleaning protocols and denture cleansing agents.<sup>25</sup> There is clearly a lack of standardized protocols imposed by dental practitioners towards denture wearing patients.<sup>26</sup> The economic constraints have also been known to play a major part questioning the affordability of simple yet effective products such as denture brush and effervescent tablets and with this being the situation, it is a far-fetched idea to impose advanced cleaning agents and techniques that have emerged in the recent times, such as AgBr-NP@CTMAB (silver bromide nanoparticles (AgBr-NPs) coated with cetyltrimethylammonium bromide),<sup>27</sup> UV-lights<sup>28</sup> and ultra-sonic devices.<sup>29</sup>

The current literature on denture cleaning modalities reveals a significant gap. There are numerous studies that investigate hygiene levels in populations that use removable partial dentures.<sup>30-33</sup> Only a few studies have been done in concern with complete dentures where the confounding factors from the natural teeth are eliminated.<sup>34-37</sup> This oversight aligns in terms of relevancy with an ageing population increasingly dependent on complete dentures. With the notion of addressing the gaps associated with literature on complete denture hygiene, this randomized control trial aims to evaluate the efficiency of mechanical, chemical, and combined (mechanical-chemical) approaches in the reduction of VSC and microbial load associated with halitosis. The null hypothesis was that there is no significant reduction in levels of VSC and microbial load in the combined approach in comparison to the mechanical and chemical groups.

## MATERIAL AND METHODS

This randomised control trial commenced upon receiving ethical clearance from the scientific and

research committee of Dayananda Sagar University (288/3-IRB-DSCDS-IEC), aimed to measure the volatile sulphur compounds and microbial load in dentures which commenced in the department of prosthodontics in Dayananda Sagar College of Dental Science, Bangalore, India. All participants involved in this study were included upon receiving a written consent. The sample size calculation was done based on the effect size data from a study by baba et al.<sup>24</sup> For the 3-arm design of the current study, ANOVA's  $f=0.53$  was derived from Cohen's  $d=1.06$ , which is a large effect size reported by babe et al, based on the comparison observed in their mechanical and combination denture cleaning protocols. the calculations were performed using G power software (Version 3.1.9.7) with  $\alpha=.05$  and  $\beta=.20$  which yielded an initial sample size of 18 participants per group. To account for potential dropouts during the duration of the study, the final sample size per group was inflated to be 25 participants per group (total  $N=75$ ) at 15% attrition rate. A strict inclusion and exclusion were employed in filtering participants to increase the accuracy of the study. Participants aged between 40 -75 years with complete dentures on upper and lower arch for a period of 6 months fabricated with PMMA were included in this study. The gender of the participants was not taken into consideration in the current study as the objective of the study emphasized on the VSC levels and microbial load in denture surface which did not have a significant influence on the data across both male and females when in confluence with key factors such as continuous denture wearing hours, diet and age.<sup>38,39</sup> It was made sure that no active oral infection, lesion or ulceration was seen in the participants included. All participants were required to demonstrate a willingness to follow the planned protocol and ability to attend the follow ups that was scheduled. Participants who had underlying systemic conditions such as diabetes mellitus, liver cirrhosis, autoimmune disorders, chronic kidney diseases and immunocompromised condition such as HIV/AIDS were excluded. Participants who had a recent history of antibiotic use with the past four weeks were also excluded. Habits such as smoking and alcohol consumption were also noted to exclude participants.

The participants (25 per group) were divided into 3 groups (group 1: mechanical, group 2- chemical, group 3- combined) and were allocated in a random manner using Microsoft Excel' s RAND() function. Group 1 (mechanical) was provided with Clinsodent denture brush (ICPA health products, India) and were instructed to use it twice daily (morning and

night) with generic tooth paste that is affordable and easily available in the market (Colgate strong teeth toothpaste, Colgate India) for 3 minutes, while Clinsodent Denture cleansing tablets composed of 480 mg of Sodium perborate monohydrate (ICPA health products, India) was used in group 2 (chemical) with instructions to soak the denture overnight in effervescent denture cleansing tablet mixed with tap water. Group 3 (combined) were instructed to use both the products in combination as per the instruction provided for both group 1 and 2. Prior to baseline evaluation, all the participants were instructed to avoid eating and drinking 3 hours prior. Base line values for Volatile sulphur compounds (VSC) in participants ' breath were evaluated and recorded using a calibrated halimeter (Model: RH-17, Interscan Corporation, USA) by placing the sampling tube one centimetre in the participants ' open mouth making sure to avoid contact with oral mucosa, saliva and tongue. The readings were noted after 1 minute once the halimeter displayed stable reading. Microbial swabs were collected from the dentures (upper and lower) of each patient (palatal surface in upper denture and lingual surface in lower denture) using a sterile cotton moistened with sterile saline for assessing the CFU (colony forming units) at baseline. Each swab was placed into a sterile tube containing 5ml of phosphate-buffered saline (PBS) and vortexed for 30 seconds to make sure fine suspension of biofilm microorganisms are isolated. The samples went through serial dilution (up to  $10^{-5}$ ). The dilution selected ( $10^{-4}$ ) were plated (100  $\mu$ L) in triplicates in blood agar with sterile spreader and placed in incubator set at 37°C for 48 hours. The colonies were counted using a digital colony counter (Model CC-01 Plus, REMI Laboratory Instruments, India). To minimise variability, the mean CFU count for each of the participants was calculated from the triplicate plates. The obtained values were denoted in CFU/mL using the formula:  $CFU/mL = (\text{number of colonies} \times \text{dilution factor}) / \text{volume plated (mL)}$ . The reduction in microbial load for each of the group was determined using the formula:  $\text{Microbial Load Reduction \%} = ((\text{Baseline CFU Count} - \text{post intervention CFU count}) / \text{baseline CFU}) \times 100$ .

After four weeks the participants were recalled for follow up to record post intervention data. The protocol followed during pre-intervention (Baseline) was repeated with no addition or modification in the protocol and sequence of collecting samples.

The data obtained from baseline and post intervention event for VSC and CFU were analyzed using IBM SPSS Statistics (Version 26.0). Mean,

standard deviation (SD) and percentage reduction was evaluated for VSC and CFU at both the events (Baseline, post intervention). Shapiro - wilk test was used to access normality of distribution of data to proceed further. The mean microbial load reduction and VSC reduction was assessed by One-way ANOVA across the three groups. To identify the significance between each group post-Tukey test was done.

## RESULTS

From February of 2024 till January of 2025, a total of 75 complete denture wearers were observed and assessed for management of halitosis. Out of these, 4 participants were excluded due to improper implementation of protocol, and 2 participants were lost to follow-up. The final sample size was rendered to be 23 participants per group (N=69). All the groups showed a significant VSC reduction in the post-intervention period. The combined protocol (group 3, n=23) yielded the highest level in terms of VSC reduction by 35% (from  $277 \pm 25$  to  $180 \pm 24$  ppb (mean)), followed by 22% VSC (from  $247 \pm 18$  to  $192 \pm 19$  ppb (mean)) reduction in the chemical method (group 2, n=23) (Table 1). The mechanical method

(group 1) showed the least VSC reduction percentage of 11% (from  $268 \pm 20$  to  $238 \pm 22$  ppb (mean)) (Fig 1) which may indicate the poor capabilities in disrupting the biofilm contributing to the VSC in intricate parts of the porous denture surface. One way ANOVA revealed that the significance in between the groups were convincing ( $p < 0.0001$ ) with Tukey post-hoc confirming the highest significance seen in combined protocol (group 3)  $P < .0001$  followed by chemical method (group 2)  $P = .0001$  and mechanical methods (group 1)  $P = .008$ .

The microbial load analysis reveals combined protocol (group 3) showing the highest reduction percentage of 39% from  $7.90 \pm .26$  to  $4.80 \pm .32$  ( $P < .0001$ ). The chemical method (group 2) data were also significant ( $P = .0002$ ) at a reduction percentage of 25% from  $7.51 \pm .24$  to  $5.60 \pm .28$  (Table 2, Fig 2). The mechanical method (group 1) was the lowest among the three group which showed 6% reduction from  $7.80 \pm .22$  to  $7.30 \pm .25$ . Despite the lowest reduction percentage among the 3 groups the data were statistically significant ( $P = .03$ ). No adverse events or deviation from the planned protocol were reported.

## TABLES

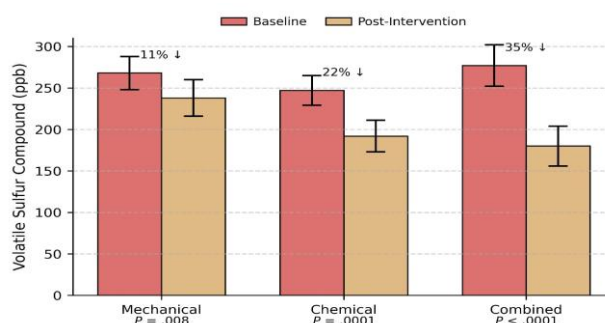
**Table 1.** Comparing the reduction percentage of volatile sulfur compound (VSCs) levels at baseline and after four weeks in parts per billion (ppb)

PROTOCOL	BASELINE	POST-INTERVENTION	REDUCTION	P-Value
Group 1-Mechanical	$268 \pm 20$	$238 \pm 22$	11%	$P = .008$
Group 2-Chemical	$247 \pm 18$	$192 \pm 19$	22%	$P = .0001$
Group 3-Combined	$277 \pm 25$	$180 \pm 24$	35%	$P < .0001$

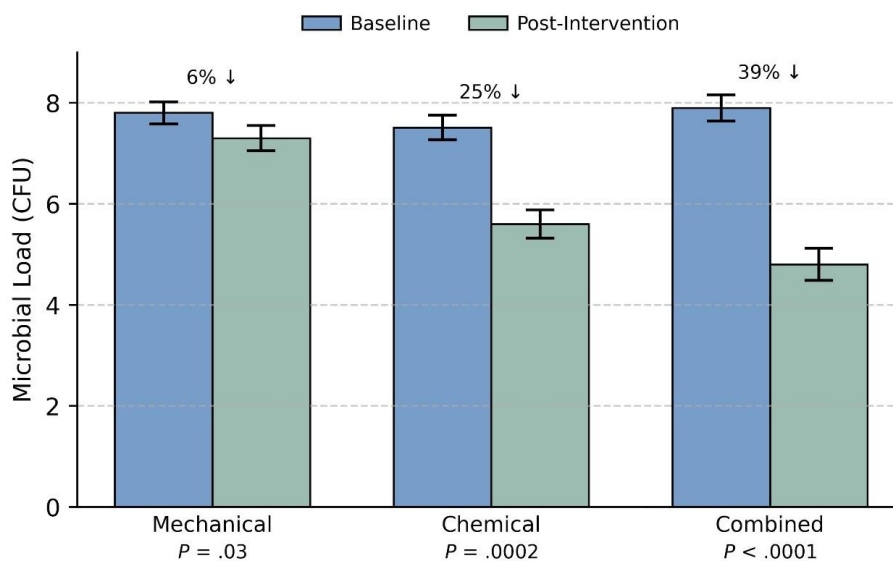
**Table 2.** Comparing the reduction percentage of microbial load as Colony Forming Units (CFU;log10) at baseline and after four weeks.

PROTOCOL	BASELINE	POST-INTERVENTION	REDUCTION	P-Value
Group 1- Mechanical	$7.80 \pm .22$	$7.30 \pm .25$	6%	$P = .03$
Group 2-Chemical	$7.51 \pm .24$	$5.60 \pm .28$	25%	$P = .0002$
Group 3-Combined	$7.90 \pm .26$	$4.80 \pm .32$	39%	$P < .0001$

## FIGURES



**Figure 1.** Percentage of reduction in Volatile sulfur compound (VSCs) levels in parts per billion (ppb) measured at baseline and post intervention (after 4 weeks) across the three groups (group 1 to 3; left to right). Error bars indicate Standard Deviation (SD; n=23 per group).



**Figure 2.** Microbial colony reduction (CFU; Colony Forming Units) from baseline measurement to post-intervention (4 weeks apart) across the three groups (group 1 to 3; left to right). Error bars indicate Standard Deviation (SD; n=23 per group).

## DISCUSSION

This study hypothesized that combining both mechanical and chemical methods of denture cleaning into one integrated protocol would result in superior efficiency than mechanical and chemical methods individually as far as the reduction of VSCs and denture microbial load is concerned in controlling halitosis, thus rejecting the null hypothesis.

The combined protocol yielded a significant result in contrast to either of the methods individually which indicated the synergistic nature of physical brushing with usage of sodium perborate tablet solution. This directly translates to decrease in halitosis which is clinically relevant for patients suffering from this condition which has a major impact on psychological aspect and general quality of life.<sup>4,6</sup> The findings in current study aligns with the already established principle of approaching halitosis with multiple treatment modalities for optimal control over biofilm formation.<sup>12,16,20,37</sup> Using a tooth brush can be a better choice for removal of debris but it is inefficient in disrupting the biofilm matrix.<sup>13,20,21</sup> In chemical methods (group 2), Clinsodent tablets containing 480mg of sodium perborate have been associated with its ability to disrupt the biofilm revealing its antimicrobial efficacy which can be inferred from the reduction of CFU values. This finding aligns with a study by Sharma et al which analyzed antibacterial and antimicrobial efficacy of effervescent tablets that are similar in composition to sodium perborate monohydrate.<sup>9</sup> This data holds valid with few other

studies that show similar effect of chemical agents such as effervescent tablets in optimal biofilm control.<sup>16,19</sup> However the lesser efficiency when compared to combined protocol (group 3) imply that standalone usage of chemical agents may not be sufficient to penetrate and eliminate biofilm in majority of the areas in a denture surface.<sup>20,40</sup> The reduction in microbial load can be directly linked to lowered risk of denture stomatitis and other prominent oral infections.<sup>20,37,41</sup> It is important to mention that the study did not focus on identifying or analyzing various stains of bacterial species or fungi but on assessing the overall impact of the different cleaning protocols in reducing the biofilm and its correlation with reduction of halitosis.

It is also important to address the lack of awareness among the Indian population on effective denture hygiene protocols where 48% of regular denture wearers were not clear on various protocols and effective techniques and solely relied on basic practices like scrubbing or rinsing under tap water.<sup>22,42</sup> This clearly shows a lack of proper communication and inconsistent guidance from dental practitioners due to a lack of standardized protocol on educating the denture wearing population on effective denture cleaning regimen<sup>11,17,22,25,30,42</sup> which can lead to lower follow-up rates.

This study laid a greater emphasis on selecting affordable and readily available denture cleaning products in the Indian market such as Clinsodent denture cleansing brush, effervescent tablets and

Colgate strong teeth toothpaste due to the fact that majority of the denture wearers in India are not economically capable of including premium and advanced denture cleaning products<sup>27-29</sup> and agents in their daily routine.<sup>11,17,18,22,23,26,42</sup> This demonstrates the efficacy on combining conventional denture cleaning techniques to act in synergy which produce significant results at a fraction of the cost of advanced premium products. For instance, Nishi et al found that there was no difference in the statistical significance between using Polident in combination with brushing and ultrasonic cleaning claiming both the methods to be effective with marginal difference.<sup>43</sup> This goes to show that raising awareness among the dental practitioners that even basic and affordable products when used in combination with guiding the denture wearing patients into a proper regimen can result in a significant improvement in the overall hygiene of the dentures which can be an effective way to control halitosis thereby enhancing patients self-esteem and confidence.

However, the limitation of this study is the four-week intervention period which may have underestimated the long-term benefits of the

combine protocol thus asserting a need for longer follow up periods. The lack of patient reported breath odor (subjective) limits the assessment of the impact on quality of life. The specific products used and the demographic characteristics of the population included in the present study limits the generalizability of the findings. Any future research should address these limitations by increasing the sample size with longer periods of interventions and subjective measures to assess the patient's perspective of improvement in halitosis. The cost effectiveness of other popular agents in denture cleaning methods must also be understood to ensure a positive impact on the patient's adherence to denture hygiene protocols.

### CONCLUSION

The following conclusions can be derived from the findings of this randomized control trial:

1. The combination of mechanical and chemical denture cleaning methods significantly outperforms individual use of either of the methods in reduction of halitosis by reduction in levels of VSCs and microbial load in the denture surface.

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