



## FREQUENCY OF DENTURE HYGIENE PRACTICES AND THEIR IMPACT ON ORAL MICROBIOME IN PATIENTS WEARING REMOVABLE PARTIAL DENTURES

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### ABSTRACT

**Background:** Denture hygiene practices are critical in maintaining oral health, particularly in patients wearing removable partial dentures..

**Objectives:** To determine the frequency of denture hygiene practices and their impact on oral microbiome in patients wearing removable partial dentures.

**Methods:** The study was conducted using a cross-sectional design, enrolling 150 patients who had been using RPDs for at least six months at Dental Department Shahida Islam Medical & Dental College, Lodhran from Jan 2021 to June 2021. Inclusion criteria for the study included adult patients aged 18-75 years who were wearing RPDs and were willing to participate in the study. All participants provided written informed consent prior to inclusion in the study. The questionnaire was designed to assess participants' demographic information, denture hygiene practices (including frequency and methods of cleaning), and any reported oral health issues. The data were analyzed using SPSS software (version 26.0).

**Results:** The majority of participants (40%) were between 51-65 years old, with a higher proportion of males (56.7%) compared to females (43.3%). Patients who cleaned their dentures less frequently (less than once daily) had significantly higher mean counts of Streptococcus mutans, Candida albicans, and Lactobacillus species compared to those who cleaned more often ( $p < 0.05$ ). The microbial load decreased with more frequent cleaning, particularly for those who cleaned their dentures after every meal. The combination of mechanical and chemical cleaning methods was associated with the lowest prevalence of oral lesions (11.1%), whereas mechanical brushing alone resulted in a higher incidence of oral lesions (25.0%).

**Conclusion:** In conclusion our study confirms that the frequency of denture cleaning has a significant impact on the prevalence of oral microorganisms. Less frequent cleaning is associated with higher counts of *Streptococcus mutans*, *Candida albicans*, and *Lactobacillus* species, highlighting the importance of regular denture maintenance.

**Keywords:** Denture hygiene, Oral microbiome, Removable partial dentures, Oral health, Denture care practices, Microbial load,

## INTRODUCTION

The use of removable partial dentures (RPDs) is a common solution for individuals who have lost one or more teeth but still retain some of their natural dentition. RPDs provide aesthetic and functional benefits, allowing patients to chew and speak more effectively while also improving their appearance. However, the introduction of a foreign appliance into the oral cavity, such as an RPD, significantly alters the oral environment.<sup>1,2</sup> This alteration can have profound effects on the oral microbiome—the community of microorganisms that reside in the mouth. Maintaining good oral hygiene practices is essential for preventing oral diseases, particularly in individuals wearing RPDs, as poor hygiene can lead to an imbalance in the oral microbiome, potentially resulting in various oral and systemic health issues.<sup>3,4</sup>

The oral microbiome plays a crucial role in maintaining oral health by preventing the colonization of pathogenic bacteria and supporting the overall balance of microbial communities. However, the presence of an RPD creates additional surfaces for microbial colonization, including areas that are not easily accessible during routine oral hygiene practices.<sup>5,6</sup> This can lead to the accumulation of biofilm, a complex structure of bacteria that adheres to surfaces in the mouth. Biofilm accumulation on dentures can result in a shift in the composition of the oral microbiome, favoring the growth of pathogenic microorganisms such as *Candida* species, which are associated with denture stomatitis and other oral infections.<sup>7</sup>

Denture hygiene practices, including the frequency and methods used for cleaning RPDs, are critical in controlling the biofilm formation and maintaining a healthy oral microbiome. Despite the importance of these practices, studies have shown that many denture wearers do not adhere to recommended cleaning protocols, often due to a lack of knowledge or awareness. Inadequate cleaning of dentures can lead to the persistence of pathogenic microorganisms on the denture surfaces, increasing the risk of oral infections and other complications such as halitosis (bad breath), dental caries, periodontal disease, and even systemic conditions like pneumonia in vulnerable populations.<sup>8</sup>

The impact of poor denture hygiene is not limited to the oral cavity; it can have systemic implications as well. The oral cavity serves as a gateway to the rest of the body, and an imbalance in the oral microbiome can lead to the translocation of harmful bacteria into the bloodstream, potentially contributing to systemic diseases such as cardiovascular disease, diabetes, and respiratory infections. Therefore, understanding the relationship between denture hygiene practices and the oral microbiome is essential for developing effective strategies to promote oral and systemic health in patients wearing RPDs.<sup>9</sup>

Research has shown that the frequency and thoroughness of denture cleaning are key factors in determining the composition of the oral microbiome. Regular cleaning of RPDs can reduce the microbial load and prevent the overgrowth of pathogenic species. However, the optimal frequency of cleaning and the most effective cleaning methods remain subjects of debate. Literature suggest that cleaning dentures twice a day with appropriate agents, such as antimicrobial mouth rinses or denture cleansers, is effective in maintaining a healthy oral microbiome. Others have proposed that more frequent cleaning, combined with mechanical brushing, may be necessary to prevent biofilm accumulation.<sup>10,11</sup>

In addition to traditional cleaning methods, emerging technologies and approaches are being explored to enhance denture hygiene and protect the oral microbiome. For example, the use of

probiotics, antimicrobial coatings, and advanced denture materials are being investigated as potential ways to reduce microbial colonization on denture surfaces. These innovations could offer new avenues for improving the oral health of denture wearers and minimizing the impact of RPDs on the oral microbiome.<sup>12</sup>

Therefore, it is essential to promote proper denture hygiene through patient education, regular follow-ups, and the exploration of new technologies that can enhance the effectiveness of cleaning practices. By doing so, healthcare providers can help ensure that patients wearing RPDs maintain a healthy oral microbiome and reduce their risk of oral and systemic diseases.

## **MATERIALS AND METHODS**

The study was conducted using a cross-sectional design, enrolling 150 patients who had been using RPDs for at least six months at Dental Department Shahida Islam Medical & Dental College, Lodhran from Jan 2021 to June 2021. Participants were recruited from the dental clinics of two major hospitals, with ethical approval obtained from the respective institutional review boards.

Inclusion criteria for the study included adult patients aged 18-75 years who were wearing RPDs and were willing to participate in the study. Exclusion criteria included patients with systemic conditions known to affect oral health (such as diabetes or immunocompromised states), those on long-term antibiotic therapy, and those who had undergone recent oral surgery. All participants provided written informed consent prior to inclusion in the study.

Data were collected through a structured questionnaire and clinical examination. The questionnaire was designed to assess participants' demographic information, denture hygiene practices (including frequency and methods of cleaning), and any reported oral health issues. The frequency of denture cleaning was categorized as follows: once daily, twice daily, after every meal, and less than once daily. The methods of denture cleaning were categorized into mechanical (brushing with or without toothpaste), chemical (use of denture cleansers or mouth rinses), and a combination of both.

The clinical examination involved the collection of oral swabs from the denture surfaces, the tongue, and the buccal mucosa. Samples were collected using sterile swabs, which were immediately placed in transport media and transported to the microbiology laboratory for analysis. Microbial analysis was conducted to identify and quantify the presence of key bacterial and fungal species known to be associated with oral health and disease, including *Streptococcus mutans*, *Candida albicans*, and *Lactobacillus* species. Culture methods were employed to isolate these microorganisms, and their identification was confirmed through biochemical tests and molecular techniques such as PCR (polymerase chain reaction).

To assess the impact of denture hygiene practices on the oral microbiome, participants were grouped based on their reported frequency of denture cleaning. The microbial profiles of these groups were compared using statistical analysis. Descriptive statistics were used to summarize the demographic data and hygiene practices, while inferential statistics, including chi-square tests and ANOVA (analysis of variance), were employed to compare microbial counts across different groups. The significance level was set at  $p < 0.05$ . The data were analyzed using SPSS software (version 26.0). The results were presented as mean microbial counts and percentages for categorical variables. The study also performed multivariate analysis to control for potential confounders, such as age, gender, and smoking status, in assessing the relationship between denture hygiene practices and the oral microbiome.

## **STUDY RESULTS**

The majority of participants (40%) were between 51-65 years old, with a higher proportion of males (56.7%) compared to females (43.3%). Most participants had been using RPDs for over a year, with 36.7% using them for 1-3 years.

**Table 1: Demographic Characteristics of the Study Population (n=150)**

Demographic Variable	Frequency (n)	Percentage (%)
<b>Age Group</b>		
18-35 years	20	13.3%
36-50 years	40	26.7%
51-65 years	60	40.0%
66-75 years	30	20.0%
<b>Gender</b>		
Male	85	56.7%
Female	65	43.3%
<b>Duration of Denture Use</b>		
6 months - 1 year	45	30.0%
1 - 3 years	55	36.7%
>3 years	50	33.3%

The majority of participants (40%) were between 51-65 years old, with a higher proportion of males (56.7%) compared to females (43.3%). Most participants had been using RPDs for over a year, with 36.7% using them for 1-3 years.

**Table 2: Frequency of Denture Hygiene Practices (n=150)**

Denture Hygiene Practice	Frequency (n)	Percentage (%)
<b>Cleaning Frequency</b>		
Once daily	30	20.0%
Twice daily	55	36.7%
After every meal	35	23.3%
Less than once daily	30	20.0%
<b>Cleaning Method</b>		
Mechanical (Brushing)	60	40.0%
Chemical (Denture Cleansers/Mouth Rinses)	45	30.0%
Combination of Mechanical and Chemical	45	30.0%

A significant proportion of participants (36.7%) cleaned their dentures twice daily, while 23.3% cleaned them after every meal. Mechanical brushing was the most common cleaning method (40%), followed by a combination of mechanical and chemical methods (30%).

**Table 3: Prevalence of Oral Microorganisms Based on Denture Cleaning Frequency (n=150)**

Microorganism	Cleaning Frequency Mean CFU/mL (SD)	p-value
<b>Streptococcus mutans</b>		
Once daily	$7.2 \times 10^4 (\pm 1.5)$	
Twice daily	$4.8 \times 10^4 (\pm 1.2)$	
After every meal	$3.5 \times 10^4 (\pm 1.0)$	
Less than once daily	$9.0 \times 10^4 (\pm 2.0)$	0.002**
<b>Candida albicans</b>		
Once daily	$2.8 \times 10^3 (\pm 0.5)$	
Twice daily	$1.5 \times 10^3 (\pm 0.3)$	
After every meal	$1.2 \times 10^3 (\pm 0.2)$	
Less than once daily	$3.5 \times 10^3 (\pm 0.6)$	0.001**
<b>Lactobacillus species</b>		

Once daily	$5.6 \times 10^4 (\pm 1.3)$	
Twice daily	$3.8 \times 10^4 (\pm 1.1)$	
After every meal	$2.9 \times 10^4 (\pm 0.9)$	
Less than once daily	$7.5 \times 10^4 (\pm 1.8)$	0.003**

\* CFU/mL: Colony-forming units per milliliter, \*\*Statistically significant ( $p < 0.05$ )

Patients who cleaned their dentures less frequently (less than once daily) had significantly higher mean counts of **Streptococcus mutans**, **Candida albicans**, and **Lactobacillus species** compared to those who cleaned more often ( $p < 0.05$ ). The microbial load decreased with more frequent cleaning, particularly for those who cleaned their dentures after every meal.

**Table 4: Correlation Between Cleaning Methods and Oral Lesions (n=150)**

Cleaning Method	Oral Lesions Present (n)	Oral Lesions Absent (n)	Percentage with Lesions (%)	p-value
Mechanical (Brushing)	15	45	25.0%	0.018**
Chemical (Denture Cleansers/Mouth Rinses)	12	33	26.7%	
Combination of Mechanical and Chemical	5	40	11.1%	

\*\*Statistically significant ( $p < 0.05$ )

The combination of mechanical and chemical cleaning methods was associated with the lowest prevalence of oral lesions (11.1%), whereas mechanical brushing alone resulted in a higher incidence of oral lesions (25.0%). The results were statistically significant ( $p = 0.018$ ), suggesting that a combination of cleaning methods is more effective in preventing oral lesions. The study found that more frequent and combined denture hygiene practices significantly reduce the microbial load in the oral cavity and lower the incidence of oral lesions in patients wearing RPDs. Regular denture cleaning, especially after every meal, was associated with healthier oral microbiome profiles, while a combination of mechanical and chemical cleaning methods was most effective in preventing oral lesions. These findings underscore the importance of patient education and adherence to recommended denture hygiene practices to maintain both oral and systemic health.

## DISCUSSION

The present study explored the impact of denture cleaning frequency on the prevalence of oral microorganisms in patients using removable partial dentures. The findings reveal that less frequent cleaning is associated with higher levels of *Streptococcus mutans*, *Candida albicans*, and *Lactobacillus species*, highlighting the crucial role of regular denture maintenance in oral health.

Our results demonstrated a mean CFU/mL of 90,000 ( $\pm 20,000$ ) for *Streptococcus mutans* in patients who cleaned their dentures less than once daily, compared to 72,000 ( $\pm 15,000$ ) for those who cleaned once daily. This difference was statistically significant ( $p = 0.002$ ). These findings align with those of Ali et al. (2020), who observed a significant increase in *Streptococcus mutans* counts in patients with infrequent denture cleaning, with their results showing similar CFU/mL values.<sup>13</sup> In addition, Smith et al. (2021) reported that inadequate denture cleaning resulted in elevated levels of *Streptococcus mutans*, supporting our observation.<sup>14</sup>

The correlation between poor denture hygiene and increased *Streptococcus mutans* is well-documented. A study by Wilson and Patel (2022) corroborated our results by demonstrating higher microbial counts in patients with suboptimal cleaning practices.<sup>15</sup> Their research highlighted the direct impact of cleaning frequency on bacterial populations, with *Streptococcus mutans* levels significantly reduced in individuals adhering to rigorous cleaning protocols.

Regarding *Candida albicans*, our study found a mean CFU/mL of 3,500 ( $\pm 600$ ) for patients with infrequent cleaning versus 2,800 ( $\pm 500$ ) for those cleaning once daily, with a statistically significant

difference ( $p = 0.001$ ). This observation is consistent with the findings of Chen et al. (2020), who reported elevated *Candida albicans* counts in patients with poor denture hygiene. Their study showed a mean CFU/mL of 3,200 for infrequent cleaners, aligning closely with our results.<sup>16</sup>

Similarly, Brown et al. (2021) demonstrated that frequent denture cleaning effectively reduced *Candida albicans* levels, supporting our conclusion that regular cleaning is crucial in managing fungal infections.<sup>17</sup> Their research found that patients cleaning their dentures daily had significantly lower *Candida albicans* counts compared to those with less frequent cleaning.

For *Lactobacillus* species, our study revealed a mean CFU/mL of 75,000 ( $\pm 18,000$ ) in patients who cleaned their dentures less than once daily, compared to 56,000 ( $\pm 13,000$ ) for those cleaning daily, with a  $p$ -value of 0.003. These results are consistent with Kumar et al. (2022), who reported that infrequent denture cleaning was associated with higher *Lactobacillus* species counts.<sup>18</sup> Their study showed similar statistics, reinforcing the importance of regular denture maintenance in controlling microbial growth.

In a study by Lee and Wong (2021), higher levels of *Lactobacillus* species were observed in patients with inadequate cleaning practices, further supporting our findings.<sup>19</sup> Their results highlighted the correlation between poor hygiene and increased counts of *Lactobacillus* species, which aligns with our data. Our results contribute to the growing body of evidence highlighting the importance of regular denture cleaning. Ali et al. (2020) found that the frequency of denture cleaning significantly influenced microbial counts, with their study showing similar trends in *Streptococcus mutans* and *Candida albicans* levels.<sup>14</sup> Their findings underscore the necessity of stringent cleaning practices to manage oral microbial populations effectively.

Additionally, a study by Nguyen et al. (2022) reported that patients with frequent denture cleaning had lower microbial loads, consistent with our observations.<sup>20</sup> Their research demonstrated that proper denture hygiene significantly reduced the prevalence of oral microorganisms, reinforcing our results.

A comprehensive review by Anderson et al. (2023) highlighted the benefits of regular denture cleaning in managing microbial populations and preventing associated oral health issues.<sup>20</sup> Their findings support our conclusion that more frequent cleaning is crucial in reducing microbial counts and maintaining oral health.

Furthermore, a study by Taylor et al. (2021) emphasized the importance of adherence to cleaning protocols in controlling microbial loads, aligning with our data.<sup>21</sup> Their research demonstrated that patients who followed recommended cleaning practices had significantly lower counts of *Streptococcus mutans*, *Candida albicans*, and *Lactobacillus* species.

## CONCLUSION

In conclusion our study confirms that the frequency of denture cleaning has a significant impact on the prevalence of oral microorganisms. Less frequent cleaning is associated with higher counts of *Streptococcus mutans*, *Candida albicans*, and *Lactobacillus* species, highlighting the importance of regular denture maintenance. These findings are consistent with previous research and contribute to the broader understanding of how cleaning frequency affects oral microbial populations. Adhering to recommended cleaning practices is essential for controlling microbial growth and ensuring optimal oral health in denture wearers.

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