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The Influence of 0.2 % Chlorhexidine Mouthwash on Oral Bacterial Load: An in Vivo Comparative Study in Tobacco and Non-Tobacco Users

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Abstract

Introduction: Saliva plays a vital role in oral health and overall well-being of an individual. It lubricates and moistens the mouth for comfortable speech and eating, helps regulate body water balance, and prevents tooth decay and infection by removing debris and bacteria. The oral microbiome in the saliva and oral cavity dictates a lot of in diagnosis of oral and systemic diseases. One of the most concerning disease is dental caries which can be caused by abnormal shift in microflora due to various systemic conditions or due to habits of the patient. Cigarette smoking and nicotine exposure affects the buffering capacity of saliva, promotes the cariogenic bacteria and reduces the commensals thereby creating an imbalance in oral microbial flora.

Objective: The purpose of this study is to evaluate the influence of 0.2 % chlorhexidine mouthwash on Oral Bacterial Load in tobacco and non-tobacco users.

Materials and methods: Among patients reported to OPD of Department of conservative dentistry and Endodontics, detailed case history and informed consent was taken and 24 patients were selected for the study. Unstimulated saliva was collected via the drooling method on day 0. Participants received a labelled mouthwash bottles and were abstained from using other mouthwashes during the study period. Participants rinsed with 10 ml of mouthwash twice daily for 30 s without water. On day 4 participants returned with mouthwash bottles and saliva was collected. Microbiological assessment and statistical analysis was done.

Results: Both the groups – tobacco and non tobacco users showed a significant decrease in the CFU unit of total bacterial count. However, Group 2 comprising of the non tobacco users showed greater reduction in bacterial count post mouthrinse.

Conclusion: Mouthrinse using 0.2% chlorhexidine significantly reduced the colony forming unit of the total bacterial count

Keywords: Saliva, Colony forming unit, oral bacterial load, chlorhexidine

Introduction

Saliva, a thick, colorless, opalescent fluid that is constantly present in the mouth of humans and other vertebrates, composed of water, mucus, proteins, mineral salts, and amylase. Saliva plays a vital role in oral health and overall well-being.[1] It lubricates and moistens the mouth for comfortable speech and eating, helps regulate body water balance, and prevents tooth decay and infection by removing debris and bacteria. Additionally, saliva contains the enzyme amylase, which breaks down carbohydrates into simpler compounds. By performing these functions, saliva facilitates essential processes like digestion, hydration, and oral hygiene, making it a crucial component of our overall health. It is probably surprising for most people to learn that saliva has been used in diagnostics for more than 2000 years. [2]

The oral microbiome in the saliva and oral cavity dictates a lot of in diagnosis of oral and systemic diseases. One of the most concerning disease is dental caries which can be caused by abnormal shift in microflora due to various systemic conditions or due to habits of the patient.[2] Cigarette smoking and nicotine exposure affects the buffering capacity of saliva promoting the cariogenic bacteria and reduces the commensals.[3]

The modern man has transformed the oral microflora due to dietary factor, lifestyle and oral hygiene factors. The inclusion of mouthwashes in the oral hygiene regimen has been welcomed by many lifestyle enthusiasts. There are numerous mouthwashes available in market, of which chlorhexidine still remains the popular choice for dentists. Chlorhexidine (CHX) has been commonly used in dental practice as antiseptic agent since 1970, due to its long-lasting antibacterial activity with a broad-spectrum of action.[6]

The objectives of this study are:

- To assess the colony forming unit pre and post mouthrinse using 0.2 % chlorhexidine mouthwash in tobacco users
- To assess the colony forming unit pre and post mouthrinse using 0.2 % chlorhexidine mouthwash in non tobacco users
- To compare colony forming unit pre and post mouthrinse after the usage of 0.2% chlorhexidine mouthwash in tobacco and non tobacco users.

Inclusion Criteria

- Individuals of age 20-40 years
- Participants with healthy periodontal conditions and not using mouthwashes in their normal oral hygiene routine.
- Self-reported current tobacco and non tobacco users were included

Exclusion Criteria

- Evidence of gingival inflammation, periodontitis, and removable or fixed prosthesis and orthodontic appliances.
- systemic disease/conditions
- Pregnant and lactating mother

- Patients on antibiotics, bisphosphonates, probiotics, steroids and non-steroidal analgesics within the past 90 days were not sought
- Recent history of oral prophylaxis
- Usage of mouthwashes in their routine oral hygiene practices

Methodology:

Sample Collection

Among patients reported to OPD of Department of conservative dentistry and Endodontics, detailed case history and informed consent was taken and 24 patients were selected for the study. Unstimulated saliva was collected via the drooling method on day 0. Unstimulated saliva were collected during early morning hours with the participants being in a fasting state. The patients were comfortably seated on a chair in a quiet room and requested to allow saliva to accumulate in the mouth for 5 continuous minutes. Patients were advised to refrain from swallowing and jaw movements. After 5-minutes, the participants drooled the saliva into a disposable plastic funnel that was attached to a gauged disposable measuring cylinder. Participants received a labelled mouthwash bottles and were abstained from using other mouthwashes during the study period. Participants rinsed with 10 ml of mouthwash twice daily for 30 s without water. On day 4 participants returned with mouthwash bottles and saliva was collected. Microbiological assessment and statistical analysis was done.

Microbiological Assessment (Salivary Bacterial Count)

- Salivary samples were code-labelled. Serial dilution with phosphate buffer saline up to 2X was done. Serial dilution with phosphate buffer saline up to 2X was done. Care was taken not to touch or contaminate the surface of agar in the culture plates and they were incubated at 37°C for 24h. Colonies were counted with a magnifying digital colony counter (Labtronics microprocessor colony counter). Each sector was observed for growth of microorganisms. Colonies were identified and counted in the sector with the largest concentration of full-size discrete colonies. The number of colony forming units (CFU) per ml from the original aliquot per sample was calculated.

Statistical Analysis:

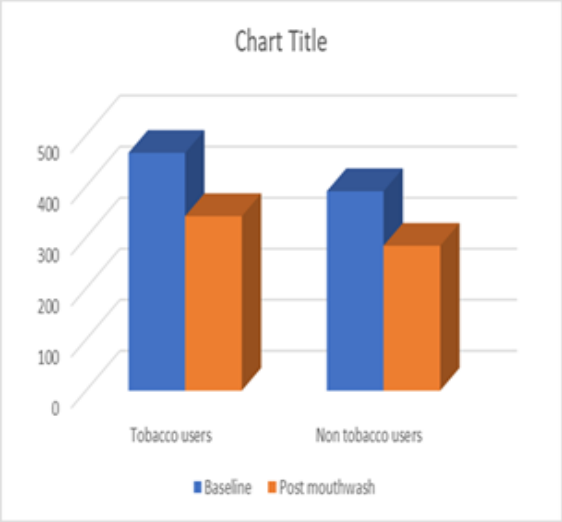
Data that were obtained were analysed using the statistical software SPSS version 27. A parametric paired student t test was used for comparing the two groups followed by post-hoc Tuckey test for in-between groups comparisons, and independent t-test for comparing two quantitative data. The level of significance was set at **P**<0.05.

Results:

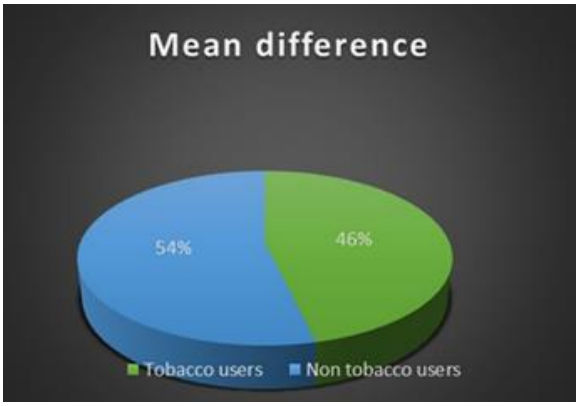
GROUP	PROCEDURE		Mean difference	Standard deviation	T test	P value
Tobacco users	Baseline	465.8	106.9	93.41	4.37	P<0.0015
	Post mouthwash	358.9				
Non Tobacco Users	Baseline	390.7	123.9	83.29	4.06	P<0.0001
	Post mouthwash	266.8				

Table 1:

Table 1 presents the comparison of oral bacterial load pre and post mouth rinse among two groups: tobacco and non tobacco users. The paired student t test shows a highly significant difference between the groups, with a p-value of less than 0.001. This indicates that the differences in colony forming unit pre and post mouth rinse among the groups are statistically significant.



Graph 1:



Graph 2:

The graph 1 shows that the decrease in the oral bacterial load pre and post mouth rinse was higher in non tobacco users than the tobacco users. However, both groups showed significant decrease in the colony forming unit count post mouthrinse.

The graph 2 shows that the mean difference was 54% in non tobacco users and 46% in tobacco users, which indicates the tobacco group showed greater counts of oral bacterial load before mouth rinse and showed a decrease in the count when compared to non tobacco users.

Discussion

Tobacco consumption has been recognized as a significant risk factor for dental caries, exacerbating the severity and prevalence of this pervasive oral health issue. This also culminates in developing periodontal disease, and other oral pathologies. The deleterious effects of tobacco on dental health are multifaceted according to Axelsson et al. The implications results in enamel erosion, microbial imbalance, delayed healing, root caries, salivary inhibition.[7]

In our study, mouthwash containing chlorhexidine was used as it is considered the gold standard owing to its superior antiplaque and antimicrobial efficacy. It is helpful to include the most efficacious and widely used products in comparative studies for greater validity. [8]Roberts et al showed that a single rinse with CHX could reduce oral flora from 50% to 90% for several hours. The substantivity of CHX is adequately documented and study showed a significant inhibition of salivary microflora count by the mouthwash. In this study, the oral bacterial load by determining the CFU unit to compare the efficacy of CHX mouthwash pre and post rinse. The chlorhexidine mouthwash is used as anti microbial agent to reduce the bacterial load and its response is dose and duration dependant.[8]

Tobacco smoking is one of the leading causes of preventable deaths worldwide. Smoking induces inflammation and consequent immune modulation.[9] To reduce the harms of continued smoking on general health and oral health, different strategies have been developing to cope with high smoking consumption worldwide.[10] Dentists need to play a vital role in preventing the damaging effects of smoking in the mouth. Cigarette smoking affects reactive free radicals and volatile aldehydes in saliva and causes a transient decline in the availability of saliva, buffering capacity (pH levels) is related to a higher risk for dental caries. Salivary buffering by carbonates significantly affects

Stephan curve, and salivary activities have a potential impact on a plaque that is the primary cause of oral disease.[11]

The results suggests that CHX mouthwash showed statistically significant values in reducing bacterial count in both groups. [12]However the magnitude of reduction was slightly higher in Non tobacco than tobacco users. Reduction in total bacterial count will be more pronounced in Non-Tobacco users compared to Tobacco users. This is due to baseline bacterial loads, oral health status and adverse effect of smoking.[12]

Non-Tobacco users typically have lower baseline bacterial counts, allowing for a greater relative reduction. They tend to have better oral health, making it easier for mouthwash to effectively reduce bacterial counts. In nicotine groups, more biofilm was formed composed of bacterial cells and extracellular polymeric substances (EPS).[13] The biofilm was more structurally formed with longer bacterial chain length and more orientated cell arrangement than the control. Tobacco users' oral environments are compromised due to smoking's harmful effects, potentially reducing mouthwash efficacy.[13]

Conclusion

In conclusion, the study demonstrates that Tobacco usage have a profound impact on oral microbes. The toxic chemicals present in tobacco, such as nicotine, formaldehyde, and acrolein, can alter the growth and diversity of oral microorganisms.[13] The mouthwashes can be included in the oral hygiene practice that can help combat oral health issues. However, quitting tobacco remains the best way to maintain a good oral health.

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