Research Article

Comparison of oral microbiota among smokers and non-smokers - A pilot study

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ABSTRACT

Background: The relationship between humans and their oral microflora begins shortly after birth and lasts a lifetime. Cigarette smoking is a public health issue. It is the major cause of oral cancer, periodontitis, color change on the teeth, halitosis, and other health implications. It brings about a drastic decrease in the commensal population of normal flora in the oral cavity leading to an increase of pathogenic microbes. The study was designed to determine the changes caused by tobacco smoking on the microbial profile and oral health conditions of cigarette smokers. **Materials and Methods:** This study included 20 adults aged 18–65 years visiting the Dental Outpatient Department of Saveetha Dental College and Hospitals, Chennai. Patients were randomly selected depending on the inclusion and exclusion criteria. All the subjects signed informed consent and filled out Fagerstrom nicotine dependence test questionnaire. From each subject, one sample which was the swab from the buccal mucosa was obtained and sent to the microbiological laboratory for analysis of the presence of any microorganisms. **Results:** *Staphylococcus* and *Bacillus* species were the most prevalent bacterial isolate, followed by *Enterococcus* and *Micrococcus* species among smokers, while *Streptococcus* was the most prevalent isolates among nonsmokers followed by *Enterococcus* and *Bacillus* species. **Conclusion:** Smokers had a diverse microbial colonization than nonsmokers. Our study suggests that smoking alters bacterial acquisition and oral mucosal colonization in favor of periodontal pathogens. Awareness should be created for the public on the health implication of smoking and poor oral practice.

KEY WORDS: Bacteria, Nicotine dependence, Oral microbiota, Smoking, Tobacco

INTRODUCTION

The mouth is an ecological niche of many microbial communities with important implications to human health and diseases. The oral cavity is made up of the lips and its inner lining, buccal mucosa, teeth, gums, the front two-thirds of the tongue, and the hard palate. The relationship between humans and their oral microflora begins shortly after birth and lasts a lifetime. The oral microbes comprise a complex community and that oral health or disease depends on the interface between the host and the microbial community as a whole.[1] The oral microbial community (the microbiota) is centrally associated to nutrition, metabolism, immunity, and endocrine balance. These microbes communicate through physical interactions called coaggregation and coadhesion, as well as other physiological and metabolic interactions. Streptococci and Actinomyces are the major initial colonizers of the tooth surface, and the interactions between them and their substrata help to establish the early biofilm community.[2]

Oral health reflects the well-being of an individual, thus maintaining oral hygiene, an essential part of ones' routine. The resident oral flora plays an important role in health and disease of the host immune system. They also provide resistance by competing for colonization sites of pathogenic microorganisms. The maintenance of proper oral hygiene can reduce oral microbial load and thus eventually helps in controlling the occurrence of oral diseases. Some pathogenic microbes such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus species, Escherichia coli, Pseudomonas aeruginosa, Candida species, Entamoeba gingivalis,*

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and *Porphyromonas gingivalis* may be present in the oral cavity due to poor oral hygiene.

Smoking and Its Ill Effects

Throughout the developing world, smoking behavior is increased worldwide despite the warnings against cancer and other related diseases and is considered as a major threat to the world health. Cigarette smoking is associated with numerous health hazards, most of which are life threatening and reduce the quality of lifestyle. The smoke which is caused due to cigarette has innumerable adverse effects on human health. As a result, smokers are at a higher risk for developing diseases such as lung and other cancers, chronic obstructive pulmonary disease, cardiovascular disease, and periodontitis.[3-5] Smoking is responsible for 75% of the cancers in the mouth.

The most common constituents of cigarette smoke are nicotine, phenol, cresol, polynuclear aromatic hydrocarbons nitrosamines, hydrazine, vinyl chloride, oxides of nitrogen, ammonia, and formaldehyde, most of which turn out to be a carcinogenic agent.Nicotine, which is the most common constituent, is a neuroendocrine stimulant and depressant, addicting drug.

Fagerstrom Test for Nicotine Dependence (FTND)

For some time, smokers have been pressured to stop smoking and it has been suggested that current smokers might be more dependent than former smokers, as less dependent smokers can quit more easily.[6] The term "hardening hypothesis" is currently being discussed, where the smoking prevalence dropped significantly over the past decades but has plateaued in recent years.[6] Most of the observational studies suggest that today's smoking population contain groups of smokers for whom it is harder to quit potentially because they have a higher degree of nicotine dependence. Thus, it becomes essential to determine the nicotine dependence of the people which would help us plan our anti-smoking campaign in a much different and essential manner.

There are numerous tests and questionnaires which are used in determining the nicotine dependence of a person. One of them is FTND, a non-invasive and easy-to-obtain self-report tool that conceptualizes dependence through physiological and behavioral symptoms. The Fagerstrom nicotine dependency test constitutes questions which enquirer the patients regarding their smoking habits such as how soon the patient smokes after he wakes up, the patient's difficulty to refrain from smoking in regions where smoking is not allowed, the average number of cigarettes does he consume in 1 day, and the difficulty he faces in quitting of smoking habits. Based on these, scores ranging from 0 to 3 would be given for the patient. At last, the overall sum of the scores would help in determining the nicotine dependence of the patient. A score under 3 usually represents that the nicotine dependence is low, a score ranging from 3 and 5 suggests that the nicotine dependence of the patient is moderate, and a score above 5 represents a very high nicotine dependence. However, little is known about the relationship between cigarette smoking, oral microbiota, and tobacco-related health hazards and nicotine dependence.

Effect of Cigarette Smoke on Oral Microbiota

Cigarette smoke contains numerous toxicants to which smokers are regularly exposed on a periodic basis.[7,8] These toxicants can potentially alter the microbial ecology of the mouth through numerous mechanisms such as antibiotic effects and oxygen deprivation.

Smoking and Oral Health Status

Periodontal disease is defined as any pathological process affecting the periodontal tissue.[9,10] It most commonly refers to inflammatory diseases affecting the marginal periodontal tissue, namely gingivitis and periodontitis.

Gingivitis is widespread, but advanced periodontitis is limited to relatively small subgroups of the population.[11,12] There are numerous risk factors associated with the development of gingivitis and periodontitis. One of the most prevalent risk factors that had been associated with an increased incidence of periodontal disease is the use of tobacco, especially cigarette smoking. Till date, knowledge about the effect of smoking on oral microbiotas is lacking. As an initial step toward understanding the role of the oral microbiota in smoking-related health outcomes, we have proposed the study to evaluate the effects of cigarette smoking on the microbiotas in the oral cavity.

MATERIALS AND METHODS

Study Sample

A cross-sectional study design was used. This study included 20 adults aged 18–65 years attending the Dental Outpatient Department of Saveetha Dental College and Hospitals, Chennai. Patients were randomly selected depending on the following criteria.

Inclusion Criteria

1. Over 18 years of age and not older than 65 years of age.
2. More than 10 natural teeth present.
3. Those individuals reported a regular smoking history for the past 1 year and maintained their smoking habits before sample collection.

Exclusion Criteria

1. Subjects diagnosed with cancer
2. Subjects reporting the use of antibiotics within the past 3 months before sample collection

3. Presence of comorbidities such as HIV, diabetes, and ischemic heart
4. Presence of malignant or potentially pre-malignant oral lesions, such as leukoplakia or erythroplakia.

Others include chronic systemic pathology, such as diabetes, other endocrine pathologies, and hematological pathologies.

Subjects were divided into two groups:
- Smokers,
- Non-smokers.

Questionnaire

A questionnaire was developed. It included questions on oral hygiene habits, smoking habits, and Fagerstrom nicotine dependent test. All the subjects chosen were asked to fill the questionnaire and the data were analyzed.

Sample Collection

All the subjects signed informed consent and filled out questionnaires. From each subject, one sample which was the swab from the buccal mucosa was obtained and sent to the microbiological laboaratory for analysis of the presence of any microorganisms. The samples were collected by following the procedure of Human Microbiome Project (http://hmpdacc.org/doc/HMP_MOP_Version12_0_072910.pdf).

The samples collected were then sent for culturing to the laboratory and the data collected were statistically analyzed by Chi-square test.

RESULTS

Figure 1 shows the age distribution of the study population.

The mean age among the subjects was 32.4 ± 11.5 years, with minimum age 18 years and maximum age 56 years. There was no statistically significant relationship between age and smoking.

The distribution of microbial isolates from smokers and non-smokers are shown in Figure 2 and Table 1. More microbes were recovered from the oral cavity of smokers than non-smokers. *Staphylococcus* and *Bacillus* species were the most prevalent bacterial isolate followed by *Enterococcus* and Micrococcus species among smokers, while *Streptococcus* was the most prevalent isolates among non-smokers followed by *Enterococcus* and *Bacillus* species. *Candida* species were found in only two of the test samples.

Fagerström Test for Nicotine Dependence

The Fagerstrom nicotine dependence questionnaire was given to all the smokers, and the results are as given below. The mean Fagerstrom nicotine test

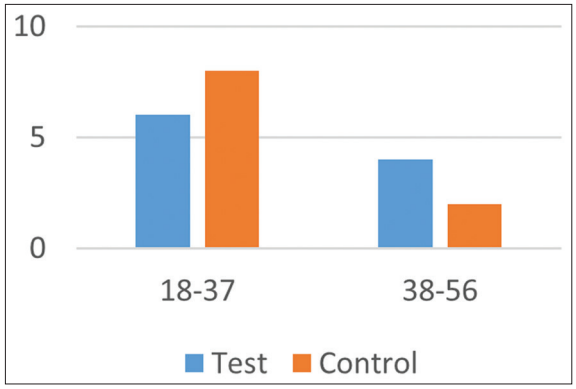

Figure 1: Age distribution of subjects

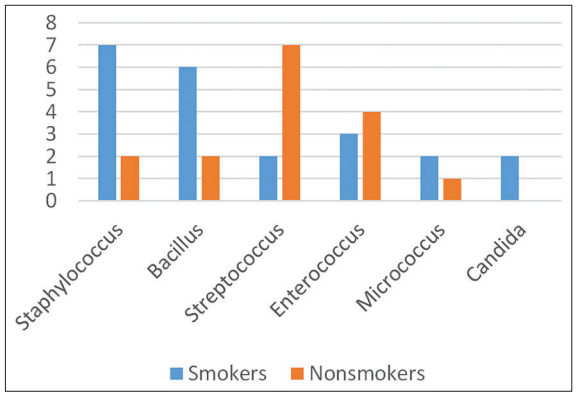

Figure 2: Distribution of microbial isolates among subjects

Table 1: Chi square test

Chi square test	Value	df	Asymp. sig. (two-sided)
Pearson Chi-square	9.317	5	0.097
Likelihood ratio	10.281	5	0.068
Linear-by-linear	0.435	1	0.509
Association number of valid cases	38		

dependency score among the subjects was found to be 2.7 ± 1.3, with minimum score being 1 and maximum score being 5. From the questionnaire, it was evident that most of the smokers were willing to quit smoking and even have tried quitting previously but have failed due to various reasons such as stress or any other social situations. One more thing, which was evident with the answers, is that most of the smokers had started smoking in the first place only by the influence of the friends and colleagues [Table 2].

DISCUSSION

The oral cavity is a very complex region in the entire human body, and it serves as a habitat for numerous microorganisms. The term " microorganisms" merely does not mean that these are harmful for the body. In fact, most of the commensalism population of microorganisms play a crucial role in protecting against the pathogenic bacteria. These commensal

Table 2: Distribution of microbial isolates among subjects

Microbial isolate group cross-tabulation			
Microbial isolates	**Group**		**Total**
	Smokers	**Non-somkers**	
Staphyloccus			
Count	7	2	9
Within group (%)	31.8	12.5	23.7
Bacillus			
Count	6	2	8
Within group (%)	27.3	12.5	21.1
Streptococcus			
Count	2	7	9
Within group (%)	9.1	43.8	23.7
Enterococcus			
Count	3	4	7
Within group (%)	13.6	25.0	18.4
Micrococcus			
Count	2	1	3
Within group (%)	9.1	6.3	7.9
Candida			
Count	2	0	2
Within group (%)	9.1	0.0	5.3
Total			
Count	2s2	16	38
Within group (%)	100.0	100.0	100.0

microorganisms prevent the adhesion of pathogenic ones to the mucous membrane. Thus, it helps a lot in maintaining the oral cavity pathogen free. Maintaining a health and hygiene oral cavity is still in hour hands.Cigarette smoking is a public health problem. It decreases the commensal population of normal flora in the oral cavity leading to an increase of pathogenic microbes.[13] Cigarette smoking could enhance microbial colonization by biofilm formation on oral epithelial cells. This may impair host immune responses against pathogens and also disrupt effective nasal mucociliary clearance.[14,15] A number of studies have reported that smoking increases the probability of extensive disease development[16,17] and an significant disruption in the oral microbiota creating an imbalance in oral environment.

In this study, higher rates of microbe were recovered from the oral cavity of smokers than non-smokers. This finding coincides with the results reported by Kubota *et al*.[19] who found that the detection rate of periodontopathic bacteria was higher in smokers. However, it is contradicting the results produced by Sreedevi *et al*.[18] who reported that there was no difference in the periodontal microbiota status between smokers and non-smokers. This difference may be due to the types of sample obtained for analysis and the technique used for analysis of the sample. In this study, oral swabs were obtained for culture while Sreedevi *et al*.[18] analyzed periodontal plaques using BANA tests.

Females were not included in the study as this habit is might not be easily admitted by most of them. Even Sreedevi *et al*.[18] who conducted a similar study in Bengaluru, India, also excluded female subjects from their study because of the same reason and to avoid potential hormone-induced microcirculatory changes.

Wetzel *et al.* isolated *Streptococcus mutans, S. Aureus,* and *Pseudomonas aeruginosa* as the most prevalent among smokers which is slightly different from the results obtained in our study. The slight variation of microbial flora among our subjects may be due to variation in oral hygiene habits.[20]

Tobacco use is associated with a range of changes to the oral mucous membranes. Although the effect of exposure to tobacco smoking was not investigated among our subjects, smokers had a higher occurrence of staying on their teeth. There was a statistically significant association between oral cavity disease and microbial isolates among smokers. Thus, it is evident that smoking has a significant effect on oral microbial acquisition and the oral cavity diseases.

The mean Fagerstrom nicotine test dependency score among the subjects was found to be 2.7 ± 1.3, with minimum score being 1 and maximum score being 5, which suggested that the nicotine dependency of these subjects only ranges from low to moderate and not very high.

Thus, there is a high probability that it would be easier to convince these subjects for quitting of smoking habits. However, FTND scores alone may not explain the nicotine dependency and the willingness of the patient to quit smoking habits. Differences in personal characteristics between smokers have been reported where socioeconomic status, educational attainment, and psychiatric comorbidities also play a crucial role.[6,21,22] These factors are probably relatively independent of the degree of tobacco dependence as measured by FTND. Thus, the FTND results can only be used as a preliminary test for the diagnosis of nicotine dependence and the willingness of the patients to quit smoking habits.

CONCLUSION

Our study showed that cigarette smoking had a significant effect on the microbiota of the buccal mucosa. Therefore, these parameters can be areas of further research with an increased sample size, which in turn may help in throwing some light on the deleterious effect of smoking on the oral microbiota which is essential part of the oral environment, thus creating an imbalance. This would help us comprehend the importance of a more profound campaign against smoking thus creating an awareness among people to enable them to improve the quality of lifestyle and to help them to live their life to the fullest.

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