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Research Article

Association of Bacterial Growth in the Oral Cavity between Tobacco Smokers and Tobacco Chewers

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ABSTRACT
Smoking is considered the major risk factor in the prevalence, extent and severity of several oral diseases. Use of tobacco alters the growth of bacteria in the oral cavity. Hence the present study examined the impact of tobacco use and its cessation on the density of oral microbial population. Study includes 90 subjects with age range 25-65 years, categorized as healthy controls (n = 30), smokers (n = 30), and tobacco chewers (n = 30). Oral bacteria in smokers, tobacco chewers, and non-tobacco users were measured using a spectrophotometer after incubation at 37ºC for a 24 h, 36 h and 48 h period. Tobacco users exhibited an increase of bacteria when compared to non-tobacco users/ healthy controls. This study revealed that tobacco chewing has a more significant effect in increasing the oral microbial population than the tobacco smoking and affect the oral hygiene.

Keywords: Smoking, tobacco chewing, bacterial growth, oral hygiene.

INTRODUCTION

The tobacco use is one of the greatest threats to global health today. Approximately one-third of the adult population in the world use tobacco in some form. Tobacco is a risk factor for oral cancer, oral cancer recurrence, adult periodontal diseases, and congenital defects such as cleft lip and palate in children whose mother smokes during pregnancy. Tobacco use suppresses the immune system's response to oral infection. Cancer of the oral cavity is high among men, where oral cancer is the eighth most common cancer in the world[1].

The impact of tobacco smoking on general health has been widely studied and is directly related to several important medical problems including cancer. Broad range of buccal changes has been noted in tobacco smokers and smokeless tobacco users. Tobacco is a delivery system for the addictive agent nicotine. Chewing of tobacco and products of betel nut are significantly contributing factors for the oral submucosal fibrosis. Therefore it was observed that in all these cases normal flora of the oral cavity was reduced and they developed submucosal fibrosis which leads to leukoplakia or cancer of the oral cavity[2].

In the past three decades, there has also been an increasing awareness of the role of tobacco use in oral health problems. Smoking is considered the major risk factor in the prevalence, extent and severity of periodontal diseases[3]. Tobacco smoke is involved in the pathogenesis of several diseases including local toxic effects in the oral cavity[4]. The noxious effects of smoke compounds justify the high incidents of periodontal diseases, caries and neoplastic diseases of oral tissues in smokers.

Cross-sectional studies have shown that smokers are two to seven times more likely to present periodontitis, compared to nonsmokers. Bacteria in dental plaque are the primary etiological agents of periodontal diseases, and elucidating the effects of smoking on oral microbial ecosystem[5]. Studies using molecular approaches for bacterial identification and characterization have demonstrated that the subgingival microbial profile associated with periodontitis in smokers is diverse and distinct from that in nonsmokers[6].

The use of smokeless tobacco is particularly common amongst south Asian communities, in particular chewing tobacco which is either chewed alone or with betel quid or paan. The use of these products can have a significant detrimental impact on the oral cavity[7]. Recent evidence also indicates that smoking cessation alters patterns of microbial colonization[8]. The purpose of our present study was to investigate the bacterial growth in the oral cavity between tobacco smokers and tobacco chewers.

MATERIALS AND METHODS

Subjects

Thirty healthy current smokers, 30 tobacco chewers and 30 nonsmokers were recruited from general population with age range 25-65 years. Data related to the subjects, medical history, family history of diseases, smoking

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Table 1: Demographic characteristics and tobacco exposure of sample population

Characteristics	Controls	Tobacco Users	
		Smokers	Chewers
N	30	30	30
Mean age (Yrs)	47.3 ± 6.3	48.5 ± 7.4	51.8 ± 9.5
Age range (Yrs)	25 - 60	25 - 63	25 - 65
Sex (M/F)	23/7	28/2	15/15
Duration of exposure(Yrs)	-	30.9 ± 6.5	24.1 ± 5.2

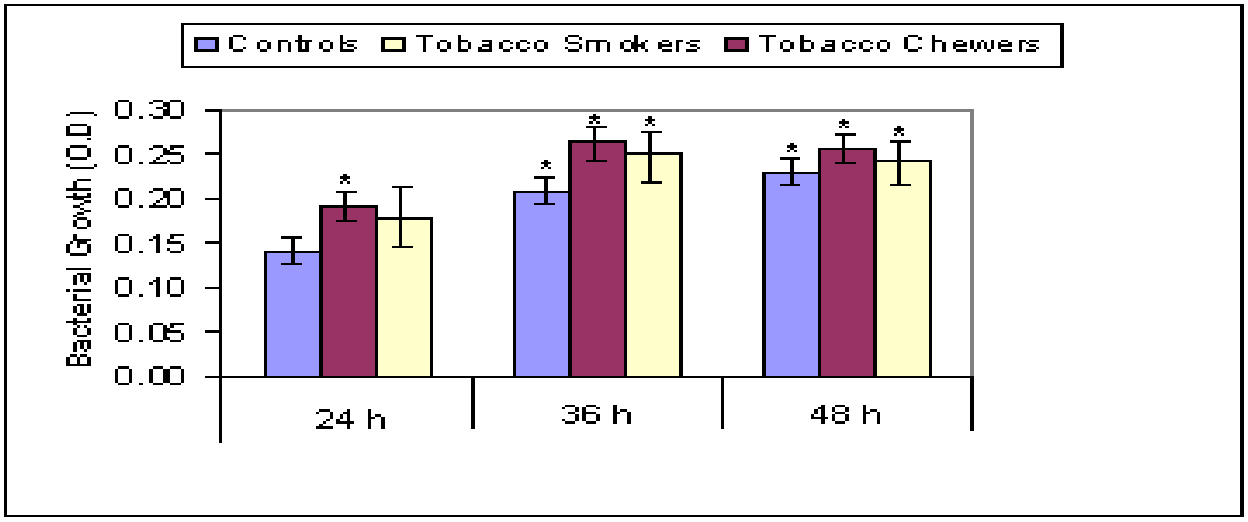

Figure 1: Mean of oral bacterial growth or concentrations at 24, 36 and 48 hrs of tobacco users and controls. The absorbance of the bacteria was measured by a spectrophotometer. Values are mean ± standard deviation, *P < 0.05.

Table: 2: Distribution of normal flora and cariogenic *Streptococci* in different tobacco user and controls.

Bacterial growth		Smokers	Chewers	Controls
Normal bacterial microflora	%	43.3%	36.6%	83.3%
	Number	13	11	25
Pathogenic bacterial flora	%	56.6%	63.3%	16.6%
	Number	17	19	5
S. mitis		21.0%	13.5%	0
S. mutans		49.8%	52.3%	0
S. sanguis		22.7%	30.2%	0
S. sobrinus		0%	8.2%	0

habits, and occupational history were obtained through questionnaire after obtaining the informed consent from the individuals. Tobacco users were further enquired regarding the type (cigarettes or beedis or any other forms; pan masala or ghutka or other chewing tobacco; snuff), smoked duration and frequency. Exclusion criteria included diabetes, HIV infection, use of immune suppressant medications, steroids and antibiotics within the last 3 months. Each person was instructed not to eat or drink anything for two hours before the appointment. The dental examination was performed by trained dentist. The institutional ethics committee approved this study. The work was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Samples

After giving informed consent, all study participants donated saliva sample, which was collected from back of the left and right cheek using individually packed sterilized swab and swabs were transferred to laboratory with in 2 hrs.

Bacterial culture

The saliva samples were cultured in both nutrient broth and plates of Mitis Salivarius Bacitracin Agar, selective media for the isolation of *Streptococcus spp*, blood agar, MacConkey agar and RCM, The inoculated mitis salivarius agar plates were incubated aerobically in a candle jar (5% to 10% CO_2). The inoculated RCM plates were incubated anaerobically for identifying the organisms. The bacterial growth of the broth culture was measured spectrophotometrically. The Streptococcus and other bacterial isolates were identified by gram's staining, colony morphology were characterized biochemically[9].

Statistical analysis

SPSS 17.0 (SPSS, Chicago, III, USA) was used for data management and analysis. Statistical evaluations were assessed using the Student's t test, and $p < 0.05$ was considered significant. The data are expressed as mean ±

standard deviation. ANOVA test was used to compare the bacterial growth among the three groups.

RESULTS

The demographic characteristics of the subjects are shown in Table 1. There were no differences between the groups except for their tobacco habits ($P < 0.05$, 2-sample *t* test).

The turbidity of bacterial growth from broth culture was measured using spectrophotometer. Higher bacterial growth resulted in higher turbidity and a higher optical density reading. At 24 h and 48 h the growth of oral bacteria obtained from smokers, tobacco chewers, and non tobacco users were significantly different ($P < 0.05$). The growths of bacteria in tobacco chewer's samples were significantly higher than smokers ($P < 0.05$). Likewise, growth of bacteria in tobacco users was significantly higher than that of controls ($P < 0.05$).

Results of this study indicate that the use of smokeless tobacco associated with increased carriage of pathogenic organisms. The smokeless tobacco in India contained varying amount of sugars which could be responsible for root caries as well as an increased amount of gingival recession in smokeless tobacco users. It has been reported that substances present in tobacco smoke alter the charge and other properties of oral epithelial cell surfaces, allowing the growth of certain pathogenic bacteria[10]. Cigarette smoke also affects the survival of specific bacterial species isolated from the oral cavity, inhibiting some species of *Neisseria* and gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumonia*[11].

Smoking decreases the salivation, which leads to dry mouth. Since saliva is instrumental in keeping a healthy balance in bacterial levels, the chances of bacterial infection increase in the oral cavity once that balance is altered. For instance, small abrasions of the teeth and gums are more likely to become open sores when the mouth is host to an unhealthy number of infections bacteria[12].

However, little is known about the effects of chronic use of tobacco on the oral microbiome. The human oral cavity harbors more than 700 different bacterial taxa, possessing relevant quantitative differences between individuals[13]. Bacterial communities differ between healthy and diseased oral cavities and, as they can cause or prevent infections, this may have a significant impact on general health [14].

Distribution of normal flora and cariogenic *Streptococci* in tobacco smokers, tobacco chewers and controls are shown in Table 2. Increased number of pathogenic bacterial microflora was found in smokers and chewers (56.6% % and 63.3%), whereas a decreased percentage of pathogenic bacterial microflora (16.6%) was found in controls. Most frequently isolated bacteria were *Strepotococcus mutans* and *S. sanguis* in both cases. Less significant pathogenic bacterial microflora was found in controls than smokers and chewers.

The opportunistic pathogens, found commonly as members of the resident flora of persons without caries and expressing their pathogenicity only under specific environmental conditions. *Streptococcus mutans* and *Streptococcus sobrinus* , two species of the 'mutans streptococci' are the most significant in human caries[15]. Adaptation response to stresses varies among different oral species and among strains of a species[16]. High carbohydrate, low salivary flow may leading to local dominance by mutans streptococci, followed by enamel demineralization and cavitation, that often involves Lactobacillus[17].

The normal flora of the oral cavity comprises of both aerobic and anaerobic organisms. Aerobic flora normally consists of *Streptococcus viridans, Diptheroids* and *Neisseria catarrhalis* .The anaerobic flora shows predominance of *Lactobacilli*, *Leptotrichia buccalis* and *Veillonella*. The common fungus which inhabits the oral cavity is *Candida albicans*. Our study observed reduced flora, some samples showed Pseudomonas *aeruginosa*, *Klebsiella* and *Candida albicans* in the culture plate.

Similar study was carried out previously [18] which showed reduction in oral microbial flora to approximately 56% as compared to control where microflora reduction was 50% in the betel leaf chewers.

Smokers have decreased levels of salivary and serum immunoglobulins which impairs their ability to fight the bacteria in the oral cavity. Smoking also alters the cells that attack bacteria which affect smoker's ability to clear pathogens[19]. The dangers posed to oral health from smoking and chewing tobacco are well documented within the dental literature but lack of knowledge of the risks is a concern. However it is vital to guarantee that public awareness of tobacco related oral diseases continues to improve the quality of life of those people who are at maximum oral cancer risk.

CONCLUSION

From the present study, we can conclude that the long-term use of tobacco especially the smokeless form can cause significant alteration in bacterial load. The alterations in bacterial count due to long-term effect of tobacco usage can render oral mucosa vulnerable to various oral and dental diseases. Therefore, tobacco chewing and smoking cessation should be considered in the treatment of caries.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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