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Effect of Tobacco Chewing on Salivary and Tongue Coating pH and Assessment of Oral Microflora in Tobacco Chewers

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ABSTRACT

Salivary functions rely on its various physicochemical characteristics and any variations under threshold levels are considered risk factors for the development of oral diseases. **Aim:** A single-blind randomized cross-sectional study was undertaken to assess the effect of chewing tobacco on pH of saliva and tongue coating and its impact on oral microflora. **Materials and Method:** Forty-five (15 controls and 30 study subjects with only tobacco chewing habits under two age groups) unstimulated salivary and tongue coating pH was assessed early in the morning before oral hygiene procedures were done and the saliva sample was inoculated for aerobic culture on Cysteine lactose electrolyte deficient agar and incubated at 37^{o}C for 24-48 hours for microbiological analyses. **Results:** The mean tongue coating pH in young and elderly tobacco chewers was found to be 7.13 and 7.13±0.70 respectively. The mean salivary pH in young and elderly tobacco chewers was found to be 8.09±0.30 and 8.19±0.54 respectively. The differences between the groups for salivary and tongue coating pH were not statistically significant ($p \geq 0.682$) ($p \geq 0.724$). The predominance of microorganisms in young tobacco chewers, was streptococcus viridans, E.coli, Klebsiella pneumonia, and Coagulase Negative Staphylococcus, whereas in elder tobacco chewers showed the predominance of streptococcus viridians, Gram-positive cocci, and Coagulase Negative Staphylococcus. **Conclusion:** As alterations in normal oral flora and salivary pH due to the long-term effect of tobacco usage makes oral mucosa acquire oral and dental diseases, tobacco usage cessation should be considered in the treatment of various oral diseases and be a part of prevention of oral health in dentistry.

INTRODUCTION

Tobacco abuse has become a global epidemic and also a serious worldwide health concern. It is estimated that by 2025 the number of smokers would rise by 1.6 billion and the number of deaths due to this habit is expected to reach 6.4 million in 2015 and 8.3 million in 2030.[1] As a substitute for a smoking habit, because of its effect on the soft tissues of the oral cavity and respiratory tract, the use of chewing tobacco has become increasingly popular. The prevalence of tobacco chewing is higher in India compared to other parts of the world. Both tobacco smoking and tobacco chewing have noticeable effects on the ecology of mouth.[2]

The tobacco products are known for their powerful parasympathetic action, that produces euphoria, and counteract fatigue. According to some studies, normal flora of the oral cavity was reduced in tobacco users and they developed submucosal fibrosis and lead to a formation of leukoplakia or cancer of the oral cavity.[3]

Saliva possesses an important impact through functions relying on its physicochemical characteristics such as flow rate, pH, and buffering capacity; so variations under threshold levels are considered risk factors for the development of oral diseases.[1]When the oral cavity is repeatedly exposed to tobacco for a long time it presumably effects & bring about changes in oral microflora.

The aim of present study was to analyze the effect of tobacco habits on salivary & tongue coating pH & the effect of tobacco on the oral microbiota.

MATERIALS AND METHOD

A single-blind randomized cross-sectional study was undertaken to assess the relationship of the pH of saliva and tongue coating in tobacco chewers and effect of chewing tobacco on oral microflora. This study was approved by the Ethical Committee Rama Dental College and Research Center. Forty-five age and sex matched, oral and systemically healthy subjects consisting of 30 study subjects with only tobacco chewing habits under two age groups (group I (18-30 years) and group II (40-55 years) and 15 controls (group III) were considered for the study. A grace period of 10years was given to assess the long-term effect of tobacco on saliva and its pH.

The exclusion criteria were subjects with medical disorders, such as diabetes mellitus, renal disease, gastrointestinal disorders, respiratory diseases, evidence of recent bronchitis, sinusitis or tonsillitis, pregnant women, patients undergoing antibiotic or other antimicrobial therapy, and those who, on pre-study clinical screening, presented a probing depth ≥4 mm, cavitated caries lesion, nasopharyngeal alterations, mouth breathers and patients with prostheses, orthodontic or dental appliances.

The nature of the study was explained and informed consent was obtained from all the subjects. All patients were seen in the morning, at 7 am., fasting for at least 8 hr and without having performed any oral hygiene procedures on the day of consultation.[4]

Each patient underwent collection of saliva. Before saliva collection, patients were kept seated for 5 min, relaxed and without talking. Unstimulated saliva was collected over a period of 5 min. Before collection, the mouth was emptied by an initial swallow.[4] The subjects were instructed to spit out the produced saliva each 30 s in a plastic sterilized airtight container.

Salivary pH was measured by a digital pH meter (EI Model 111/101 pH System, India), calibrated with standard solutions of pH 4.0 and 7.0. The electrode was washed with distilled water and dried with absorbent paper after each analysis.[5] In the same consultation, tongue coating pH was measured using pH indicator strips (pH 0-14; Merck, Darmstadt, Germany). One strip was placed on posterior tongue region, with the patient mouth opened, for 1 min. The color change in the strip indicated tongue coating pH.

Microbiological processing of the samples was carried out in the department of Microbiology, Rama Medical College, Mandana, Kanpur on the same day of sample collection. All the salivary samples were well processed in the laminar flow under aseptic precautions. Each sample for aerobic culture was inoculated on Cysteine lactose electrolyte deficient (CLED) agar and incubated at 37^{o} for 24-48 hours. Growth on CLED agar (with Andrade's indicator) was studied after 24 hours with the help of gram staining. The positive growth plates were sub-cultured in the pure form on Nutrient agar and McConkey agar plates. The microorganisms were classified into either GPC (gram positive cocci), GPB (gram positive bacillus), GNC (gram-negative cocci), GNB (gram negative bacillus) and these isolated organisms were identified by biochemical reactions.

RESULTS

The mean salivary pH was found to be 8.09±0.30 in (group I) young tobacco chewers, 8.19±0.54 in (group II) elder tobacco chewers, 8.04±0.14 in (group III) controls. The differences between the groups were not statistically significant (p≥0.682) (Table 1).

The mean tongue pH was found to be 7.13 in young tobacco chewers (group I), 7.13±0.70 in elder tobacco chewers (group II), 7.08±0.51 in controls (group III). The differences between the groups were not statistically significant (p≥0.724) (Table 1).

In the present study on comparing group I and group II, salivary and tongue coating pH showed similar results and when a type of predominant microorganism was assessed, the group I (young tobacco chewers), showed majorly streptococcus viridians followed by *E.coli*, *Klebsiella pneumonia*, Coagulase Negative Staphylococcus (CoNS). Whereas group II (elder tobacco chewers) showed the predominance of streptococcus viridians, followed by GPC and CoNS, while in group III (controls) CoNS was mostly seen followed by Gram-positive bacilli, *E. coli* (Graph 1).

DISCUSSION

Saliva is the first biological fluid that is exposed to tobacco (smoked/smokeless form), which contains numerous toxic compositions responsible for structural and functional changes in saliva.[6] Smokeless tobacco chewing deserves special attention in India because of its popularity and widespread social acceptance.[7]

A wide variety of mucosal changes has been noted in habitual users of smoked and smokeless tobacco. These changes most likely result from the many irritants, toxins, and carcinogens found naturally in cured or burned tobacco leaves, but may also arise from the mucosal drying effects, the high intraoral temperatures, intraoral pH changes, local alteration of membrane barriers and immune responses, or altered resistance to fungal and viral infections.[7]

Some of the most frequently occurring important pathological conditions of the teeth and oral cavity are strongly dependent on the pH changes. The pH in the saliva plays an important role in

the life, growth & multiplication of oral bacteria. The numbers of acidophilic bacteria are increased when the pH of the saliva is very low whereas the acid sensitive bacteria are decreased.

In addition to the presence of certain types of bacteria, the type and amount of substrate, and oxygen and pH levels influence the occurrence and severity of tongue coating (a biofilm deposit over the tongue dorsum). Hence in the present study, an attempt for the first time was done to assess the pH of tongue coating in tobacco users.[4]

The mean tongue pH was found to be more towards alkaline in all the groups in the present study. But the differences were not statistically significant. Since the pH of the tongue coating was assessed for the first time among only tobacco chewers there were no studies with data available to be correlated. In a study done by Ramesh et al (2014)[8] on two groups consisting of the first group with only tobacco smokers and the second group with combined habits of both chewing and smoking also showed alkaline pH. The mean tongue coating pH was found to be 6.80±0.86 in smokers, 6.93±1.03 in chewers & smokers, 7.00±0.37 in controls. The differences between the groups were not statistically significant.[8]

Kanwar et al. (2013)[9] observed only tobacco chewers with salivary pH of 6.7(±0.1), only smokers with pH 6.8(±0.1), controls with salivary pH 7.04(±0.1) with the results being not significant. In the present study, the mean salivary pH was found to be 8.09±0.30 in young tobacco chewers, 8.19±0.54 in elder tobacco chewers, 8.04(±0.14) in controls. The differences between the groups were also not statistically significant. The difference in the pH of saliva could be attributed to use of pH indicator strips in their study and not pH digital meter.

Grover et al. (2016)[10] assessed salivary pH in tobacco smokers and chewers among subjects aged 25–40 years. Their study indicated that a lower (acidic) salivary pH was observed in tobacco users as compared with control. Hence the authors concluded that these alterations in pH could be due to the long-term effect of tobacco use that can render oral mucosa vulnerable to various oral and dental diseases. This result is not in accordance with the present study wherein we observed an alkaline salivary and tongue coating pH. This could be attributed to the time of sample collection which was 7 a.m in the present study the time when the subjects had not consumed anything before collection of the sample since the last dinner at night. But in the Grover et al, (2016) study saliva collection was carried out between 9:00 am and 12:00 pm and

subjects were requested not to drink, eat or perform oral hygiene or chew or smoke 60 min before and during the procedure.[10]

In the present study when oral flora was assessed, young tobacco chewers showed majorly *Streptococcus viridans, E. coli, Klebsiella pneumonia*, and CoNS. Elder tobacco chewers showed predominantly Streptococcus viridians, GPC, and CoNS.

The study on gutka-chewers and smokers by Avasn et al,(2004)[5] revealed the decrease in salivation and mucus formation in gutkha chewers, which further resulted in reduction in a number of oral microflora. Aspergillus species was found in gutkha chewers and smokers. They concluded that Gutkha chewing and smoking may lead to an increase in the oral pathogens by reducing the normal symbiotic microbial flora.[5]

Saini et al. (2009)[3] assessed the type of oral microflora in subjects with habit of tobacco along with betel leaf and found streptococcus viridians to be predominant followed by *Pseudomonas aeruginosin, Klebsiella pneumonia*, and CoNS. In a study by Pavia et al. (2000)[11] used the extract of nicotine to check for its antimicrobial properties by checking the zone of inhibition when inoculated with various microorganisms. The authors observed that nicotine interfered in the growth of selected microorganisms. They emphasized that the nicotine can affect or shift the type and quantity of oral microflora or both and also intensify the penetration of these microbes into the injured mucosa. Other pros and cons could be that the few kind of microbes may be reduced in growth and proliferation making way other few other pathogenic microorganisms to grow.

In another study by Ramesh et al. (2015)[8] when assessed for the oral microflora in tobacco users, they found that in only smokers, *Enterococcus faecalis, E. coli & Klebsiella* species were mostly found, whereas in subjects with both smoking and chewing habit the predominant microorganisms found were Streptococcus viridians. The authors concluded that understanding what can change the microbiota (including mouth sites, diet, and habit) will give more information on how to study oral microbiota and tobacco-related cancers.[8]

While the viridans streptococci are generally considered to be harmless commensals, it is clear that when given the opportunity, these facultative organisms elicit proinflammatory and pro-

coagulatory effects on a variety of target cells. Also amongst various species of viridans streptococci, streptococcus sanguis and oral are the predominant species recovered from infective endocarditis cases.[12]

Coagulase Negative Staphylococcus, Gram-positive cocci represent as one of the major nosocomial pathogens due to a patient- and procedure-related changes, with *S. epidermis* and *S. hemolytic* being the most significant species. They account substantially for foreign body-related infections and infections in preterm newborns. In addition to CoNS found as food-associated saprophytes, many other CoNS species colonize the skin and mucous membranes of humans and animals and are less frequently involved in clinically manifested infections. Although, CoNS. possess fewer virulence properties the host susceptibility is much more important. Therapeutically, CoNS are challenging due to the large proportion of methicillin-resistant strains and increasing numbers of isolates with less susceptibility to glycopeptides.[13]

E. coli is a facultative anaerobic, gram-negative, microorganism with, fecal–oral transmission being the major route through which pathogenic strains of the bacterium cause disease. Most *E. coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24hrs, and sometimes fever. In rare cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and gram-negative pneumonia.[14]

Klebsiella pneumonia a facultative anaerobic, Gram-negative bacilli found in the normal flora of the mouth, skin, and intestines, can cause destructive changes to human and animal lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum. K. pneumonia accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. The limitations on the therapeutic options demand new measures for the management of Klebsiella hospital infections. Few factors such as capsules or lipopolysaccharides of Klebsiella are presently considered as promising candidates for vaccination that may serve as immunological infection control measures.[15]

From the molecular perspective, mucosal bacterial infections may influence carcinogenesis by inducing chronic inflammation in adjacent stroma leading to upregulation of cytokines and growth factors.[16] Today it is accepted that chronic inflammation resulting from low grade, persistent chemical, bacterial, viral agents that predispose formation of the preneoplastic foci and promotes tumor development.[17]

CONCLUSION

From the present study, we can conclude that alterations in normal oral flora and salivary pH due to the 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 various oral diseases be a part of health prevention in dentistry Also, future studies with a larger sample size should account for protective and contributing factors such as oral hygiene regimens and dietary pattern.

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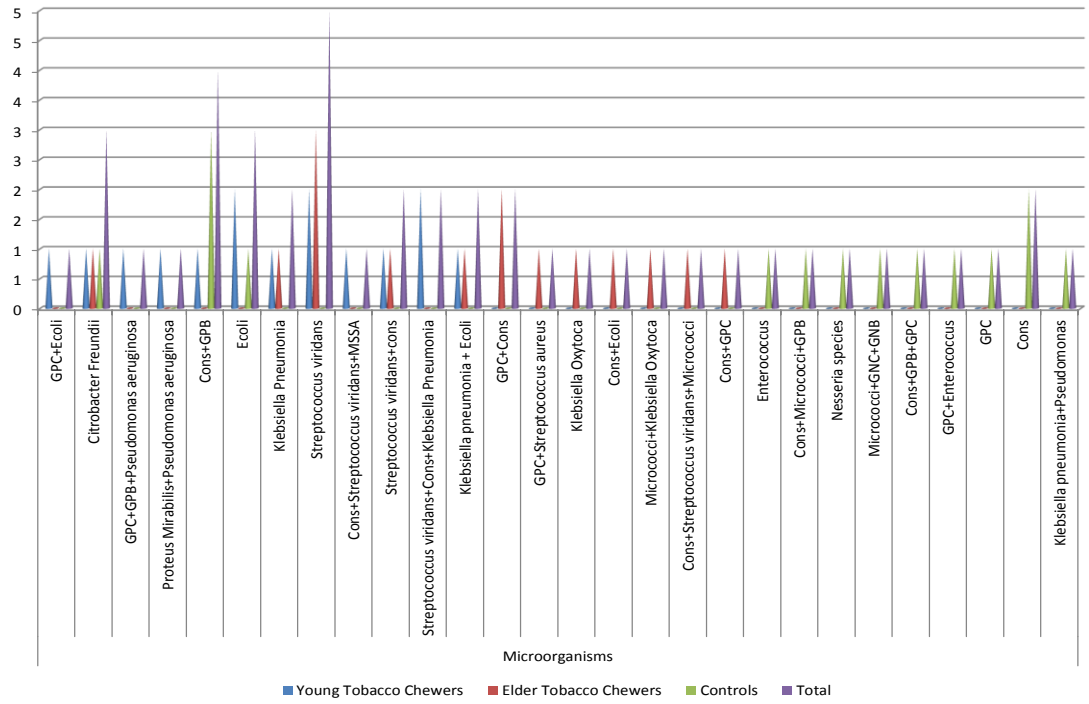
Table 1: Distribution of salivary & tongue coating pH among study subject.

	Group I (n=15)	**Group II (n=15)**	**Group III (n=15)**	**Total (n=45)**	**F value**	**P value**
Salivary pH	8.09±0.30	8.19±0.54	8.04±0.14	8.11±0.46	0.386	0.682 (NS)
Tongue pH	7.13	7.13±0.70	7.0	7.08±0.51	0.326	0.724 (NS)

The test used ANOVA, P≤0.05 is considered statistically significant.

Graph 1. Frequency of various microorganisms in study subjects



Graph 1: Shows the predominance of respective microorganisms in three different groups i.e. group I (young tobacco chewers), it includes a majority of streptococcus viridans, E.coli, Klebsiella pneumonia, CoNS. Group II (elder tobacco chewers) shows the predominance of streptococcus viridians, GPC, CoNS, whereas group III (controls) CoNS, GPB, E.coli were seen.