

*Original Research***Evaluation of Salivary and Tongue Coating pH and the Effect of Tobacco on Oral Microflora among Tobacco Users**

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ABSTRACT: Saliva is complex and important body fluid, is very essential for oral health. Saliva is the exposed to numerous toxic components in tobacco products, is responsible for structural and functional changes in oral tissues. **Aim & objectives:** A single blind randomized cross-sectional study was undertaken to assess the effect of chewing and smoking tobacco on pH of saliva and tongue coating and role of tobacco on oral microflora. **Material and methods:** Forty five unstimulated (15 controls and 15 study subjects with only tobacco smoking and 15 with both smoking and chewing habits) salivary and tongue coating pH was assessed and the saliva sample was inoculated on to the blood agar for microbiological analyses. **Results:** The mean salivary pH in group I and group II was found to be 7.66 ± 0.50 and 8.06 ± 0.67 respectively. The mean tongue coating pH group I and group II was found to be 6.80 ± 0.86 and 6.93 ± 1.03 respectively. The differences between the groups for salivary ($P \geq 0.788$) and tongue coating pH ($P \geq 0.079$) were not statistically significant. Among all the groups Coagulase Negative Staphylococci & Gram positive bacilli were predominantly. In Group I, Enterococcus faecalis, E.coli & Klebsiella species were mostly found, whereas in Group II the predominant microorganisms found were Streptococcus viridians. **Conclusion:** The pH and microbiota of mouth differs depending on the methods of collection, its analysis and the area that is tested. 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.

Key words: Saliva; Tobacco; Smoking; pH; Tongue; Oral microflora.

INTRODUCTION

Tobacco in its many forms is a risk factor for various systemic diseases, periodontal disease, and gingivitis.¹ Lack of awareness of the effects of tobacco use and the difficulty to discontinue the habit has led to the increased incidence of tobacco use. Tobacco habit encountered around the world is mainly in the form of tobacco smoking, tobacco chewing, and tobacco snuff use but in India, tobacco is used in the form of bidis (34%), cigarettes (30%), chewing tobacco (19%), hookah (9%), cigars and cheroots (5%), and snuff (2%).²

Tobacco was responsible for an estimated three million annual deaths in the world. The products of tobacco are known for its psychotropic effect, has powerful parasympathetic action, produce euphoria, and counteract fatigue. Few studies have observed that in all these tobacco users normal flora of the oral cavity was reduced and they developed submucosal fibrosis which leads to leukoplakia or cancer of the oral cavity.³

Tobacco smoke contains a major class of organic chemical compounds that includes chemical asphyxiants, irritants, ciliastic compounds, carcinogens and co-carcinogens. Its use is known to be associated with cancer of the lungs, larynx, oesophagus and lips, chronic bronchitis, emphysema, coronary artery disease.² 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

The pH in the saliva plays an important role in the life, growth & multiplication of oral bacteria. The numbers of acidophilic bacteria is increased when the pH in the saliva is very low whereas the acid sensitive bacteria are decreased.⁴

When oral cavity is repeatedly exposed to tobacco for long time it presumably effects & brings about changes in oral microflora.⁴ The aim of present study was to analyze the effect of tobacco habits on salivary & tongue coating

pH & to assess the effect of tobacco on colonizing oral microbiota.

MATERIAL AND METHODS

A single blind randomized cross-sectional study was undertaken to assess of the pH of saliva and tongue coating in subjects with tobacco habits and find the effects of these products on normal 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, 15 subjects with only tobacco smoking habits (group 1) and 15 subjects having habit of consuming both smokeless and smoking tobacco (group 2) and 15 controls (group 3)

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.⁴

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 a.m., fasting for at least 8 h and without having performed any oral hygiene procedures on the day of consultation⁴.

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. 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. 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 with the mouth opened, for 1 min. The color change in the strip indicated tongue coating pH.⁴

Microbiological estimation method:

Microbiological processing of the samples was carried out at the department of microbiology Rama Medical College, Mandana, Kanpur on the same day of sample collection. All the salivary samples well processed in the laminar flow under aseptic precautions. Each sample for aerobic was streaked on blood agar plates and incubated at 37°C for 24-48 hrs. in aerobic culture, and in anaerobic jar with gas pack for five days. Growth on CLED (Cystine lactose electrolyte deficient) agar (with Andriade's indicator) (Fig 1) studied after 24 hrs. With the help of gram staining into GPC (gram positive cocci), GPB (gram positive bacillus), GNC (gram negative cocci), or GNB (gram negative bacillus) and these isolated organisms were identified by biochemical reactions.



Figure 1: CLED agar showing bacterial growth of colonies

ANAEROBIC ORGANISMS: These were only studied for growth and identified by only gram staining (according to CLSI guidelines

2014). Data were analyzed statistically by one-way ANOVA. A significance level of 5% was set for all analyses.

RESULTS:

The mean salivary pH was found to be 7.66±0.50 in (group 1) smokers, 8.06±0.67 in (group 2) chewer & smokers, 8.03±0.36 in (group 3) controls. The differences between the groups were not statistically significant ($P \geq 0.788$) (Table 1).

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

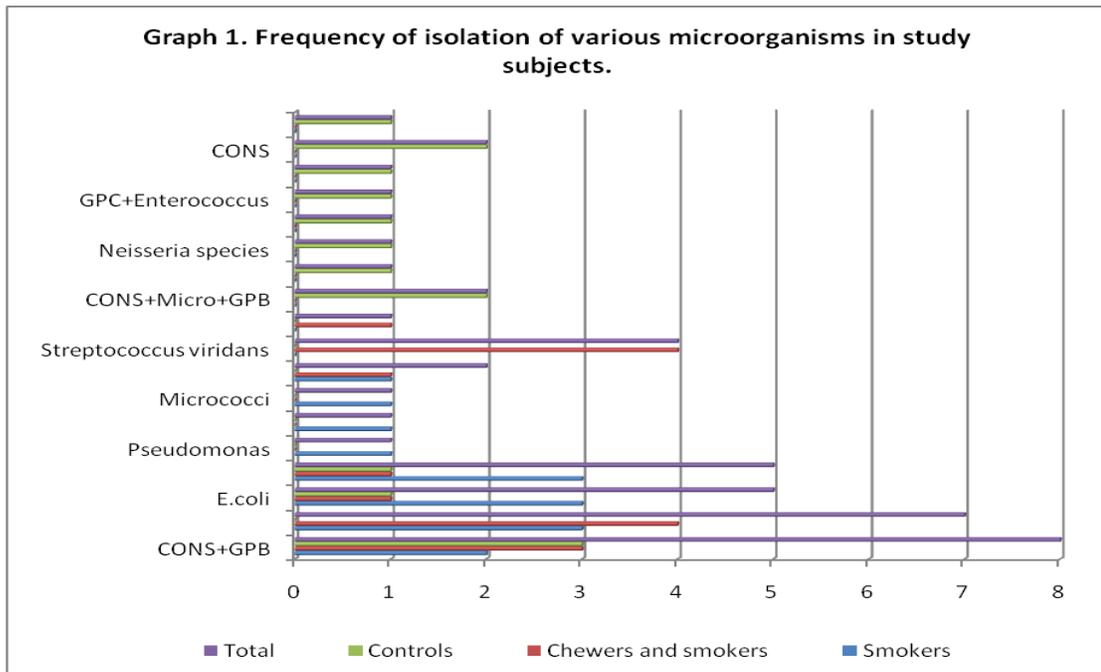
	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	Total (n=45)	F value	P value
Salivary pH	7.66±0.50	8.06±0.67	8.03±0.36	7.92±0.55	2.701	0.788
Tongue pH	6.80±0.86	6.93±1.03	7.00±0.37	6.91±0.79	0.239	0.079

Test used ANOVA, $P \leq 0.05$ is considered statistically significant.

The mean tongue coating pH was found to be 6.80±0.86 in (group 1) smokers, 6.93±1.03 in (group 2) chewer & smokers, 7.00±0.37 in (group 3) controls. The difference between the groups were not statistically significant ($P \geq 0.079$) (Table 1).

In Group 1 subjects the predominant microorganisms seen were enterococcus faecalis, E.coli & Klebsiella were as in Group 2 habit the predominant microorganisms found were Streptococcus viridans followed by Klebsiella pneumonia. Few microorganisms like micrococci, K.oxytoca & pseudomonas were seen in Group 1 but absent in Group 2 (Graph 1). Subjects in (group 3) control group showed predominantly CONS, GPB & micrococci (Graph 1).

When the microflora in all the groups were assessed Coagulase Negative Staphylococci (CONS) & Gram positive bacilli (GPB) was predominantly seen followed by Klebsiella pneumonia (Graph 1).



Graph I : Shows the distribution of oral microflora in the study groups (1 & 2) and control group (3).

DISCUSSION

Use of tobacco in various forms and its interaction is known to cause abnormality in salivary pH, flow rate as well as the oral micro-flora. Alterations in salivary pH have a significant impact on oral and dental health and can be used for the diagnosis of a wide range of diseases. Reports have suggested that over longer time periods smokers had a lower pH in stimulated whole saliva however another report showed no difference.⁶

In the present study unstimulated salivary pH was towards alkaline in both smokers (7.66±0.50), and in smokers & chewers (8.06±0.67). Singh et al (2015)⁵ observed smokers with salivary pH 6.30(±0.36), controls pH 7.10(±0.24) and result was found to be significant, but this slight difference can be explained as to the saliva sample collection done by Singh et al (2015)⁵ was when the subjects reported at any time of the day and pH strips were used for pH estimation. Hence the additional factors may have brought about this difference. Khan et al (2010) observed a lower salivary pH in smokers than in non-smokers.⁷

Fenoll-Palomares *et al.* (2004)⁸ reported a mean salivary pH of 6.7 ± 0.27 in smokers which was lower in controls (6.8 ± 0.29). No statistical difference was seen. Similarly, Rooban *et al.* (2006)⁹ also observed a lower salivary pH in smokers than controls with the pH of 6.48 ± 0.36 and 6.59 ± 0.56 respectively, but the difference was statistically significant ($P = 0.03$).

On the contrary, the study conducted by Al-Weheb (2005)¹⁰ showed that the mean salivary pH was higher in smokers that is, 7.32 as compared to nonsmokers that is, 7.27. There were no studies clearly mentioning about subjects using both types of tobacco and assessing salivary pH in them. Hence comparison was not established with results of present study. The assessment of salivary pH by various methods and between populations would be some of the limitations.

The mean tongue coating pH was found to be 6.80 ± 0.86 in smokers, 6.93 ± 1.03 in chewer & smokers, 7.00 ± 0.37 in controls. The difference between the groups were not statistically significant ($P \geq 0.079$). As there were no studies done, in which tongue coating pH being assessed in tobacco users, comparison of results of present study could not be done.

The normal flora of the oral cavity comprises of both aerobic and anaerobic organisms. Aerobic flora normally consists of - *Streptococcus viridans*, Coagulase Negative Staphylococci (CONS), Diptheroids and *Neisseria catarrhalis*. The anaerobic flora shows predominance of Lactobacilli, *Leptotrichia buccalis* and *Veillonella*. The count of normal flora is more than one lakh bacteria/ml of saliva (CFU-colony forming unit). In the present study subjects with tobacco habits showed predominantly enterococcus faecalis, E.coli & klebsiella species and streptococcus viridans were more frequently found in subjects with a habit of both chewing and smoking.³

Pavia et al (2000)¹¹ has shown decreased activity of Streptococci and other oral commensals in smokers, whereas Lie et al (2001)¹² failed to show any differences between smokers & non-smokers. Zonuz et al (2008) reported that the growth of *Streptococcus mutans* and *S. sanguis*, two common oral bacteria, was stimulated by cigarette smoke.¹³ In contrast, Ertel et al.(1991) showed that cigarette smoke inhibited the growth of Gram positive organisms, e.g., *S. pneumoniae* and *S. aureus*, but had little effect on Gram negative enteric bacteria such as *Klebsiella*, *Enterobacter* and *Pseudomonas*. Consistent with this observation, they reported that smokers have a propensity to develop heavy Gram negative colonization of the oral cavity relative to non-smokers. Bagaitkar et al (2008)¹⁴

Although there are few bacteria's that has been linked to cause various types of cancers in humans. No such direct link is established in oral cancers.¹⁵ It has been suggested that

specific oral bacteria play a part in carcinogenesis, either through induction of chronic inflammation or by interference, either directly or indirectly, with eukaryotic cell cycle and signaling pathways, or by metabolism of potentially carcinogenic substances like acetaldehyde causing mutagenesis.¹⁶

CONCLUSION: The alterations in normal oral flora and salivary pH due to 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 oral disease. However, the microbiota in a person's mouth differs depending on the methods of collection and the part of the mouth that is tested. 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.

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