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### **Effect of Long Term Tobacco Use on Unstimulated Salivary Ph And Buffering Capacity**

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#### **ABSTRACT**

**Background:** It is firmly established that tobacco use is a primary cause of many oral diseases and adverse oral conditions. The negative impact relates not only to smoking but use of smokeless tobacco. Saliva is the first biological fluid that is exposed to tobacco which contains numerous toxic compositions responsible for structural and functional changes in saliva. Alterations in salivary pH and buffering capacity have a significant impact on oral and dental health. There are several studies concerning the effect of chewing tobacco and smoking on salivary secretion, though, long-term effect of tobacco use on pH and buffering capacity is still not clear.

**Aims and Objectives:** The aim of this study is to analyze and compare the long- term effects of tobacco on salivary pH and buffering capacity between tobacco chewers, smokers, and controls.

**Materials and Methods:** Subjects will be divided equally into 4 groups; tobacco smokers (group A), chewers (group B), chewers+ smokers (group C) and controls (group D). Saliva of each subject will be collected under resting condition. The salivary pH and buffering capacity will be measured using GC Saliva-Check Buffer kit.

**Statistical Analysis:** Data will be analyzed using the Statistical Package for Social Service (SPSS) computer software. Unpaired Student's t-test, one-way ANOVA will be applied to assess the difference between groups.

**Results:** Within its limitations, the results of the present study suggest that pH and buffering capacity is lower (acidic) in tobacco chewers, chewers+ smokers and tobacco smokers. While comparing the 3 groups, groups in which tobacco chewing habit is present showed more acidic pH.

**KEY WORDS:** Tobacco, salivary pH, buffering capacity

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## **INTRODUCTION**

The epidemic of tobacco use is one of the greatest threats to global health today <sup>1</sup>. Approximately one-third of the adult population in the world use tobacco in some form and of whom half will die prematurely <sup>2</sup>. According to the World Health Organization (WHO) estimates, globally, there were 100 million premature deaths due to tobacco in the 20<sup>th</sup> century, and if the current trends of tobacco use continue, this number is expected to rise to 1 billion in the 21<sup>st</sup> century <sup>1</sup>. In addition to several other chronic diseases, tobacco use is a primary cause of many oral diseases and adverse oral conditions. Various clinical and epidemiological evidence have been documented regarding the adverse effects of tobacco on oral health <sup>3</sup>. The adverse effects of cigarette smoking and other smokeless forms are numerous and use of tobacco has been associated with oral mucosa, gingival diseases and dental alterations <sup>4</sup>.

Saliva is a complex and important body fluid which is very essential for oral health <sup>5</sup>. It plays a critical role in oral homeostasis because it modulates the ecosystem within the oral cavity <sup>6</sup>. Taking this into account, quantitative and/or qualitative alterations in salivary secretion may lead to local (caries, oral mucositis, candidiasis, oral infections, chewing disorders) or extraoral (dysphagia, halitosis, weight loss) adverse effects <sup>7,8,9</sup>. Saliva is the first biological fluid that is exposed to cigarette smoke, containing numerous toxic compositions responsible for structural and functional changes in saliva. Alterations in salivary pH and buffering capacity have a significant impact on oral and dental health <sup>10</sup>. Several studies of resting salivary pH estimate a range of 6.5–7.9 <sup>12</sup>.

Approximately 600 million people use arecanut worldwide in some form and is the fourth most commonly used psychoactive substance <sup>12</sup>. Arecanut contains four major alkaloids: Arecaidine, arecoline, guvacine and guvacoline. In the presence of lime (calcium oxide which turns to alkali calcium hydroxide in aqueous form), arecoline and guvacoline are largely hydrolyzed into arecaidine and guvacine, respectively <sup>13</sup>. The main ingredient of tobacco is nicotine and nicotine acts on certain cholinergic receptors in the brain and other organs causing neural activation leading to altered salivary secretion <sup>14</sup>.

There are several studies concerning the effect of chewing tobacco and smoking on salivary secretion, though, long-term effect of tobacco use on pH is still not clear. Due to the paucity of literature on the influence of tobacco use on pH, this study was undertaken to analyze and compare the long-term effect of tobacco on pH in tobacco chewers, tobacco smokers and control.

## **AIM & OBJECTIVE**

The aim of this study is to analyze and compare the long-term effects of tobacco on salivary pH and buffering capacity between tobacco chewers, smokers, and controls.

## **MATERIALS AND METHODS**

The present study consisted of 64 subjects (males and females ) within the age group of 25-40 years referred to KVG Dental College & Hospital were randomly selected and equally divided into four groups of 16 subjects each .

**Group A:** Tobacco smokers

**Group B:** Tobacco chewers

**Group C:** Tobacco chewer + smokers

**Group D:** Control

## **INCLUSION CRITERIA**

- Males and females of age between 25 and 40 years
- Consumption of tobacco (smoked and smokeless form) for minimum period of around 5 years.
- Subjects volunteering to take part in study.

## **EXCLUSION CRITERIA**

- Age over 40 years.
- Alcohol consumption.
- History of any other habits (tongue thrusting, mouth breathing, bruxism).
- Denture wearers
- Pregnant and postmenopausal women.
- History of radiotherapy.
- Uncooperative patients.
- Patients with systemic or salivary gland diseases or under any drug therapy.

Informed consent was obtained from each subject for saliva collection. Thorough history was taken along with detailed oral examination. Collection of saliva each subject was done under resting condition.

The pH and buffering capacity of saliva was measured using GC Saliva Buffer Kit (GC India).

## **SALIVA COLLECTION**

Saliva collection was done under resting conditions to obtain unstimulated saliva. Saliva collection was carried out between 9.00 am and 1.00 pm to avoid diurnal variations. Subjects were requested not to drink, eat or perform oral hygiene or chew or smoke 60 min before procedure. Subjects are then seated on the dental chair and asked to spit 2–3 times into an 1 min in a disposable

container. The patient was instructed not to speak, chew or perform any other activities during the procedure (figure 1, 2).

Testing of salivary pH and buffering capacity was done immediately after collection of the samples using GC Saliva Buffer Kit.



**Fig 1: Collection of saliva sample**



**Fig 2: Collected saliva sample**

### **TESTING OF SALIVARY pH**

A drop of saliva sample collected is transferred to a litmus paper. After 10 seconds the color of the paper is compared with the value in the pH scale and the value is recorded (figure 3, 4).



**Fig 3: Testing salivary pH**



**Fig 4: Comparing value with pH indicator**

### **TESTING OF SALIVARY BUFFERING CAPACITY**

3 drops of saliva sample collected is transferred to the 3 segments of the testing paper. After 2 minutes the color in the 3 segments is recorded (figure 5). Values are added and buffering capacity is recorded as Normal or Low.



Fig 3: Testing salivary buffering capacity

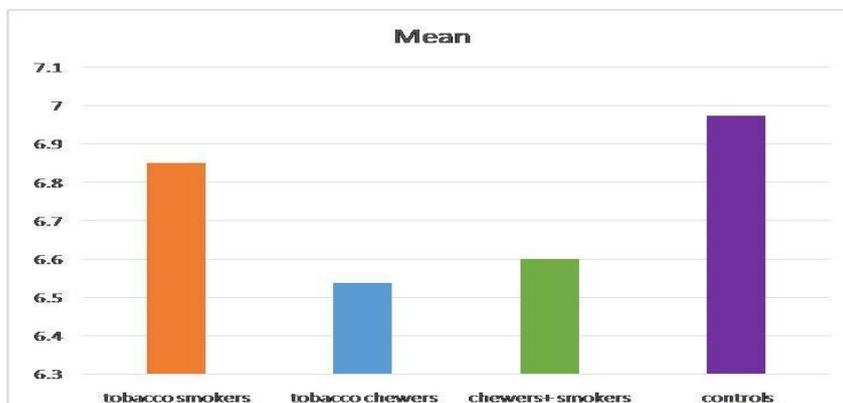
## STATISTICAL ANALYSIS

Data was analyzed using the Statistical Package for Social Service (SPSS) computer software. Unpaired Student's *t*-test, one-way ANOVA was applied to assess the pH difference between different groups.  $P < 0.05$  was considered as statistically significant. The confidence of 95% was considered.

## RESULTS

The subjects in our study were present in the age group of 25–40 years. Group A, B and C subjects consume tobacco for minimum of around 5 years. The mean pH scores of saliva in four distinct groups showed that pH scores were maximum in the control group while it was least in tobacco chewers group (graph 1).

When unpaired *t*-test was applied for comparison of pH scores of saliva between different groups, results showed a significant difference between pairs of groups at 0.05 level of significance, i.e. ( $P < 0.05$ ) (table 1).



Graph 1- mean pH scores of different groups

GROUP	N	t value	p value
TOBACCO SMOKERS	16	3.03	P = 0.0413**
CONTROL	16		
TOBACCO CHEWERS	16	6.7524	P< 0.0001**
CONTROL	16		
TOBACCO SMOKERS+ CHEWERS	16	4.52	P< 0.0001**
CONTROL	16		

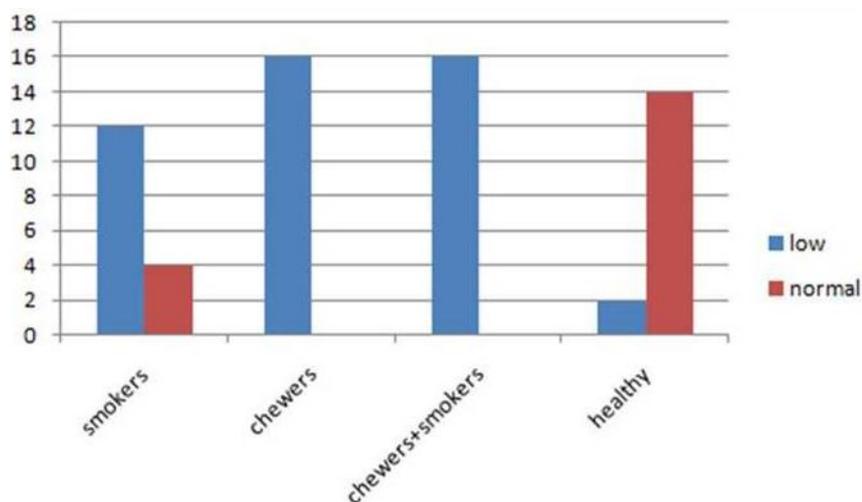
**Table 1- comparing values of different groups to control\*\* statistically**

When one way ANOVA test was applied for comparing the pH scores of saliva among four groups, it showed a significant difference in pH scores of saliva among four groups (table 2).

Source	SS	df	MSS	F	P
Between groups	2.0469	3	0.6823	19.61	< 0.0001**
Within Group	2.0875	60	0.0348		
Total	4.1344	63			

**Table 2- intergroup comparison of pH values \*\* statistically significant**

The mean scores for buffering capacity in four distinct groups showed normal buffering capacity in the control group while it was significantly low in tobacco using groups (graph 2).



**Graph 2- mean buffering capacity scores of different groups**

When one way ANOVA test was applied for comparing the buffering capacity of saliva among four groups, it showed a significant difference in buffering capacity of saliva among four groups (table 3).

Source	SS	df	MSS	F	P
Between groups	8.1875	3	2.729167	34.47	< 0.0001**
Within Group	4.75	60	0.079167		
Total	12.9375	63			

**Table 3- intergroup comparison of buffering capacity\*\* statistically significant**

## DISCUSSION

Saliva is a complex and important body fluid which is very essential for oral health <sup>5</sup>. Saliva is required for protecting the oral mucosa, teeth remineralization, digestion, taste sensation, pH balance and phonation. It includes a variety of electrolytes, peptides, glycoproteins and lipids which have antimicrobial, antioxidant, tissue repair and buffering properties <sup>15</sup>. Saliva is the first biological

fluid that is exposed to cigarette smoke, which contains numerous toxic compositions responsible for structural and functional changes in saliva<sup>10</sup>.

From the inferences of the present study it is clear that tobacco users have more acidic pH and lower buffering capacity as compared to non tobacco users which may make them more prone dental caries. On comparing tobacco chewers to smokers, the chewers had more acidic pH and lower buffering capacity as compared to tobacco smokers & the difference were extremely statistically significant.

The decrease in salivary pH and buffering capacity is mostly attributed to the decreased salivary flow rate in smokers which reduces the bicarbonate ion concentration in saliva and hence reducing pH and buffering capacity, in addition to this Lime present in mostly all forms of chewing tobacco also reacts with the available bicarbonate ions leading to loss of bicarbonate ions available. The alteration in electrolytes and ions alters the pH as they interact with the buffering systems of saliva.

Voelker et al in his study found relationship between caries risk and smoking, buffering capacity and smoking, and stimulated salivary pH and smoking were concluded. No significance difference between *S. mutans* and smoking were noted from the preliminary results<sup>16</sup>.

Grover et al in 2015 in his study observed that a lower (acidic) salivary pH was observed in tobacco users as compared with control. These alterations in pH due to the long-term effect of tobacco use can render oral mucosa vulnerable to various oral and dental diseases<sup>4</sup>. Khan *et al.* in 2010 also observed a lower salivary pH in smokers than in nonsmokers which was consistent with the findings of the present study<sup>17</sup>. Rooban *et al.* 2008 observed a mean pH of 6.77 in nonchewers and those who chew tobacco, the mean pH turns acidic<sup>18</sup>.

But in contrast Reddy *et al.* and Alpana Kanwar *et al.* observed no difference in salivary pH between the chewers and nonchewers. This difference could be due to the amount of tobacco, lime and other components. The role of lime has been a source of concern. Lime (calcium oxide in aqueous forms calcium hydroxide) could cause a free radical injury or the high alkaline content probably reacts with the salivary buffering systems and alters the pH<sup>19, 20</sup>.

A salivary pH of 7.0 usually indicates a healthy dental and periodontal situation. At this pH, there is a low incidence of dental decay and little or no calculus. A saliva pH below 7.0 usually indicates acidemia (abnormal acidity of the blood). If a chronic condition exists, the mouth is more susceptible to dental decay, halitosis and periodontitis. Chronic acidemia can be a causative factor for a multitude of diseases affecting the whole body<sup>21</sup>.

## **CONCLUSION**

Within its limitations, the results of the present study suggest that pH and buffering capacity is lower (acidic) in tobacco chewers, chewers+ smokers and tobacco smokers. While comparing the 3 groups, groups in which tobacco chewing habit is present showed more acidic pH. This is, to the best of our knowledge, the first study which has reported on the pH & Buffering capacity of saliva in tobacco users..

With a high prevalence of tobacco consumption in different forms, the oral health of the population is at risk, and oral health programmes to increase the awareness of the public to the health hazards of tobacco consumption need to be implemented.

Moreover, due to the high prevalence of smokeless tobacco use in this population, there is a need for further studies with an improved study design to better understand the effects of the different forms of tobacco products on pH, buffering capacity and various other parameters.

### ***Declaration of patient consent***

Consent from the patient is obtained before the collection of samples. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be recorded. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

### **Conflicts of interest**

There are no conflicts of interest.

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