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Comparative Evaluation and Correlation of Salivary Total Antioxidant Capacity with Dental Caries among Smokers and Tobacco Chewers in North Karnataka Region of Indian Sub Population - A Clinico Biochemical Study

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Abstract

Background: Tobacco chewing and smoking influence salivary antioxidant levels and therefore their effect on dental caries incidence needs to be evaluated.

Objectives: To correlate the effect of smoking and tobacco chewing on salivary pH and total antioxidant capacity (TAC) in individuals with and without dental caries in North Karnataka region of India.

Methods: About 180 male patients aged 20-60 years were chosen and allotted to three groups (n=60). Group 1: Non-smokers and chewers. Group 2: Only tobacco chewers and Group 3: Only smokers. Each group was further split into two subgroups (n=30) A: with dental caries, B: without dental caries. Dental caries was assessed by WHO-recommended DMFT (Decayed, Missing, Filled, Teeth) criteria and salivary samples were obtained by spitting method. Salivary pH was determined and supernatants from centrifuged samples were subjected to ferric reducing antioxidant power (FRAP) assay for estimating TAC levels. The one-way analysis of variance (ANOVA) and Post Hoc Tukey tests were used for statistical analysis (P <0.05).

Results: Higher caries rate with lower pH and decreased salivary TAC levels were found in tobacco chewers and smokers with and without dental caries compared to non-tobacco chewers and non-smokers with significant difference.

Conclusion: Tobacco chewers and smokers showed decreased salivary TAC levels with lower pH and high caries rate compared to individuals without these habits.

Keywords: Antioxidant capacity, Dental caries, Saliva, Smoking, Tobacco chewing

Introduction

Dental caries affects people of all ages worldwide and its occurrence has been linked to changes in saliva, including those to its flow rate, pH, viscosity, and salivary components, such as imbalances in levels of free radicals (FR), reactive oxygen species (ROS), and antioxidants.[1]

The usage of tobacco, either chewed or smoked, is harmful to human health and has been associated with occurrence of oral disorders such as the malignant and precancerous lesions, periodontal disease, and dental caries.[2] The World Health Organization (WHO) estimated that smoking causes more than seven million deaths per year and the tobacco products cost hundreds of billions of dollars worldwide.[3] Several hazardous compounds, including nitrous oxide, carbon monoxide, nicotine, tar, cadmium, methanol etc., are found in tobacco. These substances and their metabolites interact with biological molecules in the human body, forming reactive oxygen and nitrogen species (ROS/RNS), free radicals (FR), and initiate the chain reaction of radicals. Antioxidants are responsible for stabilizing and deactivating the FR. Most of the body fluids consist of antioxidants, which play a significant role in body's defense system. Oxidative stress is caused by imbalance between ROS, RNS, free radicals, and antioxidants leading to damage to the tissues and cells that result in disease or infection.[4-6]

Along with its other functions, such as lubrication, antibacterial activity, and buffering capacity, saliva also serves as a source of antioxidants and helps in maintaining the redox balance. Saliva has been employed as a diagnostic tool because of its non-invasive collection procedure and as the antioxidant content of saliva and blood correlate well.[5] Since salivary antioxidants are the initial line of defense that come into contact during tobacco chewing and smoking and as it is reported that saliva plays an important role in anti-cariogenic activity, saliva can be considered as the most suitable sample to measure antioxidant capacity.[3,5] Low levels of total antioxidant capacity (TAC) have been reported among tobacco smokers and chewers; however, literature is scarce regarding comparison of and correlation of salivary TAC levels with dental caries among tobacco chewers and smokers. Therefore, this study was undertaken to fill the gap in the existing literature.

The current study's objectives were to (i) assess the impact of smoking and tobacco use on the pH and TAC of saliva in individuals with and without dental caries and (ii) compare the salivary TAC levels with prevalence of dental caries among smokers and tobacco chewers in the North Karnataka region of the Indian subpopulation.

Material and Methods

This study was carried out in accordance with the institutional ethical clearance (Reference No. IEC/2020-21/28). For sample size estimation, a power calculation was conducted by using the variance statistical test using G*Power software version 3.1 (Heinrich Hein University, Dusseldorf, Germany) with 80% power, standard deviation=1.19 and α=0.05 level of significance, and total sample size was determined to be 180.

About 180 male patients between the ages of 20 and 60 years visiting the outpatient department of our institute were chosen for the study and written informed consent was obtained. The inclusion criteria considered for the study were, individuals who never smoked or chewed tobacco (control), participants with either tobacco chewing habit or a habit of smoking every day for at least ten years and subjects who met WHO standards for caries evaluation. The study excluded participants with both habits of smoking and chewing tobacco, periodontal diseases, oral mucosal lesions, systematic diseases, other conditions that alter salivary secretions, and those on radiotherapy, chemotherapy, participants with history of chronic alcohol intake, and use of antioxidant medications during the previous three months. The study participants were then split into three groups (n=60 each):

Group 1: No history of tobacco chewing and smoking (Control)

Group 2: History of only tobacco chewing

Group 3: History of only smoking

Two subgroups (n=30 each) were obtained from each group as A: with dental caries and B: without dental caries.

The World Health Organization's (WHO) DMFT (Decayed, Missing, Filled, Teeth) scoring criteria were used to assess dental caries. The subjects were advised to rinse their mouth with chlorhexidine mouthwash. The smooth and occlusal surfaces of the teeth were cleaned with sterile cotton, dried, examined using mouth-mirror and probe under proper illumination and the DMFT scores were recorded.

The saliva samples were collected by spitting method. Participants were advised not to eat or drink (water being the only exception) for at least one hour prior to the sample collection and were advised not to smoke, chew tobacco, or gum. Unstimulated saliva samples were collected between 9 and 11 am to minimize circadian errors.

The individuals were instructed to rinse their mouths with distilled water, sit comfortably with their eyes open, their head slightly tipped forward, with restricted orofacial movements and allowed to rest for five-minutes and were directed to spit the saliva into a sterile container every 60 seconds for 10 minutes and about 8-10 mL of saliva was collected.[7]

The salivary pH was recorded immediately after collecting saliva to avoid any deterioration of the sample in each group, as per Baliga *et al.*[8] For the pH measurement, a single electrode digital pH meter (Systronics Model 335, Ahmedabad, India) was used. The electrode was immersed in 0.1 N hydrochloric acid for the entire night, and the pH meter was calibrated before the test using freshly prepared pH 7 and pH 4 buffer solutions and then stored in distilled water. The electrode was gently dried using a sterile tissue paper and then inserted in a 10 mL glass beaker holding 3 mL saliva sample. The electrode tip was again gently rinsed with distilled water, and it was then submerged in the distilled water when not in use.

For salivary TAC analysis, about 5 mL of saliva samples were centrifuged for 10 minutes at 4°C at 4000 rpm in a microcentrifuge (Remi, model RM-12C, India) to eliminate cell debris. The resultant supernatants were collected in saliva containers until further use.

The salivary TAC was assessed by using Ferric Reducing Antioxidant Power (FRAP) assay, according to Shankar *et al.*[9] The saliva sample was mixed thoroughly with 2.5 mL of 20 mM sodium phosphate buffer (pH 6.6) using a magnetic stirrer (Borosil Model 100MS000115000, India), and then it was incubated (Kemi Model - KIS 1, India) for 20 minutes at 50°C. Then, 2.5 mL each of 1% potassium ferricyanide and 1% trichloroacetic acid were added, and the mixture was kept for five minutes. The absorbance of sample was read at 700 nm in a visible spectrophotometer (Systronics model 168, Ahmedabad, India), compared to the standard reference ascorbic acid and data were recorded.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) software of version 22 (IBM Statistics, Chicago, USA), was used to analyze the obtained data using One-way Anova and post Hoc Tukey tests at $P \leq 0.05$ level of significance.

Results

In group 1, the mean DMFT score was found to be lowest with greater pH in subgroup 1B (control), whereas salivary TAC levels were found to be highest in subgroup 1A, followed by subgroup 1B. Salivary TAC levels and pH were found to be lowest with high DMFT scores in tobacco chewers (group 2) and smokers (group 3) compared to non-smokers & non-tobacco chewers (group 1). In groups 2 and 3, salivary TAC levels and pH were less in caries group (subgroup A) compared to subjects without caries (subgroup B) ($P < 0.05$) (Table1).

Table 1: Mean of DMFT scores, pH and salivary TAC values and *P* value using one-way ANOVA for all groups

Group	Sub group	DMFT Score	pH	TAC value (µM/mL)	*P* value
1	A	4.0333 ±0.9643	6.6950 ±0.1909	568.3420 ±7.5118	<0.05 (S)
	B	0±0	7.2637 ±0.1791	452.0610 ±7.0165	
2	A	5.4333 ±1.1943	5.4923 ±0.0510	202.9667 ±2.6068	
	B	0±0	5.8583 ±0.0441	247.3936 ±1.83335	
3	A	5.6667 ±1.2954	5.9883 ±0.0552	261.1313 ±1.3966	
	B	0±0	6.3693 ±0.0449	293.4600 ±2.6647	

S- significant difference

Multiple comparisons between groups showed significant differences between all the groups in terms of pH and TAC values. For DMFT score, significant difference between all the groups was noted with no significant difference between the subgroup B of all groups as well as between subgroups 2A and 3A (Table 2).

Table 2: Multiple comparison by Post hoc Tukey test between the groups for DMFT score, pH and TAC values

Comparison groups	DMFT Score		pH		TAC value (µM/mL)	
	Difference	*P* value	Difference	*P* value	Difference	*P* value
Subgroup 1A and Subgroup 1B	4.0333	0.0000(S)	0.5687	0.0000(S)	116.2810	0.0000(S)
Subgroup 1A and Subgroup 2A	1.4000	0.0000(S)	1.2027	0.0000(S)	365.3753	0.0000(S)
Subgroup 1A and Subgroup 2B	4.0333	0.0000(S)	0.8367	0.0000(S)	320.9483	0.0000(S)
Subgroup 1A and Subgroup 3A	1.6333	0.0000(S)	0.7067	0.0000(S)	307.2107	0.0000(S)
Subgroup 1A and Subgroup 3B	4.0333	0.0000(S)	0.3257	0.0000(S)	274.8820	0.0000(S)
Subgroup 1B and Subgroup 2A	5.4333	0.0000(S)	1.7713	0.0000(S)	249.0943	0.0000(S)
Subgroup 1B and Subgroup 2B	0	1.0000(NS)	1.4053	0.0000(S)	204.6673	0.0000(S)
Subgroup 1B and Subgroup 3A	5.6667	0.0000(S)	1.2753	0.0000(S)	190.9297	0.0000(S)
Subgroup 1B and Subgroup 3B	0	1.0000(NS)	0.8943	0.0000(S)	158.6010	0.0000(S)
Subgroup 2A and Subgroup 2B	5.4333	0.0000(S)	0.3660	0.0000(S)	44.4270	0.0000(S)
Subgroup 2A and Subgroup 3A	2.3333	0.8799(NS)	0.4960	0.0000(S)	58.1647	0.0000(S)
Subgroup 2A and Subgroup 3B	5.4333	0.0000(S)	0.8770	0.0000(S)	90.4933	0.0000(S)
Subgroup 2B and Subgroup 3A	5.6667	0.0000(S)	0.1300	0.0002(S)	13.7377	0.0000(S)
Subgroup 2B and Subgroup 3B	0	1.0000(NS)	0.5110	0.0000(S)	46.0663	0.0000(S)
Subgroup 3A and Subgroup 3B	5.6667	0.0000(S)	0.3810	0.0000(S)	32.3287	0.0000(S)

S- significant difference, NS-no significant difference.

Discussion

Dental caries is the most prevalent chronic illness affecting people worldwide and therefore necessitates research for its prevention. Saliva contains a variety of biological components that help in remineralization and protect enamel, dentin, and cementum from the formation of caries, and it is dependent on the quantity and levels of its contents.[1,10] The varied composition of saliva affects the dental caries occurrence and association was found between various components of saliva and dental caries.[11]

Antioxidants are chemicals that when present in low quantities relative to an oxidizable substrate, considerably slow down or prevent that substrate's oxidation. In healthy human physiology, ROS and anti-oxidant defense capacity are in dynamic equilibrium. Oxidative stress develops when this equilibrium shifts in favor of ROS. In oral cavity, they help in controlling the oral bacteria that cause dental plaque accumulation leading to occurrence of dental caries and periodontal diseases.[12] Since salivary antioxidants are first line defense mechanism among tobacco chewers and smokers, estimation of antioxidants in such individuals and their correlation with dental caries is essential.[13]

Salivary TAC was assessed in the current study utilizing the FRAP assay. It is a modern technique for assessing antioxidant capacity. The principle is based on the observation that a colored ferrous-tripyridyltriazine complex results from the conversion of ferric to ferrous ions at low pH.[14]

In the present study, the results showed that the salivary TAC levels in tobacco chewers and smokers (groups

2 and 3) were less compared to controls (subgroup 1,) which is in line with other studies by Shetty AV *et al.*[15] and Shwetha S *et al.,*[16] who reported that salivary TAC levels and pH were lowest in tobacco chewers compared to smokers. This could be due to the decreased salivary flow rate in tobacco chewers compared to smokers. In addition, lower levels of antioxidants among smokers and tobacco chewers emphasize the impact of smoking and tobacco use in the etiology of oral diseases. This might be caused by greater levels of free radicals, resulting in oxidative stress and depletion of body's antioxidant reserves.

In the present study, salivary TAC levels were higher in non-smoker/non-tobacco chewers with caries compared to similar subjects without caries, which is in accordance with the findings of Shetty AV *et al.,*[15] and Hegde MN *et al.,*[17] who suggested that the reported increase could be due to increased levels of proteins and cariogenic activity and change in antioxidant levels in response to an infection or illness. In the present study, mean DMFT score was found highest among the tobacco chewers and smokers compared to controls. This is due to less salivary flow, lower pH and lower TAC levels in these individuals compared to the non-tobacco users.[15]

In present study, among non-smokers and non-tobacco chewers, pH was lower in caries group compared to non caries group. However, salivary pH was lowest in the tobacco chewers and smokers compared to controls, which is similar to the study conducted by Kanwar A *et al.*[18] This could be attributed to the alteration in electrolytes and ions as they interact with the buffering systems of saliva and low pH is also associated with high caries rate.[15,19]

According to Vellappally S *et al.,*[20] tobacco usage either in chewing or smoking forms is associated with high dental caries occurrence and the mean score of decayed teeth was found to be highest among tobacco chewers (6.96) compared to smokers (6.44). The present study results are in line with above study with mean DMFT score found to be highest in the tobacco chewers and smokers compared to controls. This is due to reduced salivary flow and lower TAC levels in these individuals compared to the individuals without habits.[16]

In the present study, compared to controls with and without caries, tobacco chewers and smokers showed a reduced salivary total antioxidant capacity and a higher rate of dental caries. This emphasizes the role of tobacco usage in the pathogenesis of dental caries.[15]

Clinical significance

Saliva is a simple diagnostic tool for estimating dental caries and therefore can aid in the implementation of dental caries prevention strategies. It is recommended to incorporate antioxidant rich food supplements, toothpastes and mouth rinses containing antioxidants for tobacco users. The findings of the current study may serve as guidelines for future investigations into the antioxidant capacity of tobacco chewers to prevent harmful consequences related to dental caries.

Limitations

The duration, frequency of smoking, type and amount of tobacco chewing were not considered in the present study. Therefore, study protocols with more research variables and fewer confounding variables are needed in the future.

Conclusion

Tobacco chewers and smokers had lower salivary TAC levels, lower pH and high caries rate compared to individuals without these habits both with and without dental caries.

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Conflict of interest

None

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