ORIGINAL ARTICLE

Salivary oxidative stress level among tobacco chewers and smokers: A comparative Study

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ABSTRACT

Tobacco contains various toxic contents which produce oxygen-free radicals that damage oral tissues. Since saliva encounters tobacco, it has antioxidant defense system and also can serve as a biomarker for oral diseases. Thus, the present study aims to evaluate salivary oxidative stress levels among smokers and chewers. Unstimulated saliva from 240 males who visited tobacco cessation clinic for the first time was collected. Standard protocol was followed to collect saliva and assess salivary antioxidant levels from 80 participants with the habit of smoking, smokeless, and both (smoking and smokeless) tobacco users. The collected data were statistically analyzed. The mean salivary superoxide dismutase and malonyldialdehyde levels were significantly high for participants with both habits ($P < 0.000$). There was a significant high reduction of glutathione peroxidase and catalase in participants with both habits ($P < 0.000$). Both smoking and smokeless tobacco modify salivary antioxidant activity. The estimation of salivary oxidative stress can serve as a diagnostic and prognostic biomarker for oral tissue damage and dysplasia. Furthermore, they can function as early biomarkers in preventing dysplastic changes in the oral cavity.

Key words: Antioxidant stress, catalase, malonyldialdehyde, saliva, smokers, tobacco chewers

INTRODUCTION

About 24.3% and 25.9% of the adults are current smokers and tobacco chewers in India.[1] This increased use of tobacco has led to an increase in the prevalence of noncommunicable diseases such as ischemic heart disease, cancers, diabetes, and chronic respiratory diseases. India spends nearly $27.5 billion for treating smoking and smokeless tobacco-related diseases among people aged ≥35 years, encountering a massive economic burden.[2] Both smoking and smokeless tobacco use attributed to 3500 death/day in India.[3]

Tobacco products contain 5000 toxic substance.[4] There is a structural analogy between nicotine and the neurotransmitter acetylcholine (Ach). Thus, nicotine combines with receptors of Ach easily and endeavors actions similar to Ach, which up tights mental and physical arousal, several emotional aspects, learning, and memory. This mechanism makes one addicted to tobacco.[5,6] Evidence reports that absorption and retention of nicotine in the bloodstream are twice in oral smokeless tobacco to smoke form.[7]

These toxic substances produce oxidative stress causing tissue damage and apoptosis (programmed cell death).[8] Oxidative stress is the system's inability to compensate the harmful effects of excessive production of reactive oxygen

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species (ROS) such as peroxides, superoxide, and hydroxyl radicals.[9] Imbalance in the rate of production of ROS and rate of clearance by endogenous antioxidants (superoxide dismutase [SOD], catalase [CAT], and Glutathione peroxidase [GSH-Px]) is the reason for cellular and extracellular damage.[9] ROS are highly reactive chemicals formed from O_2 which is toxic to all aerobic species. On exposure to tobacco, this O_2^- increases the levels of antioxidant enzymes mentioned earlier.[10] The highly reactive O_2^- is then converted to H_2O_2 by SOD which in turn is converted into molecular oxygen and water by CAT and GSH-Px as shown in Figure 1.[11] The ROS causes plasma membrane injury and cell death by oxidation and formation of lipid peroxidase on reaction with fatty acids.[11] On reaction with proteins and DNA, ROS by oxidation causes loss of enzyme activity and abnormal folding of proteins and mutation of DNA, respectively.[12]

Noninvasive diagnostic body fluid to rendezvous tobacco is saliva. Peroxidase and uric acid systems of saliva help in fighting against ROS, thereby presenting saliva as a diagnostic and preventive factor.[13,14] SOD fights against the ROS produced on tobacco use. Thus, the present study aims to estimate and compare salivary antioxidants such as SOD, CAT, GSH-Px, and malonyldialdehyde (MDA) levels among smokers and chewers.

MATERIALS AND METHODS

About 240 male patients with ages ranging from 25 to 55 years were selected using the convenience sampling method from the tobacco cessation counseling clinic of the author's institution. Eighty patients having the habit of smoking were enrolled in Group 1; 80 patients having the habit of using smokeless tobacco were enrolled in Group 2; and 80 patients having the habit of using both smoke and smokeless tobacco were enrolled in Group 3. All healthy current tobacco users with no use of any antibiotic or anti-inflammatory and antioxidant drugs at least for the past 6 months were recruited. Written informed consent was accomplished from the potential patients after explaining the purpose of this research. Cognitive-behavioral therapy had been given to all the patients recruited for the study. Research ethical clearance to execute the study was accomplished by the authors' Institution Scientific Review Board (IHEC/SDC/PHD/21/132).

Salivary sample collection

Unstimulated salivary samples from all the participants were collected. Participants were asked not to eat/drink anything or use tobacco in any form 1 h before sample collection. The salivary samples were collected from 9 a.m. to 11 a.m. to prevent variations due to circadian rhythm. The study participants rinse their mouth with 15 mL of pure water to clear away debris. They were insisted to pool saliva in the floor of their mouth and drool it into the sterile containers. Supernatant saliva was aspirated after centrifugation and used for biochemical assay.[15]

Superoxide dismutase

SOD for the salivary samples was assessed using Misra and Fridovich standard procedure.[16] The units of SOD in salivary samples had been expressed as units/mL. The principle of this assay is that SOD inhibits the self-oxidation of adrenaline to adrenochrome at pH 10.2.

Glutathione peroxidase

GSH-Px level of the collected salivary samples was assessed using Rotruck in 1973 described in text book on Enzymatic basis of Detoxication.[17] Their levels were measured in a spectrophotometer at 412 nm.

Catalase

CAT assay of salivary samples was carried out using the

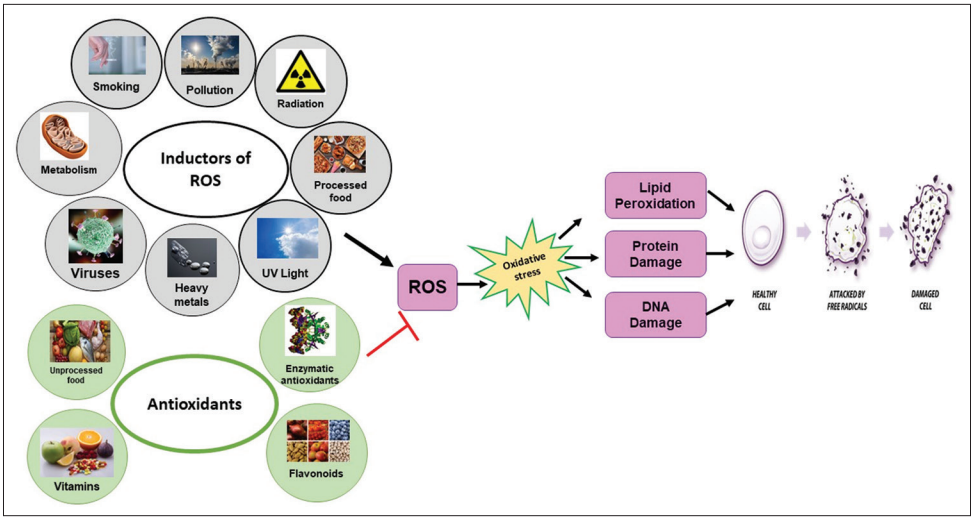

Figure 1: Production of reactive oxygen species and its effect

directions given in the ELISA kit (SINNOWA Medical Science and Technology, Jiangsu, China). The signal produced to load bound analyte and its intensity at 450 nm was assessed and conveyed in units of U/ml.

Malonyldialdehyde

MDA analysis of salivary samples was measured using the method described by Stalnaya and Garishvili (1997).[18] The obtained readings were then compared with a series of standard solutions of 1,1,3,3-tetraethoxypropane and conveyed as micromoles per milliliter (μmol/mL).

Statistical analysis

Obtained data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 23.0 IBM, Chicago, Illinois, United states. The normality of the data was assessed using Kolmogorov–Smirnov test. A one-way ANOVA test was carried out to compare the mean of salivary oxidative stress levels among the groups. Pairwise comparison was carried out with Tukey's Honest Significant Difference test. $P < 0.05$ was considered to be statistically significant.

RESULTS

The mean age of participants in Groups 1, 2, and 3 was 37.58 ± 6.79, 39.29 ± 7.45, and 38.13 ± 7.91, respectively. The mean SOD among participants of Groups 1, 2, and 3 was 28.78 ± 9.184, 39.67 ± 11.892, and 45.29 ± 12.131, respectively, with a significant difference between the groups ($P = 0.000$) as shown in Table 1. A significant difference in mean GSH-Px was elucidated between the groups ($P = 0.000$) shown in Table 2. The mean CAT among the participants in Groups 1, 2, and 3 was 7.51 ± 1.26, 6.12 ± 0.94, and 5.03 ± 0.64, respectively, with a significant difference ($P = 0.028$) as shown in Table 3. There is a significant difference in mean MDA levels among the groups ($P = 0.017$) shown in Table 4. Tukey's *post hoc* test reported a significant difference in mean SOD, GSH-Px, CAT, and MDA between the groups ($P < 0.05$). The mean levels of SOD, GSH-Px, CAT, and MDA levels of all three groups were shown in Figures 2 and 3.

Table 1: Mean comparison of superoxide dismutase units/ml among the study participants

Group	Mean±SD	*F*	*P*
1	28.78±9.184	72.546	0.000
2	39.67±11.89		
3	45.29±12.13		
Pairwise comparison by Tukey's Honest Significant Difference test			
Comparison	**Mean difference**	***P***	**95% CI**
Group 1 versus Group 2	−10.89	0.018	−14.52–−4.268
Group 2 versus Group 3	−5.62	0.007	−9.467–−1.277
Group 3 versus Group 1	16.51	0.001	9.583–19.48

SD: Standard deviation, CI: Confidence interval, HSD: Honest Significant Difference

Table 2: Mean comparison of salivary glutathione peroxidase among the study participants

Group	Mean±SD	*F*	*P*
1	1.78±0.042	81.752	0.000
2	0.56±0.006		
3	0.07±0.001		
Pairwise comparison by Tukey's Honest Significant Difference test			
Comparison	**Mean difference**	***P***	**95% CI**
Group 1 versus Group 2	1.22	0.004	0.56–2.89
Group 2 versus Group 3	0.49	0.035	0.08–1.64
Group 1 versus Group 3	1.71	0.000	0.91–2.59

SD: Standard deviation, CI: Confidence interval, HSD: Honest Significant Difference

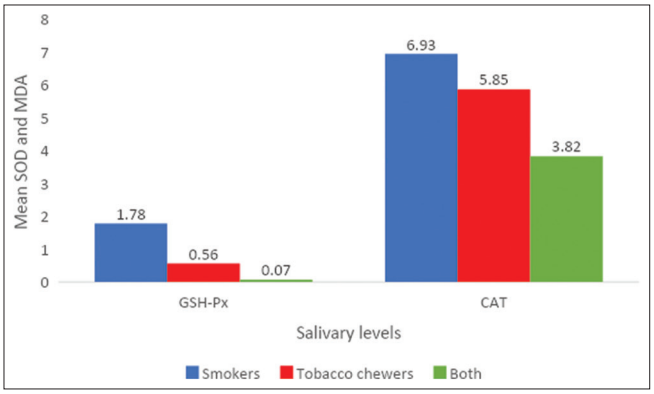

Figure 2: Mean level of SOD and MDA levels among the study participants. SOD: Superoxide dismutase. MDA: Malonyldialdehyde

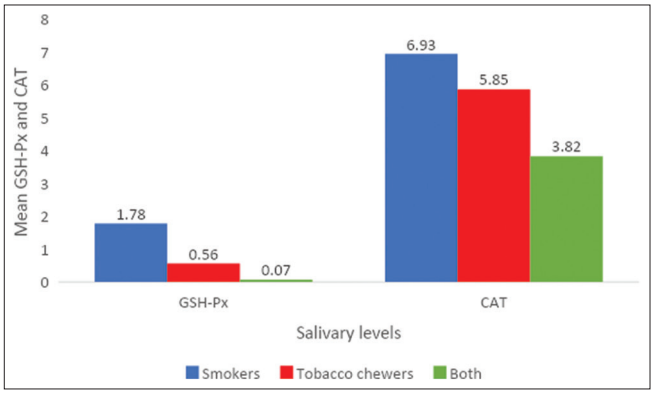

Figure 3: Mean level of GSH-Px and CAT levels among the study participants. GSH-Px: Glutathione peroxidase, CAT: Catalase

DISCUSSION

An imbalance in the rate of production of ROS and rate of

Table 3: Mean comparison of salivary catalase U/ml among the study participants

Group	Mean±SD	*F*	*P*
1	6.93±0.531	83.167	0.000
2	5.85±0.473		
3	3.82±0.432		
Pairwise comparison by Tukey's Honest Significant Difference test			
Comparison	**Mean difference**	***P***	**95% CI**
Group 1 versus Group 2	1.08	0.001	0.09–2.47
Group 2 versus Group 3	2.03	0.028	1.27–3.52
Group 1 versus Group 3	3.13	0.000	1.58–4.26

SD: Standard deviation, CI: Confidence interval, HSD: Honest Significant Difference

Table 4: Mean comparison of malonyldialdehyde µmol/mL among the study participants

Group	Mean±SD	*F*	*P*
1	17.56±4.125	85.249	0.000
2	28.94±5.278		
3	34.21±5.648		
Pairwise comparison by Tukey's Honest Significant Difference test			
Comparison	**Mean difference**	***P***	**95% CI**
Group 1 versus Group 2	−11.38	0.006	−8.62−−2.89
Group 2 versus Group 3	−5.27	0.012	−9.34−−3.49
Group 3 versus Group 1	16.65	0.000	11.48–22.23

SD: Standard deviation, CI: Confidence interval, HSD: Honest Significant Difference

clearance by endogenous antioxidants produce oxidative stress. This oxidative stress and free radical reactions contribute as etiology of numerous systemic diseases.[19-21] ROS react with lipids, proteins, and DNA/RNA by oxidation to cause protein damage and mutation of DNA.[22] The first biological fluid to encounter tobacco is saliva. The prime objective of the present study was to estimate and compare the salivary oxidative stress levels among smokers, smokeless, and both (smoking and smokeless) tobacco users. To our knowledge, this is the first study to evaluate the antioxidant level among tobacco users in both forms.

The present study results showed a significant increase in the mean SOD levels among participants who used both forms of tobacco (45.29 ± 12.13) compared to smokers and smokeless tobacco users. On comparison of mean GSH-Px, it was found that there is a significant decrease among the participants who have both habits (0.07 ± 0.001). Similarly, there is a significant high decrease in CAT among participants who used both forms of tobacco (3.82 ± 0.432). A significant high increase in MDA levels among participants with both habits was elucidated (34.21 ± 5.648).

A previous studies showed a statistically significant rise in salivary SOD among smokers compared to nonsmokers.[23,24] The results of the present study are in consistency with the abovementioned studies. In contrast, a case–control study among khat chewers showed no significant change in the SOD and GSH-Px levels between chewers and nonchewers.[25] A case–control study among tobacco chewers showed a significant increase and decrease in the salivary MDA and CAT levels compared to controls.[26] These results are found to be similar to the results of the present study. In contrast, a previous study among 200 smokers and nonsmokers showed no significant difference in salivary CAT levels.[27] The reason behind this difference could be attributed to the concentration of toxic constituents in different forms of smokeless tobacco.

One of the biomarkers for lipid peroxidation is MDA which is found to be higher in both forms of tobacco users. Salivary MDA levels are found to be increased in other pathological conditions such as periodontitis, cardiovascular disease, oral potentially malignant disorders, and oral cancer.[28-30] High lipid peroxidation by both forms of tobacco is responsible for increased MDA levels in individuals with both habits. The increase in the salivary CAT can be due to peroxidation of proteins and oxidation of DNA in both form users.[31] Increased oxidative stress by ROS and improper functioning of antioxidant defense might be the contributing etiology for cancer-related oral diseases.

Although the present study made an effort for stringent consideration of eligibility criteria, the findings could be limited, since we failed to control the confounders such as gingival, periodontal disease, and diet which influence salivary oxidative stress levels. Furthermore, we failed to consider the duration and intensity of habits which might be directly proportional. Further longitudinal studies controlling confounding factors are needed to assess the clinical impact of increased salivary oxidative stress as a diagnostic, prognostic, and preventive biomarker.

CONCLUSION

There is a marked increase in the salivary SOD, GSH-Px, CAT, and MDA levels among smokeless and both forms of tobacco users. People with both habits are at high risk for potentially malignant disorders, cancer, periodontitis, and dental caries. Awareness programs need to be targeted in this context to reduce the oral cancer burden of the country by early diagnosis of dysplastic changes.

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Conflicts of interest
There are no conflicts of interest.

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