# ACTIVITY OF SALIVARY SUPEROXIDE DISMUTASE IN SMOKELESS TOBACCO CONSUMERS AND NON-CONSUMERS– A BIOCHEMICAL STUDY

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**ABSTRACT**

**Background and objectives:** The habit of smokeless tobacco chewing is one of the known risk factors for oral cancer. Most likely, the antioxidant defense system in dealing with free radicals induced by smokeless tobacco and prevention of oral cancer is important. Saliva is the first biological fluid that encounters the harmful products of smokeless tobacco and has antioxidant defence system able to counter toxic activities of free radical species. The aim of this study was to compare the levels of superoxide dismutase (SOD) in saliva of smokeless tobacco consumers and non- consumers.

**Methods:** In this study, un-stimulated saliva of 100 subjects (50 smokeless tobacco consumers and 50 non-consumers) was collected. The activity of super oxide dismutase

enzyme was measured by standard biochemical methods (Marklund method) and the obtained data was analyzed by student's unpaired 't'test.

**Results & Conclusion:** The mean activity of super oxide dismutase was significantly higher in the smokeless tobacco consumers group compared to non-consumers ($p<0.001$). The results of this study demonstrate that consumption of smokeless tobacco leads to increased activity of salivary super oxide dismutase. Thus, evaluating the variations in the level of SOD activity in smokeless tobacco consumers might be useful for estimating the level of oxidative stress caused. Thereby, helping in patients education regarding the ill-effects of smokeless tobacco and determining the evolution and progress of various oral diseases.

**Key Words:** Antioxidants; Saliva; Smokeless tobacco; Superoxide dismutase.

## Introduction

Tobacco use kills nearly six million people worldwide each year. According to the World Health Organization (WHO) estimates, globally, there were 100 million premature deaths due to tobacco in the 20$^{th}$century, and if the current trends of tobacco use continues, this number is expected to rise to 1 billion in the 21$^{st}$ century. India′s tobacco problem is very complex, with a large use of a variety of smoking forms and an array of smokeless tobacco products. Many of these products aremanufactured as cottage and small-scale industries using varying mixtures and widelydiffering processes of manufacturing. Bidis are mostly manufactured in the unorganized sector while cigarettes are mainly manufactured in large-scale industries.[1]

Smokeless tobacco use was associated with cancers of the lip, oral cavity, pharynx, digestive, respiratory and intra-thoracic organs. Various studies have shown a significant association of chewing tobacco and oral cancer with direct relationbetween quantity and duration of use. India has one of the highest rates of oral cancer in the world, with over 50% attributable

to smokeless tobacco use. There is sufficient evidence in humans for the carcinogenicity of smokeless tobacco. Several studies have established a causal association between use of smokeless tobacco and cancers of oral cavity, esophagus and pancreas. Smokeless tobacco causes acute increase in blood pressure and heart rate, and has been associated with a small increase of cardiovascular disease risk. Effects on insulin sensitivity, glucose tolerance and the risk for diabetes from smokeless tobacco use are plausible. The use of smokeless tobacco causes reproductive and developmental toxicity and its use during pregnancy increases the risks for pre-eclampsia and premature birth, causes increased placental weight and reduces mean birth weight. Smokeless tobacco use by men causes reduced semen volume, reduced sperm count, reduced sperm motility and an increased frequency of abnormal spermatozoa.[1]

Saliva is the first biological medium encountered during tobacco use, and anti oxidant enzymes are present in the saliva. Aerobic organisms possess antioxidant defensesystems that deal with Reactive Oxygen Species (ROS) produced as a consequence ofaerobic respiration and substrate oxidation. In the process of normal cellularmetabolism, oxygen undergoes a series of univalent reductions, leading sequentially to the production of oxygen ($O_2$), hydrogen peroxide ($H_2O_2$ ), and water ($H_2O$).[2] Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicalsor by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals. Antioxidant enzymes are, therefore, absolutely critical for maintaining optimal cellular and systemic health and well-being.[3]

Tobacco consumption generates free radicals and results in increased oxidative stress and lipid peroxidation. Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation unless checked byantioxidants. The first line defence

antioxidants are Superoxide Dismutase (SOD), GPx (glutathione peroxidise) and Catalase (CAT).[4] The major cellular defence against anion $O_2$ and peroxynitrite is a group of oxidoreductases known as SODs, which catalyze the dismutation of anion $O_2$ into oxygen and $H_2O_2$. In mammals, there are three isoforms of SOD (SOD1 [CuZnSOD]; SOD2 [MnSOD]; SOD3 [EC-SOD]), andeach is a product of distinct genes and distinct subcellular localization, but catalyzes the same reaction. This distinct subcellular location of these SOD isoforms is particularly important for compartmentalized redox signaling. The mechanism of dismutation of $O_2$ to $H_2O_2$ by SOD involves alternate reduction and reoxidation of a redox active transition metal, such as copper (Cu) and manganese (Mn) at the active site of the enzyme.[2] SOD are basically, metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation.[3] To summarize, SOD plays an important protective role by decreasing the toxic effects of free radicals.[5] Hence, SOD activity is compared in the saliva of smokeless tobacco consumers and non-consumers in this study.

**Aims & Objectives**

To compare the activity of superoxide dismutase, in the salivary level of the smokeless tobacco consumers and non-consumers and level of the salivary superoxide dismutase in the smokeless tobacco consumers and non-smokeless tobacco non-consumers.

**Materials & Methodology**

Present study was conducted in Rajkiya medical college, Orai, Jalaun. Data was collected from out patients visiting to the department of Dentistry collaboration with the department of biochemistry. After informing the patients about the study and taking written consent from the patients. In this study followed inclusion criteria as followed, Individuals consuming smokeless tobacco for a period more than one year will be considered for study group and Healthy Individuals who do not consume smokeless tobacco will be considered for control group.

The sample for the present study comprised of 100 patient of both gender age between 20 to 70 years, was divided into two groups (study and control) with 50 patients each. The patients were divided into the following groups, Group I: 50 healthy individuals who do not consume smokeless tobacco (controlgroup) and Group II: 50 individuals who consume smokeless tobacco for a period more than one year (study group). After obtaining the informed consent, patients were examined properly. Their medical history and habit history were recorded in a case history format which was specifically designed for the study purpose. Oral cavity was examined thoroughly for any oral manifestations.

The patients were asked to avoid eating, drinking, and brushing 2 hours beforesampling. Patients were asked to rinse the mouth thoroughly before collecting the sample. All samples were collected between 9am to 11am. During sample collection, patients were seated upright in the dental chair. Then, approximately 5ml of un-stimulated saliva was collected by spitting method in a sterileplastic container. In the laboratory, the saliva was centrifuged for 10 minutes at the speed of 4000 rpm. The supernatant was separated and maintained at $-40^0$c and later analyzed for sod activity. SOD activity measured by Modified Marklund method.

**Results**

The habit of smokeless tobacco chewing is one of the known risk factors for oral cancer. Antioxidant defense system in dealing with free radicals induced by the habit of chewing tobacco is important in preventing precancerous lesions and oral cancer. In this study, the activity of super oxide dismutase is compared in the saliva oftobacco consumers and non-consumers. The present study was conducted on a total of 100 subjects visiting the department of Dentistry in our institution after obtaining consent from the patients. The patients were further divided equally intotwo groups as group 1 (tobacco chewers) and group 2 (non-chewers). Each group contains 50 patients. The entire set of study values obtained were tabulated, categorized

and subjected to statistical analysis using students unpaired't' test and p value <0.05 was considered significant.

In the study, Group 1 denotes patients who chew tobacco (n=50), with minimum age of the patient being 21 years and maximum age of the patient being 67 years with the mean value of 34.44 and standard deviation of 13.23 Group 2 denotes patients whoare non-chewers (n=50), with minimum age of the patient being 20 years and maximum age of the patient being 59 years, with mean value 38.24 and standard deviation 08.85. **(Table 1)** Also, it has been noted that the salivary antioxidant defense system appears to be less efficient with age. And it was also found that there was no significant difference between genders of two groups.

| Group | Number | Mean | Standard Deviation | Minimum Age (years) | Maximum Age (years) |
|---|---|---|---|---|---|
| Group 1 | 50 | 34.44 | 13.23 | 21 | 67 |
| Group 2 | 50 | 38.24 | 08.85 | 20 | 59 |
| P value | *P-value* is .094791. The result is *not* significant at $p < .05$ | | | | |

Table 1: Age distribution

In our study, Group 1 patients who chew tobacco, with mean value of SOD activity being 3094.732 and standard deviation of 292.35. And Group 2 patients who are non-chewers, with mean value of SOD activity being 2028.514 and standard deviation 481.29. As the standard deviation is very highly significant, the presence of superoxide antioxidant is increased in tobacco chewers when compared to non chewers. **(Table 2)**

| Parameter | Groups | N | Mean | Std. Deviation | P value |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| SOD Activity | Group 1 | 50 | 3094.73 | 292.35 | <0.00001 |
| | Group 2 | 50 | 2028.51 | 481.29 | |

**Table 2-** SOD Activity in comparison between Group 1 and Group 2.

**Discussion:**

Tobacco chewing produces various oxygen free radicals, which are considered as the main causes of damage to bio-molecules when exposed to tobacco. Saliva is the first biological fluid that encounters chewing form of tobacco and has an anti-oxidant defense system able to counter toxic activities of free radical species.[6] The salivary antioxidant system has been drawing increased attention in recent years.[7] The present study showed that the activity of superoxide dismutase in saliva was significantly higher in tobacco chewers (3094.73) compared with non-chewers (2028.51), where p= 0.00001. The increased levels this enzyme, as a component of the antioxidant defense system in saliva, reduces the damaging effects of free radicals produced by the consumption of tobacco.[8] Oxidant toxicity caused by smokeless tobacco may lead to increase antioxidant enzymes such as SOD. Superoxide dismutase converts super oxide an ion to hydrogen peroxide (H2O2) which then is removed by glutathione peroxidase (GPX) or catalase [8] Saliva, in addition to its cleansing and lubricating properties, constitutes a first line of defence against free radical-mediated oxidative stress.[9] There was no significant difference between age and gender of the two groups in the present study. Also, it has been noted that the salivary antioxidant defense system, appears to be less efficient with age.[10] Study by Farhad mollashahi L et al [9], was performed on enzymatic antioxidants such as SOD in saliva of tobacco consumers, which correlates with our study. Here, the activity of superoxide dismutase in saliva was significantly higher in paan (paan with tobacco) consumers compared with non-consumers. And, it is difficult to compare the results of the study with other studies as very less studies were conductedin this regard. This issue may be considered as a limitation of our study.

Our study was similar to that of a study by Patel B P, et al [11], who showed that erythrocyte SOD was lowered and GPx was elevated in tobacco users compared to non tobacco users. But the results of this study, inconsistent with our study. Our study is in accordance with a study by Kanehira et al [12], in which a comparison of salivary antioxidant enzyme levels in elderly smokers and nonsmokers showed a significant increase in the SOD level among the smokers. It shows that detoxification activity of free radicals might be deteriorated in the oral cavity of elderly smokers, and that as the duration of tobacco chewing habit increases the level of antioxidant superoxide dismutase falls. The results of the present study are consistent with the research of Baharvand et al [7] and Saggu TK et al[ 6] who found that smoking increases the activity of salivary superoxide dismutase.

In another study by Abdolsamadi et al [10], the activities of salivary superoxide dismutase, glutathione peroxidase, and peroxidase enzymes in smokers were significantly lower in smokers compared to those in non-smokers, which was inconsistent with our study. The difference between these results and ours may be due to the measurement of this enzyme in subjects with periodontal disease. In a pilot study by Naga Sarisha et al[13], smokers, smokeless tobacco users, and controls were explored for erythrocyte antioxidant enzymes-Superoxide dismutase (SOD) and Glutathione Peroxidase (GPx) where mean SOD levels were decreased in cases compared to control group. On the other hand, similar to our study Shetty et al [14], reported a higher activityof salivary SOD in patients with leukoplakia and oral cancer compared to the controls suggesting SOD level as a biomarker for oral cancers. This can be due to the fact that the majority of participants with leukoplakia and oral cancers were smokers. Salivary SOD is studied in the saliva of smokers, but existing literature shows that only one study has been carried out to evaluate salivary SOD activity in tobacco chewers. Thus we have conducted a study to evaluate for the salivary SOD activity intobacco chewers in comparison to non chewers

is carried out to know its activity against oxidants, to support further studies in this regard and to provide literature with useful data. This study emphasizes the importance of saliva as an easy non-invasive tool in diagnosing patients who are more prone to precancerous lesions and conditions, and its importance in patient education and motivation programs for tobacco cessation. The discrepancies between the results of previous studies and the present study might be attributed to differences in sample sizes, the number of cigarettes smoked, subject inclusion and exclusion criteria, environmental conditions, and social habits. Based on the results of the present study, the use of tobacco products decreases the anti-oxidative activity of the saliva with time. However, further studies with larger sample sizes and different doses of smoking and chewing tobacco is necessary to find a relationship between tobacco use and salivary antioxidant capacity and antioxidant.

**Conclusion:**

The present study carried out in bundalekhand population, enlightens us to the possible relationship between superoxide dismutase enzyme levels, oxidative stress and tobacco habit. This study showed that antioxidant salivary superoxide dismutase enzyme activity in tobacco chewers is higher in comparison to non-chewers. This is because of body's defense mechanism in initial stages. In initial or early stages these antioxidant levels increases thereby showing an evidence of endogenous activity. But as the duration of the habit increases there is decrease in the body's defense mechanism, and the level of superoxide dismutase starts to fall. And whenthis happens clinical manifestations start appearing. Tobacco habit coupled with the lesion would probably have synergistic effect in lowering the bodies' antioxidant status paving way for an enhanced oxidative stress. This study emphasizes the importance of saliva as an easy non-invasive tool in diagnosing patients who are more prone to precancerous lesions and conditions, and its importance in patient education and motivation programs for tobaccocessation.

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