Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr ICV 2018= 82.06

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Assessment of superoxide dismutase levels in saliva among tobacco and non-tobacco users - A cross sectional study

Jayanta Saikia[1], Balaji Pachipulusu[2], Poornima Govindaraju[3], Dipshikha Das[4]

[1]Post graduate student (MDS 2nd year), [2]Professor, [3]Senior lecturer Department of Oral Medicine and Radiology, Rajarajeswari Dental College & Hospital, Ramohali cross, Mysore Road, Bangalore – 560074, Karnataka, India; [4]Post Graduate Student (MDS 2nd year), Department of Public Health Dentistry, I.T.S Centre for Dental Studies and Research, Delhi- Meerut Road, Murad Nagar, Ghaziabad - 201206, Uttar Pradesh, India.

***ABSTRACT*:**

Objective: To estimate the level of salivary superoxide dismutase, To determine the differences in the level of salivary superoxide dismutase among tobacco chewers, smokers, combined users and non-tobacco users, To evaluate alteration in the levels of SOD in association with patient age, tobacco usage (smokeless, smokers & combined users) duration and frequency, also to see the prevalence of common oral mucosal lesions among the study groups. **Methods**: Saliva samples are collected randomly from eighty patients (aged 20–60 years) after taking complete demographic details and habit history and signed informed consent who visited the Department of Oral Medicine and Radiology. The SOD levels were analysed using spectrophotometric method. **Results**: Among 80 study samples, Group 4 (non-tobacco users) had a higher mean score of SOD levels 2.28 followed by group 1(smokers) with a mean score 1.53, group 2(smokeless tobacco users) with a mean of 1.47 and the least SOD level was seen in case of Group 3(Smokeless and smokers) with a mean score of 1.18. The salivary SOD levels had a statistically significant negative correlation with age (- 0.364**) of the patients, duration (-0.786**) and frequency (-0.735**) (** Correlation is significant at 0.01 level) of tobacco use among patients which signifies that with a gradual increase in the age, duration and frequency of tobacco use there is a downfall of the salivary super oxide dismutase levels. Study subjects consuming tobacco in both smokeless as well as smoking forms had a higher mean score of oral mucosal lesions, the most common being tobacco pouch keratosis (40%), for smokeless forms tobacco pouch keratosis followed by OSMF was more common, in case of smokers smoker's palate was more common followed by leukoplakia, whereas no such premalignant lesions were assessed among non-tobacco users. There is no significant statistical difference in the level of SOD among the gender. **Conclusion**: The current study suggests that there is a reduction in the level of salivary superoxide dismutase enzyme level among tobacco users with increased frequency and duration of the habit as compared to non-tobacco users, also a weak reduction in the SOD enzyme was noted with the increase in age. Study subjects consuming tobacco in both smokeless as well as smoking forms had a higher mean score of oral mucosal lesions, the most common being tobacco pouch keratosis.

Key words: Antioxidant, Oro-mucosal lesions, Salivary superoxide dismutase, Saliva, Tobacco.

Received: 14 October, 2019 Revised: 14 November, 2019 Accepted: 15 November, 2019

Corresponding author: Dr. Dr Jayanta Saikia, Post graduate student (MDS 2nd year), Department of Oral Medicine and Radiology, Rajarajeswari Dental College & Hospital, Ramohali cross, Mysore Road, Bangalore – 560074, Karnataka, India

This article may be cited as: Saikia J, Pachipulusu B, Govindaraju P, Das D. Assessment of superoxide dismutase levels in saliva among tobacco and non-tobacco users - A cross sectional study. J Adv Med Dent Scie Res 2019;7(12): 66-72.

INTRODUCTION

The word cancer Latinised from Greek word 'Karkinos' meaning crabs referred by Hippocrates, the earliest known description of cancer appears in several papyri from ancient Egypt, the Edwin Smith Papyrus was written around 1600BC and contains a description of cancer. Cancer is the second most leading cause of mortality in economically developed countries and the third most leading cause of death in developing countries. Oral cancer accounts for approximately 30-40% of all cancer in India [1]. According to the Global Adult Tobacco Survey (GATS) 2016-17, nearly 42.4% of men, 14.2% of women and 28.6% of all adults currently use tobacco [2], the Indian data suggest that the relative risk of developing oral cancer is 2.82 for smokers and 5.98

for chewers. 80% of oral cancers progress from precancerous lesions and about 2-12% of precancers are transformed into cancer [3]. The World Health Organization predicts that tobacco deaths in India may exceed 1.5 million annually by 2020 [4].

Tobacco usage is a harmful habit that generates free radicals and results in increased oxidative stress and lipid peroxidation which causes adverse effects on oral health and plays a most important role in cancer, potentially premalignant oral epithelial lesions, periodontal disease, and tooth loss.

Saliva is the first fluid that is exposed to tobacco and its antioxidant system plays an important role in anti-cancer potential. The human body has non-enzymatic and enzymatic antioxidant defense mechanisms to neutralize the harmful effect of ROS. The non-enzymatic antioxidants include reduced glutathione, albumin, vitamins A, C, and E, uric acid, bilirubin, lactoferrin, ceruloplasmin, transferrin, and haptoglobin. The enzymatic antioxidants include glutathione peroxidase, superoxide dismutase, and catalase. [5]

A fragile balance exists between the pro-oxidant mechanism of tissue damage and antioxidant defence repair system, if the balance is shifted towards ROS activity, significant tissue destruction occurs due to cell break down and DNA mutation leading to Potentially Premalignant Oral Epithelial Lesions (PPOEL). [5]

In the present study, we assessed the level of superoxide dismutase level (SOD), SOD enzyme is thought to play an important role in protecting the cell against the potentially deleterious effects of reactive oxygen species (ROS) by catalysing the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. [5]

As it is known Saliva acts as a mirror of a body's health as it contains hormones, proteins, enzymes which are often measured in standard blood tests to detect any disease. However, unlike blood, collection of saliva is easy, less painful to patients, less infectious for healthcare provider during handling and nearly all analytes that are in blood are also present in saliva. [6] Assessment of the level of SOD in saliva of tobacco chewers, smokers and healthy control group can put together a glorious way in understanding the risk of oral cancer due to both smokable & chewable tobacco form consumption and variation of SOD levels along with age of participants, frequency and duration of tobacco use and assess the common PPOEL among different tobacco habit groups.

With the above introduction, the aim of the present study is to assess the level of SOD enzyme among tobacco and non-tobacco users.

MATERIALS AND METHODS

A cross-sectional study was carried out in the Department of Oral Medicine and Radiology in a Dental College in South India to assess the effect of tobacco chewing and smoking on the level of SOD enzyme. Study subjects were selected from the people visiting the Department of Oral Medicine and Radiology. Eighty patients (aged 20–60 years) with a history of tobacco usage and non-tobacco users who came to the Department of Oral Medicine and Radiology. Ethical clearance for the study was obtained from the ethical committee, prior to the initiative of the study (RRDCHet/03omr/2019).

The subjects were fully informed about the nature of the study, and signed consent was obtained from them followed by recording the demographic details along with the oral mucosal lesions if diagnosed. Inclusion criteria were: Tobacco and Non-tobacco users in the age group of 20-60 years, cigarette smokers with a smoking history of at least one cigarette a day for not less than one year, 1-year history of chewing a 10-g tobacco packet daily. Exclusion criteria were: Subjects below the age of 20 years old, Subjects having systemic conditions, under vitamin supplements, antimicrobial and anti-inflammatory drugs during the previous 3 months, Subjects who had undergone radiation therapy.

METHOD OF COLLECTION

Saliva samples are collected randomly from eighty patients (aged 20–60 years) after taking complete habit history and informed consent who visited the Department of Oral Medicine and Radiology.

ARMAMENTARIUM

Kidney tray, Mouth mirror, Straight probe, Periodontal probe, Explorer, Tweezer, Cotton holder, Gloves, Mouth mask, Sterile container

COLLECTION OF SALIVA

The participants were asked to refrain from consuming any food or beverages for 1 h prior to the saliva collection. The tobacco users were also asked not to use tobacco for 1 h prior to the collection of saliva, Whole saliva was collected under non-stimulatory conditions in a quiet room, during this period, the participants were instructed to flush the mouth with 100 mL of distilled water, and they were seated in a relaxed position. After a few minutes of relaxation, they were trained to avoid swallowing saliva and asked to lean forward and spit all the saliva they produced in 5 min into a sterile plastic container. Following collection, the samples were centrifuged for approximately 5 min at 3000 revolutions per minute (rpm). The samples were then stored at a low temperature for analysis. The SOD levels were analysed by spectrophotometric method. [7]

LABORATORY ANALYSIS

Reagents:

1. 0.067 M Potassium phosphate buffer pH-7.8
2. 0.1 M EDTA
3. 0.12M Riboflavin
4. 0.015M Nitro blue Tetrazolium (NBT)

0.1 ml of saliva samples were mixed with 3 ml of phosphate buffer, 0.2 ml of EDTA, 0.1 ml of NBT and incubated the tubes in a lightbox providing uniform light intensity. (A foil-lined box approximately 4' long X 8" X 6" with an internally mounted 40 W fluorescent bulb has been used) for 5-8 minutes to achieve a standard temperature (Fig.2) .0.1ml of Riboflavin was added. Standard was taken as 3 ml of phosphate buffer, 0.2 ml of EDTA, 0.1 ml of NBT. Incubated the samples for 12 minutes in a lightbox (Fig.3) and measured OD at 560 nm using Spectrophotometer (Fig.4)

STATISTICAL ANALYSIS

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses, One-way ANOVA test followed by Tukey's post hoc analysis, Pearson Correlation test, Chi square test and Independent t sample test. The level of significance will be set at $P<0.05$

RESULTS:

A total of 80 subjects participated in the study with 20 each in smoker group, tobacco chewer group, smoker + tobacco chewer group and control group with a mean age of 37.6±9.06. The mean duration of smoking was 1.8±0.9 years and mean duration of tobacco chewing was 2 ±1.05 years and 3±1.33 years for combined tobacco smokers and chewers respectively.

Among 80 study samples, Group 4 (non-tobacco users) had a higher mean score of SOD levels 2.28 followed by group 1(smokers) with a mean score 1.53, group 2(smokeless tobacco users) with a mean of 1.47 and the least SOD level was seen in case of Group 3(Smokeless and smokers) with a mean score of 1.18 (Table 1).

The salivary SOD levels had a statistically significant negative correlation with age (- 0.364**) of the patients, duration (-0.786**) and frequency (-0.735**) (** Correlation is significant at 0.01 level) of tobacco use among patients which signifies that with a gradual increase in the age, duration and frequency of tobacco use there is a downfall of the salivary super oxide dismutase levels (Table 2).

Study subjects consuming tobacco in both smokeless as well as smoking forms had a higher mean score of oral mucosal lesions, the most common being tobacco pouch keratosis (40%), for smokeless forms tobacco pouch keratosis followed by OSMF was more common, in case of smokers smoker's palate was more common followed by leukoplakia, whereas no such premalignant lesions were assessed among non-tobacco users (Table 3). There is no significant statistical difference in the level of SOD among the gender (Table 4).

TABLE 1: COMPARISON OF SALIVARY SUPER OXIDE DISMUTASE LEVELS AMONG FOUR STUDY GROUPS

Descriptives

sod levels	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	20	1.5320	.28147	.06294	1.4003	1.6637	1.10	2.07
2	20	1.4700	.50482	.11288	1.2337	1.7063	1.10	3.30
3	20	1.1845	.28596	.06394	1.0507	1.3183	.70	1.90
4	20	2.2895	.46081	.10304	2.0738	2.5052	1.50	3.00
Total	80	1.6190	.56589	.06327	1.4931	1.7449	.70	3.30

ANOVA

sod levels	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.363	3	4.454	28.362	.000
Within Groups	11.936	76	.157		
Total	25.298	79			

Multiple Comparisons

sod levels

Tukey HSD

(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.06200	.12532	.960	-.2672	.3912
	3	.34750*	.12532	.035	.0183	.6767
	4	-.75750*	.12532	.000	-1.0867	-.4283
2	1	-.06200	.12532	.960	-.3912	.2672
	3	.28550	.12532	.112	-.0437	.6147
	4	-.81950*	.12532	.000	-1.1487	-.4903
3	1	-.34750*	.12532	.035	-.6767	-.0183
	2	-.28550	.12532	.112	-.6147	.0437
	4	-1.10500*	.12532	.000	-1.4342	-.7758
4	1	.75750*	.12532	.000	.4283	1.0867
	2	.81950*	.12532	.000	.4903	1.1487
	3	1.10500*	.12532	.000	.7758	1.4342

*. The mean difference is significant at the 0.05 level.

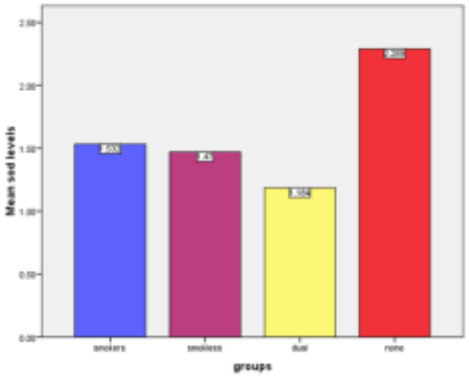


TABLE 2: CORRELATION OF SALIVARY SUPER OXIDE DISMUTASE LEVELS AMONG STUDY SUBJECTS WITH AGE, DURATION AND FREQUENCY OF HABITS IN EACH GROUP

Correlations

			sod levels	duration	frequency	age
Spearman's rho	sod levels	Correlation Coefficient	1.000	-.786**	-.735**	-.364**
		Sig. (2-tailed)	.	.000	.000	.001
		N	80	80	80	80
	duration	Correlation Coefficient	-.786**	1.000	.727**	.377**
		Sig. (2-tailed)	.000	.	.000	.001
		N	80	80	80	80
	frequency	Correlation Coefficient	-.735**	.727**	1.000	.366**
		Sig. (2-tailed)	.000	.000	.	.001
		N	80	80	80	80
	age	Correlation Coefficient	-.364**	.377**	.366**	1.000
		Sig. (2-tailed)	.001	.001	.001	.
		N	80	80	80	80

**. Correlation is significant at the 0.01 level (2-tailed).

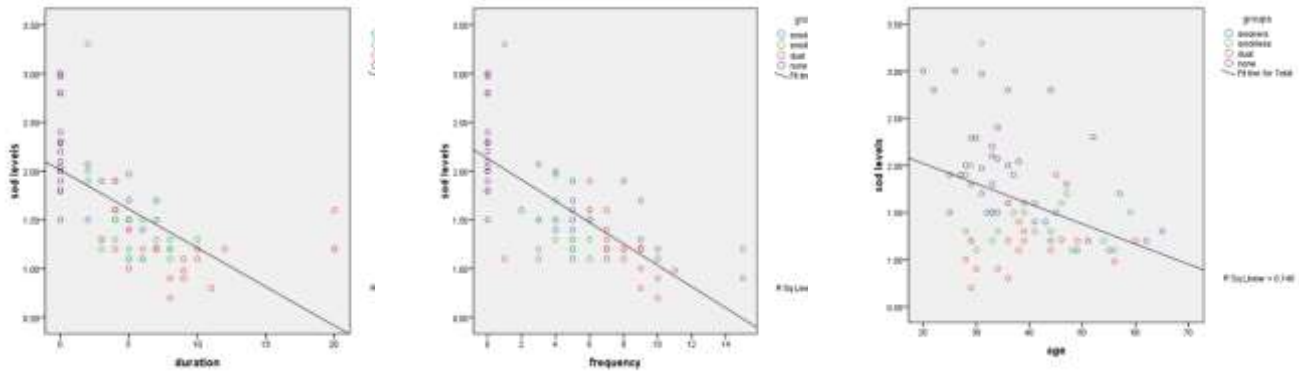


TABLE 3: COMPARISON OF ORAL MUCOSAL LESION AMONG FOUR THE STUDY GROUPS

groups * oml Crosstabulation

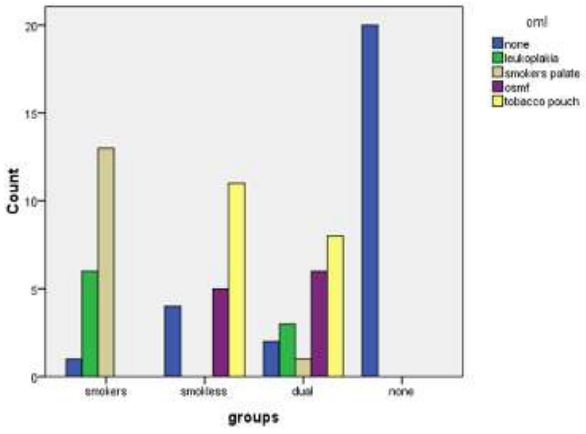
			oml					Total
			none	leukoplakia	smokers palate	osmf	tobacco pouch	
groups	smokers	Count	1	6	13	0	0	20
		% within groups	5.0%	30.0%	65.0%	.0%	.0%	100.0%
	smokless	Count	4	0	0	5	11	20
		% within groups	20.0%	.0%	.0%	25.0%	55.0%	100.0%
	dual	Count	2	3	1	6	8	20
		% within groups	10.0%	15.0%	5.0%	30.0%	40.0%	100.0%
	none	Count	20	0	0	0	0	20
		% within groups	100.0%	.0%	.0%	.0%	.0%	100.0%
Total		Count	27	9	14	11	19	80
		% within groups	33.8%	11.2%	17.5%	13.8%	23.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.121E2[a]	12	.000
Likelihood Ratio	117.840	12	.000
Linear-by-Linear Association	9.799	1	.002
N of Valid Cases	80		

a. 16 cells (80.0%) have expected count less than 5. The minimum expected count is 2.25.

Bar Chart



COMPARISON OF SOD LEVELS AMONG MALES AND FEMALES

Group Statistics

	gender	N	Mean	Std. Deviation	Std. Error Mean
sod levels	male	65	1.5817	.54064	.06706
	female	15	1.7807	.66058	.17056

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
sod levels	Equal variances assumed	1.707	.195	-1.232	78	.222	-.19897	.16157	-.52063	.12268
	Equal variances not assumed			-1.086	18.566	.292	-.19897	.18327	-.58317	.18522

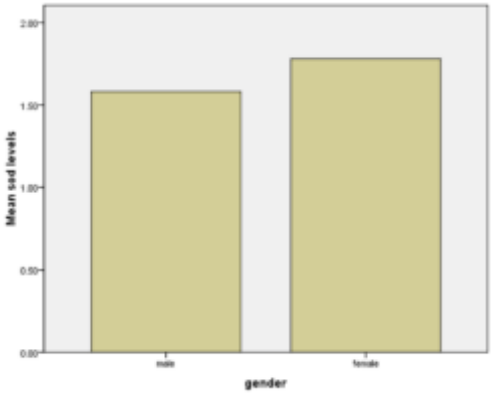


Fig.1: Saliva Samples

Fig.2: Incubation of samples

Fig.3: Incubation of samples in light box

Fig. 4: Spectrophotometer

DISCUSSION

Free radical an atom or group of atoms with at least one unpaired electron in there outer ring, in our body it is usually an oxygen molecule that has lost an electron and stabilize itself by stealing an electron from a nearby molecule by doing so it leads to cell breakdown, tissue damage and DNA mutations and hence are the known possible culprit for developing PPOEL. Antioxidants can neutralize these free radicals by scavenging them and donating their electron to them without turning itself into a free radical and help them to stabilize.

Saliva is the first body fluid which encounters the cigarette smoking and tobacco chewing, Antioxidant system of saliva plays a significant role in the anticariogenic and antibacterial effect of saliva.

In the present study, it was observed that SOD levels reduced as age advanced and there was an obvious reduction in the SOD enzyme levels among tobacco users and non-tobacco users, the least enzyme levels were found in the combined habit group (Smokers + Smokeless) followed by the Smokeless and the Smoker group. There was also a significant reduction of the antioxidant enzyme, with an increase in the duration and frequency of tobacco usage, similar to the results of earlier studies, S. Reddy et al(2012) [7], Sirisha et al.(2013) [8], Shwetha et al (2018) [1] However, few studies Baharvand et al (2010) [9], H.D. Jenifer et al (2015) [5] noted higher level of SOD enzyme levels among smokers.

Manifestation of tobacco associated oral mucosal lesions varies with the form of tobacco usage, in the present study the common lesions that were observed in conventional smoking group are smoker's palate and leukoplakia, for smokeless group tobacco pouch keratosis, OSMF and Leukoplakia, for combined users (Smoke + Smokeless) Tobacco pouch keratosis followed by OSMF and smoker's palate. Almost similar results were seen in Shyam Sundar Behura et al (2015) [10], Naveen KB et al (2016) [11] study.

To the best of our knowledge, this is the first study simultaneously evaluating the SOD enzyme levels in saliva of smokers, smokeless tobacco users and combined users along with the common oral mucosal lesions associated with various tobacco users.

CONCLUSION

The least SOD enzyme level was for combined habit (smokers + smokeless) users followed by Smokeless users and Smokers compared to non-tobacco users. This indication of the decreased salivary antioxidants in the tobacco chewers and smokers accentuates the role of smoking and tobacco chewing in the pathogenesis of PPOEL and cancers. As salivary antioxidants in smokers and chewers were significantly decreased, it is recommended to integrate antioxidants in food supplements, tooth pastes and mouth rinses in order to prevent the harmful effects of tobacco.

The use of saliva as a "diagnostic tool" is an upcoming area of research. It offers the advantage over serum as the collection process of saliva is noninvasive, simple and cost efficient.

REFERENCES

1. Sankepally Shwetha et al., Influence of Tobacco Chewing and Smoking on the Salivary Total Antioxidant Power-A Clinical Comparative Study. Journal of Clinical and Diagnostic Research. 2018 Jun, Vol-12(6): ZC09-ZC12.
2. Global Adult Tobacco Survey FACT SHEET |INDIA 2016-17.
3. Garg KN, Raj V, Chandra S. Trends in frequency and duration of tobacco habit in relation to potentially malignant lesion: A 3 years retrospective study.J Oral Maxillofac Pathol 2013;17:201206
4. Rani M, Bonu S, Jha P, *et al* Tobacco use in India: prevalence and predictors of smoking and chewing in a national cross sectional household survey *Tobacco Control* 2003;12:e4
5. Jenifer HD et al. The influence of cigarette smoking on blood and salivary superoxide dismutase enzyme levels among smokers and non-smokers-A cross sectional study. J Trad Comp Med 2015; 1-6.
6. John T McDevitt. Saliva as the next best diagnostic tool. J Biochem. [10] 2006;45(2):23-25
7. Reddy S et al. Influence of cigarette smoking on blood and salivary super oxide dismutase levels among smokers and non-smokers. J Invest Clin Dent (2012); 3: 298–303.
8. Naga Sirisha CV, Manohar RM. Study of antioxidant enzymes superoxide dismutase and glutathione peroxidase levels in tobacco chewers and smokers: A pilot study. J Can Res Ther 2013;9:210-4.
9. Baharvand M et al. Comparison of Superoxide Dismutase Activity in Saliva of Smokers and Nonsmokers. South Med J 2010;103(5):425-27.
10. Shyam Sundar Behura et al., Oral Mucosal Lesions – A Case-Control Study. Journal of Clinical and Diagnostic Research. 2015 Jul, Vol-9(7): ZC17-ZC22.
11. Naveen-Kumar B, Tatapudi R, Sudhakara-Reddy R, Alapati S, Pavani K, Sai-Praveen KN. Various forms of tobacco usage and its associated oral mucosal lesions. J Clin Exp Dent. 2016;8(2):e172-7.