

ORIGINAL ARTICLE

EVALUATION OF SERUM ANTIOXIDANT ENZYMES IN ORAL SUBMUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA: A CLINICAL AND BIOCHEMICAL STUDY

Sanjay B. Nyamati¹, Annapoorna H.B²., Juhi Tripathi³, Neha Sinha³, Shraddha Roy³, Ruchika Agrawal³

¹Principal, Professor & Head, ²Reader, ³Post graduate Student, Department of Oral Medicine and Radiology, Triveni Institute of Dental Sciences, Hospital and Research Centre, Bilaspur, Chhatisgarh


ABSTRACT:

Background Present study was aimed to estimate the levels of glutathione peroxidase, malondialdehyde (MDA), superoxide dismutase (SOD) in oral submucous fibrosis (OSMF), and oral squamous cell carcinoma patients. **Materials and Methods:** Blood samples were collected from 30 patients who were divided into three groups: Group I as control with 10 normal individuals, group II with histopathologically confirmed different cases of OSMF, and group III with histopathologically confirmed cases of oral squamous cell carcinoma (OSCC). Results obtained were statistically analyzed. **Results:** Superoxide dismutase (SOD) and Malondialdehyde (MDA) was demonstrated significantly more in case of control group in comparison to OSMF and OSCC group whereas Glutathione peroxidase (GPx) was found significantly higher in OSF and OSCC group. Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) was found to be significantly more in OSF group in comparison to OSCC patients and levels of MDA was observed higher in OSCC patients. The P-value was calculated and the results were highly significant. **Conclusion:** SOD, GPx, MDA can be used as valid biomarkers for patients who are at risk of developing cancer.

Key words: MDA, oral squamous cell carcinoma, oral submucous fibrosis, SOD.

Corresponding Author: Dr. Sanjay B. Nyamati, Principal, Professor & Head, Department of Oral Medicine and Radiology, Triveni Institute of Dental Sciences, Hospital and Research Centre, Bilaspur, Chhatisgarh

This article may be cited as: Nyamati SB, HB Annapoorna., Tripathi J, Sinha N, Roy S, Agrawal R Evaluation of serum antioxidant enzymes in oral submucous fibrosis and oral squamous cell carcinoma: A clinical and biochemical study. J Adv Med Dent Sci Res 2016;4(4):83-87.

Access this article online	
Quick Response Code 	Website: www.jamdsr.com
	DOI: 10.21276/jamdsr.2016.4.4.18

INTRODUCTION

Oral malignancies are debilitating disorders of the oral cavity. Etiopathogenesis of these disorders is multifactorial. The prognosis of these disorders depends upon early diagnosis and management.¹

Imbalances between the oxidant-antioxidant status have been implicated in the pathogenesis of premalignant and malignant lesions. The extent of

oxidative damage caused by ROS directly depends on body antioxidant defense mechanism.²

Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are the three major enzymatic antioxidant defense systems responsible for scavenging free radicals and nascent oxygen.³

Antioxidant enzymes catalyze decomposition of ROS. Redox modulation is seen by distinctive changes in the activities of these enzyme systems in oxidative stress. Thus, an overall balance between production

and removal of ROS may be more important in various cancers including OSCC.⁴

Literature reports reveal reduction in the levels of enzymatic antioxidant superoxide dismutase (SOD), glutathione peroxidase and an increase in antioxidants like malondialdehyde (MDA) in these disorders.⁵

Despite therapeutic and diagnostic advances, the rate at which oral pre-cancerous and cancerous lesions are spreading is alarming. This highlights the need for continued efforts to discover suitable biomarkers for early diagnosis. In spite of high prevalence of OSF in India and their potential to undergo malignant transformation, the antioxidant status of these individuals has not been widely investigated.⁶ Keeping this in mind, this study was undertaken to evaluate and compare the bio-chemical alterations in the sera of OSF, oral cancer patients and healthy subjects.

MATERIALS AND METHODS

Ethical clearance was taken from Institutions Ethical Committee before the commencement of study. A written consent from all the subjects were taken who were involved in the study.

Blood samples were collected from 30 patients who who reported to the Department of Oral Medicine and Radiology. They were divided into three groups: Group I as control with 10 normal individuals, group II with histopathologically confirmed different cases of OSMF, and group III with histopathologically confirmed cases of oral squamous cell carcinoma (OSCC).

METHOD OF BLOOD COLLECTION

Venous blood samples were collected using heparin as an anticoagulant for the estimation of SOD and GPX. 5ml of whole blood was centrifuged for 10 minutes at 3000 rpm, plasma was separated and erythrocytes were washed four times with 3ml of 0.9% NaCl solution, and centrifuged for 10 minutes at 3000 rpm after each wash.

Washed and centrifuged erythrocytes were made up to 2.0 ml with cold redistilled water and mixed and left to stand at 4° C for 15 minutes. The haemolysate was diluted with 0.01 mmol/liter phosphate buffer pH 7.0, so that the 5 fold inhibition was between 30% and 60%. SOD estimation was based on the method of Suttle et al where as glutathione peroxidase was estimated by the method of Paglia and Valentine.⁷ Both the enzymes SOD & GPX were determined by

Ransel anti-oxidant enzyme kit provided by Randox and samples were processed on Ciba corning express plus autoanalyzer for spectrometry. MDA was calculated by the method given by Kei Satoh in 1978 in which trichloroacetic acid was added to the mixture which was centrifuged at 3300 rpm and measured at 530 nm.⁸

STATISTICAL ANALYSIS

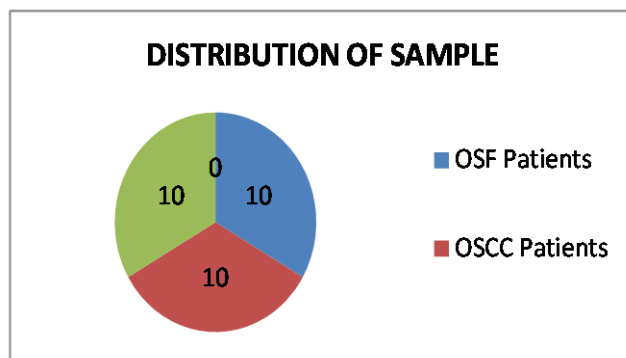
The software used for the statistical analysis was Statistical Package for Social Sciences (SPSS) version 16.0. Chi-square test was used to find the level of significance (P-value), where P < 0.001 was considered to be highly significant.

RESULTS

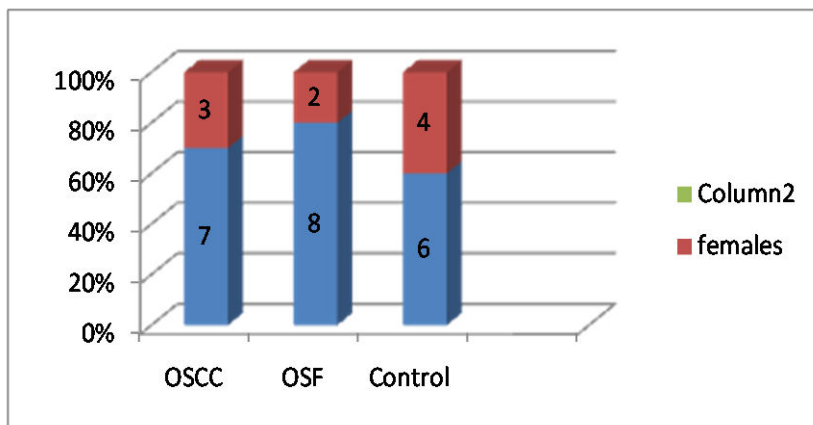
The mean age in OSF, oral cancer and control group was found to be 34.33 ± 8.5 , 55.73 ± 8.09 and 45.43 ± 6.09 years, which reflects the subject population mostly being affected. Gender wise distribution of subjects enrolled were shown in graph 1 where OSCC include 7 males and 3 females, OSF group include 8 males and 2 females and control enrolled 6 males and 4 females.(Graph 1 and 2)

Superoxide dismutase (SOD) and Malondialdehyde (MDA) was demonstrated significantly more in case of control group in comparison to OSMF and OSCC group whereas Glutathione peroxidase (GPx) was found significantly higher in OSF and OSCC group. (Table 1 and 2)

Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) was found to be significantly more in OSF group in comparison to OSCC patients and levels of MDA was observed higher in OSCC patients. (Table 3)



Graph 1: Distribution of sample in different groups.



Graph 2: Gender wise distribution of sample

Table 1: Comparison of values in OSMF and the control group

Enzymes	OSF patients	Control group	P value
Superoxide dismutase (SOD)	102.55±20.42	194.35 ±14.28	0.000 (Highly significant)
Glutathione peroxidase (GPx)	26.03±5.46	60.46±12.45	0.000 (Highly significant)
Malondialdehyde (MDA)	8.82±1.22	3.40±0.56	0.025(Significant)

Table 2: Comparison of values in OSCC and the control group

Enzymes	OSCC patients	Control group	P value
Superoxide dismutase (SOD)	47.55±10.32	194.35 ±14.28	0.000 (Highly significant)
Glutathione peroxidase (GPx)	12.42±1.42	60.46±12.45	0.000 (Highly significant)
Malondialdehyde (MDA)	13.22±2.40	3.40±0.56	0.0123(Significant)

Table 3: Comparison of values in OSMF and OSCC

Enzymes	OSCC patients	OSFgroup	P value
Superoxide dismutase (SOD)	47.55±10.32	102.55±20.42	0.000 (Highly significant)
Glutathione peroxidase (GPx)	12.42±1.42	26.03±5.46	0.025 (Significant)
Malondialdehyde (MDA)	13.22±2.40	8.82±1.22	0.0021(Significant)

DISCUSSION

This study was aimed to estimate the levels of glutathione peroxidase, malondialdehyde (MDA), superoxide distmutase (SOD) in oral submucous fibrosis (OSMF), and oral squamous cell carcinoma patients.

A male proclivity is observed in the present study groups affected by OSF and OSCC.

OSF and OSCC has multifactorial etiology but habit chewing and smoking tobacco has been implicated as most important causative factors responsible for it. The fundamental hypothesis is, free radicals damage the cellular materials, which would result in triggering or transforming normal cells into malignant ones. But, the magnitude of such damage is dependent on the body's defense mechanism, which is

mediated by various cellular antioxidants. The two verified mechanisms favoring radical alteration of ROS metabolism in cancer cells are production of huge amounts of ROS compared with non-neoplastic cells and suppression of antioxidant system.⁹

Antioxidant enzymes such as E-SOD and GPx can directly counterbalance the oxidant attack and protect the cells against DNA damage. Superoxide dismutase is a decisive antioxidant enzyme in aerobic cells, which is responsible for the elimination of superoxide radicals. E-SOD converts two toxic species: Superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) into water. This diminishes the toxic effects of superoxide radical and other radicals formed by secondary reactions. Glutathione peroxidase (GPx) is a selenocysteine - dependent enzyme. GPx in cells is the most important hydrogen peroxide (H_2O_2) scavenging enzyme.²

Superoxide dismutase (SOD) and Malondialdehyde (MDA) was demonstrated significantly more in case of control group in comparison to OSMF and OSCC group whereas Glutathione peroxidase (GPx) was found significantly higher in OSF and OSCC group. Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) was found to be significantly more in OSF group in comparison to OSCC patients and levels of MDA was observed higher in OSCC patients.

Similar results were obtained by Manoharan et al³, Kumar et al⁵ and in oral cancer patients when compared to healthy patients and the reports suggested that host tumor cells in oral cancer patients seize essential nutrients from the circulation to meet the damage caused by the growing tumor.

Gurudath et al⁶ in 2012 described SOD as a decisive antioxidant enzyme in aerobic cells that is responsible for the elimination of superoxide radicals. Gurudath et al., in their study of oral cancer group, showed a statistically significant ($P < 0.001$) decrease in the level of SOD when compared to the control group and also found it to be at the lowest level among all groups.⁶

In this study, oral cancer group showed a statistically significant ($P < 0.001$) decrease in levels of mean SOD and GPx when compared to the control group and also the lowest levels amongst the study groups. This suggests that lower antioxidant enzymes activity in oral cancer patients might be due to the depletion of the antioxidant defense system that occurs as the consequence of overwhelming free radicals by the elevated levels of lipid peroxides. GPx levels were

low suggesting that most cancer cell types couldn't detoxify hydrogen peroxide.

Malondialdehyde, a product of lipid peroxidation is a toxic compound that reacts with DNA to form covalently-bonded adducts with deoxyadenosine and deoxyguanosine, an event that can cause a mutagenic transformation within the DNA by altering their chemical behavior and possibly contributing to carcinogenesis and mutagenesis.

The mean MDA level gradually increased when healthy individuals, potentially malignant and OSCC patients were compared, which was statistically highly significant ($P < 0.001$ Chole et al.⁹ found that serum MDA levels were increased in OSSC as compared to healthy controls due to increased oxidative stress of the body.). Khanna et al¹⁰ found that serum MDA levels were highest in oral cancer patients compared to controls ($P < 0.001$). In a similar study,

Further studies with a larger sample should be carried out to validate our findings. If our results are confirmed, trials with antioxidant agents may be helpful in preventing the development of carcinoma in oral submucous fibrosis.

CONCLUSION

Evaluation of lipid peroxidation and antioxidant enzyme levels are a subject of interest for their possible role in many cancerous conditions, and serve as the back bone of cellular antioxidant defense mechanism and assess the degree of oxidative damage of the disease. So, they can be used as valid biomarkers for patients who are at risk of developing cancer. Further long term studies are required with larger sample size in order to assess the pre-treatment and post treatment effect on serum levels of SOD, GPx and MDA which includes different grades with varying age groups and duration of OSF.

REFERENCES

1. Scully C, Bagan J. Oral squamous cell carcinoma overview. *Oral Oncol.* 2009;45:301-8.
2. Gokul S, Patil VS, Jailkhani R, Hallikeri K, Kattappagari KK. Oxidant-antioxidant status in blood and tumor tissue of oral squamous cell carcinoma patients. *Oral Dis* 2010;16:29-33.
3. Manoharan S, Kolanjiappan K, Suresh K, Panjamurthy K. Lipid peroxidation and antioxidants status in patients with oral squamous cell carcinoma. *Indian J Med Res* 2005;122:529-34.

4. Yokoe H, Nomura H, Yamano Y, Fushimi K, Sakamoto Y, Ogawara K, *et al.* Characterization of intracellular superoxide dismutase alterations in premalignant and malignant lesions of the oral cavity: Correlation with lymph node metastasis. *J Cancer Res Clin Oncol* 2009;135:1625-33
5. Kumar PP, Bharathan S, Nagini S. Oxidant antioxidant status in patients with oral squamous cell carcinoma in tissues and plasma — A Biochemical Analysis on 30 Samples. *Asian J Exp Biol Sci* 2011;2:316-26.
6. Gurudath S, Naik MR, Ganapathy KS, Guruprasad Y, Sujatha D, Pai A. Superoxide dismutase and glutathione peroxidase in oral submucous fibrosis, oral leukoplakia, and oral cancer: A comparative study. *J Orofac Sci* 2012;4:114-9.
7. Paglia DE, Valentine WN (1967): Studies on the quantitative and qualitative characterization of erythrocyte GPX, *J Lab And Clin Med*: 158-64
8. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 1978;90:37-43.
9. Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum malondialdehydes in oral cancer and precancer and its association with healthy individual, gender, alcohol, and tobacco abuse. *J Cancer Res Ther* 2010;6:487-91.
10. Khanna R, Thapa PB, Khanna HD, Khanna S, Khanna AK, Shukla HS. Lipid peroxidation and antioxidants enzyme status in oral carcinoma patients. *Kathmandu Univ Med J (KUMJ)* 2005;3:334-9

Source of support: Nil

Conflict of interest: None declared

This work is licensed under CC BY: *Creative Commons Attribution 3.0 License*.