

Oral submucous fibrosis: A quantitative assessment of serum malondialdehyde, superoxide dismutase and correlation with clinical staging

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Abstract

Background: Oral submucous fibrosis (OSMF) is a progressive disorder affecting the oral mucosa. OSMF predominantly seen in South-east Asian countries. There are some biochemicals parameters which are modify in oral submucous fibrosis; this alteration can be used as a tool for diseases progress and avert malignant transformation.

Aims and Objectives: The aim of this study is to evaluate the serum malondialdehyde (malondialdehyde [MDA]), and Superoxide dismutase (SOD) in oral sub mucous fibrosis cases and compare clinical stages.

Materials and Methods: Thirty cases of clinical and histopathological established oral submucous fibrosis and thirty cases of nonsymptomatic features of oral submucous fibrosis preferred as controls. Venous blood was collected and separation of serum for estimation of MDA and SOD levels was done using an ultraviolet spectrophotometer.

Statistical Analysis: Data were analyzed using SPSS software using Student's *t*-test and Kruskal–Wallis ANOVA test.

Results: Serum MDA levels were elevated when clinical staging increases, where as SOD levels were decreased when clinical stage increases when compared with control cases and it showed statically significant.

Conclusion: Estimation of serum MDA and SOD in patients with OSMF, we can assess the degree of oxidative damage of the disease. This can be used as an early diagnostic tool for preventing malignant transformation of oral submucous fibrosis.

Keywords: Clinical staging, malondialdehyde, oral submucous fibrosis, superoxide dismutase

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INTRODUCTION

Oral submucous fibrosis (OSMF) is an insidious, chronic disease affecting any part of the oral cavity, sometimes pharynx and esophagus.^[1] It is characterized by mucosal rigidity of varying intensity due to the fibro-elastic transformation of the juxta-epithelial layer, resulting in a progressive inability to open the mouth.^[2] The first complete description of disease was defined by Schwartz (1952) as “Atrophia Idiopathica (tropica) Mucosae Oris” on five Indian women in Kenya.^[3] However, the existence of such a disease and its presentation in oral cavity was also evident in ancient Indians, which was documented by Sushruta who described it as “VIDARI,” a disease of mouth and throat in which progressive narrowing of mouth opening, depigmentation of oral mucosa and pain on taking food were noted.^[4]

The etiology of oral submucous fibrosis (OSMF) is still perplexing the dental research scholars. Chili consumption, nutritional deficiency, areca nut chewing, genetic susceptibility, altered salivary constituent, autoimmunity and collagen disorders have been suggested to be involved in the pathogenesis of this condition.^[2] However, liberation of arecoline in chewed areca nut is regarded as the main etiological agent for oral submucous fibrosis (OSMF).^[5] The disease has a very specific predilection to races, geographic areas and individuals.^[6] Indians and South East Asians are most predominantly affected by the disease.^[7] Further, persistent habits can lead to malignant transformation, it will vary because of immune status of individuals.^[8] At present, oral submucous fibrosis (OSMF) is considered as a potential malignant disorder, with malignant transformation rates as high as 7.6% have been reported from the Indian subcontinent over a 17 years period cohort study.^[9] This malignant transformation to oral squamous cell carcinoma in oral submucous fibrosis (OSMF) was first recorded by Paymaster in 1956,^[10] which paved the way for extensive exploration in this field toward identifying the factors that predispose to this condition.

Central for all chemical carcinogenesis is relied on the formation of DNA adduct and thereby causing DNA damage.^[11] Cellular responses to DNA damage in mammalian cells include DNA repair, cytotoxicity, apoptosis, mutagenesis and transformation to malignancy. These processes are either fundamental to maintaining the integrity of the cell or they set the cell on a path to mortality or malignancy.^[12] Thus, the analysis of interactions between carcinogens and DNA and their biological consequences of these reactions necessitate for better understanding the early stages of the carcinogenic process.

There exists evidence for reactive oxygen species (ROS)-induced lipid peroxidation on chewing tobacco, pan masala, etc.^[13] This ROS-induced lipid peroxidation may characteristically produce deoxynucleic acid malondialdehyde (DNA-MDA) adduct, and there by results in loss of cellular homeostasis.^[14] Therefore, the levels of MDA in serum/tissue/saliva of OSMF patients mark the extent of cellular damage caused by lipid peroxidation occurring in these individuals and may act as a marker for biomonitoring cellular damage caused by free radicals in these individuals. Further, the scenario may be exaggerated if there is a scarcity of antioxidant enzymes such as superoxide dismutase (SOD) as these enzymes protect cells from damage caused through ROS during normal metabolism and also prevent accumulation of ROS after exposure to oxidative stress.

There are some exist studies which indicate the excessive production of ROS and thereby causing increased levels of MDA^[15] and reduced the antioxidant enzyme levels such as SOD in OSMF.^[16] However, to our present knowledge, there exists few studies in which they compare the levels of serum MDA and SOD in each of the clinical stages of OSMF. Hence, the present study was designed to estimate serum MDA and SOD clinical stages of OSMF patients and to compare the same with control group to assess the utility of MDA-SOD levels as a prognostic tool with high validity and reliability.

MATERIALS AND METHODS

A total of sixty participants attended the Department of Oral and Maxillofacial Pathology, Sibar Institute of Dental Sciences, constituted the study groups for this case-control study. The study was approved by the Institutional Ethical Committee. The study cases were divided into two groups, namely, control group (Group I) comprised thirty healthy volunteers without any dilatory habits, history of malignancy or any systemic illness or on any medications and case group (Group II) comprised thirty cases of oral submucous fibrosis with no history of malignancy or any systemic illness other than that for oral submucous fibrosis. All the necessary details of case history such as age, gender, habits, extent of the lesion were recorded, and specifically for case group, clinical staging was performed and categorized into three clinical stages on the basis of mouth opening as described by Ranganathan *et al.* Biopsy was performed after obtaining informed consent from patients. In case group, only those with all clinical and histopathological details were alone recruited for the study.

Histopathological confirmation of OSMF

All the biopsied specimen were processed and stained with hematoxylin and eosin stain with standard procedure. The diagnosis of oral submucous fibrosis was made using the criteria defined by Pindborg and Sirsat criteria.

Quantification of malondialdehyde and superoxide dismutase

A 5 ml of fasting venous blood was collected from antecubital vein with the help of vacutainer under aseptic conditions from all OSMF patients and controls. The collected blood was centrifuged at 3000 rpm for 2 min to separate serum; MDA was measured using the standard procedure. MDA reactive with Thiobarbituric acid at 100°C in acidic medium to give pink color complex. The intensity of color of MDA–TBA complex was measured at 535 nm using spectrophotometer againsts a reagent blank solution. The concentration of MDA was calculated using the molar extinction coefficient of the MDA–TBA complex ($1.50 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$).

SOD was measured using standard procedure; 0.05 ml of serum was mixed with 2.85 ml of Tris buffer and 0.1 ml of pyrogallol. The optical density was obtained using spectrophotometer at 420 nm. Calculation of SOD was done using the formula and expressed as units/ml of serum $C-T/50 \times 1000$.

Statistical analysis

The obtained results were tabulated accordingly and subjected to statistical analysis using software SPSS 20.0. The Student's test was performed to compare mean MDA levels/SOD levels in case group and control group. Kruskal–Wallis ANOVA test was used to compare the three grades of OSMF in case group with corresponding MDA (nmol/dl) levels. For pair-wise comparison of three grades of oral submucous fibrosis with SOD (units/ml) in case group, Mann–Whitney U-test was used. The strength between various stages of OSMF with SOD and MDA was assessed using Spearman's rank correlation analysis. Karl Pearson's correlation was used to assess the direct or inverse relationship between MDA and SOD levels with respect to OSMF group and control group.

RESULTS

A total of 60 cases out of which 27 (90%) males and 3 (10%) females framed a control group (Group I), whereas 28 (93.3%) males and 2 (6.7%) females constituted OSMF group (Group II). The mean age of presentation of OMF participants was 34.1 ± 8 (mean \pm standard deviation [SD]), whereas in controlled participants, mean age was 34.1 ± 8 (mean \pm SD) [Table 1]. The

mean serum MDA levels and mean serum SOD levels in healthy volunteers was $204.2 \pm 3 \text{ nmol/dl}$ (Mean \pm SD) and $260.1 \pm 7 \text{ u/ml}$ (Mean \pm SD), respectively, whereas in OSMF group, serum MDA levels was $437.8 \pm 8 \text{ nmol/dl}$ (Mean \pm SD) and mean serum SOD was $82.7 \pm 26.16 \text{ u/ml}$ (Mean \pm SD). Comparison of MDA and SOD levels between healthy volunteers and OSMF participants using Student's *t*-test was statistically significant with $P < 0.05$ [Table 2].

OSMF participants in Stage III demonstrated the highest mean serum MDA level of $502.3 \pm 6 \text{ nmol/dl}$ (Mean \pm SD) followed by OSMF participants with Stage II with mean serum MDA levels of $398.25 \pm 7 \text{ nmol/dl}$ (mean \pm SD) and OSMF cases with Stage I mean serum MDA level of $367.9 \pm 2 \text{ nMol/dl}$ (mean \pm SD). Whereas, SOD levels were high in OSMF cases with Stage I with mean serum SOD level of $123.4 \pm 4 \text{ u/ml}$ (mean \pm SD), In OSMF cases, with Stage II mean serum SOD level was $88.93 \pm 23.86 \text{ u/ml}$ (mean \pm SD) and OSMF participants with Stage III mean serum SOD level was $67.7 \pm 20.57 \text{ u/ml}$ (mean \pm SD) [Table 3].

The correlation between various stages of OSMF with SOD (per/ml) and MDA (nmol/dl) by Spearman's rank correlation method indicated a significant ($P = 0.0010$) moderate inverse relationship between various grades of OSMF with SOD as Spearman $R (r_s)$ was 0.5700 and a highly significant ($P = 0.00001$) direct strong relationship

Table 1: Distribution and mean and standard deviation of male and females in control and oral submucous fibrosis groups (n=30)

Gender	Control (%)	Mean \pm SD	OSMF (%)	Mean \pm SD
Male	27 (90.0)	34.1 \pm 8	28 (93.3)	33.8 \pm 7
Female	3 (10.0)	26.6 \pm 0	2 (6.7)	44.5 \pm 4
Total	30 (100)	33.4 \pm 7	30 (100)	34.6 \pm 7

SD: Standard deviation, OSMF: Oral submucous fibrosis

Table 2: Comparison of mean malondialdehyde (nmol/dl) and superoxide dismutase (n/ml) in control and oral submucous fibrosis

Values	Mean \pm SD		P
	Control	OSMF	
MDA	204.2 \pm 3	437.8 \pm 8	0.0001*
SOD	260.1 \pm 7	82.7 \pm 2	0.0001*

* $P < 0.05$. MDA: Malondialdehyde, SOD: Superoxide dismutase, OSMF: Oral submucous fibrosis, SD: Standard deviation

Table 3: Comparison of mean malondialdehyde (nmol/dl) and superoxide dismutase (n/ml) with clinical staging in oral submucous fibrosis

Values	Stage I	Stage II	Stage III	P
MDA	367.9 \pm 2	398.2 \pm 7	502.3 \pm 6	0.001*
SOD	123.4 \pm 4	88.9 \pm 2	67.7 \pm 2	0.001*

* $P < 0.05$. MDA: Malondialdehyde, SOD: Super oxide dismutase

between various grades of OSMF with MDA as Spearman R (r_s) was 0.6725 [Table 4].

Further, using Karl Pearson's correlation between mean MDA and SOD levels in control group showed stastically insignificant ($P=0.63$), where as in oral sub mucous fibrosis cases the mean MDA and SOD it showed stastically highly significant ($P=0.0015$). These findings in the present study indicated for decreased levels of mean serum SOD level and increased mean serum MDA level with increasing clinical stages of OSMF [Table 5].

DISCUSSION

Oral submucous fibrosis (OSMF) is a chronic disease affecting the mucosa of the oral cavity. The oral mucosa is damaged by the oxidative stress caused by the ROS.^[17]

A number of compounds and enzymes may function to protect cellular components from oxidative damages.^[18] The major antioxidant defense system comprised SOD and guaiacol peroxidase appears to be responsible for scavenging free radicals and nascent oxygen.^[19] In normal cells, there is an intricate pro-oxidant and antioxidant balance. In oxidative stress conditions, this balance shifts toward pro-oxidants. If the oxidant species production is increased with concomitant prolonged and massive stress, it can result in serious cell damage.^[20] Extensive studies with biologic materials have shown clearly that the reactive-free radicals are able to produce chemical modifications in the cells and damage the proteins, lipids, carbohydrates and nucleotides.^[21] To overcome these consequences, cells have antioxidant defense systems which scavenge the

free oxygen radicals and suppress free radical chain and lipid peroxidation. Antioxidants play an important role in carcinogenesis.^[22] Given the established precancerous nature of oral submucous fibrosis, the present study estimated the levels of lipid peroxidation products MDA and antioxidant enzymes SOD in blood samples of patients diagnosed with oral submucous fibrosis.

Further, imbalance between pro- oxidants and antioxidants in normal cells will causes alterations in functions of cell. If the oxidant species production is increased with concomitant prolonged and massive stress, it can result in serious cell damage.^[23] Extensive studies with biologic materials such as serum and plasma have shown clearly that reactive free radicals are able to produce chemical modifications in cell wall, cytoplasm. This may finally result in alterations in the structural components such as protein, lipids, carbohydrates and nucleotides and leads to abnormal in cellular function.^[24]

Soma *et al.* in 2004 and Shishir Ram Shetty *et al.* in 2012 in their study establish that there is significantly augmented plasma levels of MDA in patients with oral submucous fibrosis when compared to that of controls group.^[16,23] In our observations, there is elevation in serum MDA levels in patients with oral submucous fibrosis when compared with controls and MDA level was increased when the clinical stage increases.

Uikey *et al.* in 2003 and Gurudath *et al.* in 2012 have shown that SOD levels were decreased in oral submucous fibrosis patients when compared with controls. This observation was statistically significant.^[24,25] Our findings were inconsistent with their observations. Metkari *et al.* in 2007 showed that the mean level of MDA was elevated and the mean levels of SOD were decreased in oral submucous fibrosis patients, whereas mean MDA was decreased and SOD levels were elevated in control participants. MDA levels were increased when the clinical stage increases and SOD levels were decreased when the clinical stage was increased.^[25,26] In the present study, observations are similar with previse studies.

Table 4: Correlation between stages with superoxide dismutase (n/ml) and malondialdehyde (nmol/dl) by Spearman's rank correlation method

Variables	n	Spearman R	t	P
Between grades and SOD (/ml)	30	-0.5700	-3.6712	0.0010*
Between grades and MDA (nmol/dl)	30	0.6725	4.8080	0.00001*

* $P < 0.05$. MDA: Malondialdehyde, SOD: Superoxide dismutase

Table 5: Correlation between superoxide dismutase (n/ml) and malondialdehyde (nmol/dl) in control and oral submucous fibrosis group by Karl Pearson's correlation method

Groups	Correlation between SOD (n/ml) and MDA (nmol/dl)		
	r	t	P
Control	0.0891	0.4732	0.6397
OSMF	-0.5535	-3.5166	0.0015*

* $P \leq 0.05$, OSMF: Oral submucous fibrosis, MDA: Malondialdehyde, SOD: Superoxide dismutase

CONCLUSION

Oxidative stress will plays an important role for transformation of malignancy. The estimation of lipid peroxidation and antioxidants in the serum of patients with oral submucous fibrosis and control can assess the degree of damage of the cells and progression of diseases. Further, treatment can be improved once correcting the underlying deficiency of antioxidants, this may be helpful for the successful management. By estimating oxidative

stress in the early stages of oral submucous fibrosis, we can prevent malignant transformation. However, the present study is unique in its way by demonstrating statistically significant moderate inverse relationship between mean serum MDA levels and SOD levels with respect to oral submucous fibrosis group.

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Conflicts of interest

There are no conflicts of interest.

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