ORIGINAL RESEARCH

# An estimation of serum malondialdehyde, superoxide dismutase and vitamin A in oral submucous fibrosis and its clinicopathologic correlation

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**ABSTRACT**

Recently, there has been growing interest in studies that concern with reactive oxygen species in various diseases. Several studies have shown the role of oxidant-antioxidants system in the causation and progression of various types of cancer including oral cancer. However, considering its high prevalence in India and the potential to undergo malignant transformation oral submucous fibrosis (OSF) has not been widely investigated with respect to lipid peroxidation and antioxidants. This definitely has developed a responsibility over the oral pathologists to find out the exact role of lipid peroxidation and antioxidants in OSF.

With this view in mind, the present study was undertaken and an attempt was made to correlate the serum levels of lipid peroxidation product malondialdehyde (MDA), antioxidants superoxide dismutase (SOD) and vitamin A in relation to clinical and histopathological grading of OSF. The progressively increased MDA and progressively decreased SOD and vitamin A levels has positive correlation with clinical grades of OSF.

***Key words:*** Antioxidants, malondialdehyde, oral submucous fibrosis, superoxide dismutase, vitamin A



## INTRODUCTION

Oral submucous fibrosis (OSF) is a premalignant[1] and crippling condition[2] of the oral mucosa. It has been reported mainly in the Indian population and established in the Indian literature since the time of Sushruta.[3] The etiopathogenesis of OSF believed to be multi-factorial includes areca nut chewing, ingestion of chillies, genetic and immunologic processes, nutritional deficiencies and other factors.[4]

The diagnosis and prognosis of OSF can be established by means of biopsy, which is an invasive, time-consuming procedure and causes psychological trauma to some patients. It also presents some limitations in precancerous condition (but not in precancerous lesion), as in these cases it is important to select the more appropriate site for biopsy where histopathological changes would be present. Apart from these limitations routine histopathologic examination is subjective and it depends upon the individual experience and assessment of microscopic examination.[5]

Thus the need of the hour is that the test ought to be simple, less invasive, less time consuming, easy for interpretation, economical and yet quite confirmatory for its diagnosis and prognosis. Apart from routine histopathology other diagnostic and prognostic methods such as biochemical investigations are of prime importance because of their advantages such as simplicity, less invasiveness, less time consuming, comparatively economical etc. It may also useful to monitor the response of therapy. Moreover, biochemical alteration reflects tissue changes at cellular level.

The epidemiological and experimental studies have implicated reactive oxygen species (ROS) induced lipid peroxidation i.e., malondialdehyde (MDA) in the development of precancer and cancer. The extent of oxidative damage caused by ROS can be exacerbated by a decreased efficiency of antioxidant defense mechanisms of the body.[6] Enzymatic antioxidant superoxide dismutase (SOD) plays a key role in detoxification of superoxide anion radical and hence diminishes the toxic effects of this radical and other free radicals such as hydrogen peroxide and hydroxyl radical from secondary reactions. Nonenzymatic antioxidant vitamin A has a stabilizing effect on the mucous membranes. The deficiency of vitamin A causes loss of mucous secreting cells and epithelial atrophy resulting in mucosal irritation.[7] Thus measurement of lipid peroxidation product and antioxidants is valuable in OSF to assess tissue burden because they reflect the bioavailability of

antioxidants as well as their increased utilization to scavenge lipid peroxidation products.[6]

In spite of its high prevalence in India and the potential to undergo malignant transformation, OSF has not been widely investigated with respect to lipid peroxidation and antioxidants. Moreover, to the best of our knowledge literature on lipid peroxidation and antioxidants in relation to histopathological grading of OSF is either not easily available or may be scanty.

With this view in mind, the present study was undertaken to estimate the serum levels of lipid peroxidation product MDA, antioxidants SOD and vitamin A in relation to clinical and histopathological grading of OSF.

## MATERIALS AND METHODS

The present study was carried out during the period from March 2005 to March 2006.

### Selection of cases

Of the routine OPD patients reporting to Department of Oral Pathology, Government Dental College and Hospital, Aurangabad, subjects clinically suspicious of OSF were selected. The relevant history of each patient was recorded. The patients were grouped clinically into four grades based on severity.[8] Only those patients who had not any systemic diseases and/or not received any therapy for OSF prior to study were subjected to punch biopsy from buccal mucosa. After confirmation of OSF with histopathology, they were included in the OSF group. The histopathological grading was done into four grades according to Sirsat and Pindborg.[9] Equal number of age and sex matched healthy subjects without any tissue abuse habits and without any clinically obvious oral lesions or systemic diseases were selected as the control group. The subjects for the study were grouped as follows:

Group 1 (OSF): 40 patients having OSF.
Group 2 (Control): 40 healthy subjects.

### Collection of blood sample

Under all aseptic precautions about 5 ml fasting venous blood was collected from antecubital vein of each individual into plain sterile bulb. The sample was then allowed to clot at room temperature for about two hours and was then centrifuged at 3000 rpm for 10 min, to separate the serum. Immediately this serum was used for estimation of MDA, SOD and vitamin A levels.

The estimation of MDA in serum was done by the method of Kei Satoh.[10] The color produced by the reaction of thiobarbituric acid with MDA was measured at 530 nm with the help of spectrophotometer. The results were expressed as nmol/ml.

SOD was assayed by the method of Marklund and Marklund[11] modified by Nandi *et al.*[12] This method is based on the ability of SOD to inhibit autoxidation of pyrogallol under specific conditions. Reading was taken at 420 nm and expressed as units/ml.

Vitamin A was estimated by the method of Sing *et al.*[13] at 525 nm and the results were expressed as µg/ml. The results obtained were tabulated, analyzed and interpreted.

### Statistical analysis

The data were analyzed with Student's independent 't' test and one-way analysis of variance (ANOVA) test. All statistical analyses were performed with the program Statistical Package for the Social Science (SPSS 8.0 windows, version 8.0) and *P* value of < 0.05 was accepted as statistically significant.

## RESULTS

In the present study, the levels of MDA, SOD and vitamin A were compared between the OSF group and the control group. Comparision of MDA, SOD and vitamin A among the OSF group and control group showed a statistically significant increased levels of malondialdehyde and decreased levels of superoxide dismutase and vitamin A among OSF groups [Table 1].

The levels of MDA, SOD and vitamin A within different clinical grades of OSF were compared [Table 2]. The statistical evaluation of the comparison was done using the one-way ANOVA test. From the statistical analysis it was observed

**Table 1: Comparison of malondialdehyde, superoxide dismutase and vitamin A between oral submucous fibrosis group and control group**

| | Malondialdehyde | | Superoxide dismutase | | Vitamin A | |
|---|---|---|---|---|---|---|
| | OSF | Control | OSF | Control | OSF | Control |
| Mean±SD | 9.9± 1.21 | 5.47± 1.19 | 2.46± 0.256 | 3.46± 0.273 | 18.22± 2.247 | 37.85± 5.151 |
| t value | 16.40 | | 16.93 | | 22.08 | |
| *P* value | 0.000 | | 0.000 | | 0.000 | |
| Statistical significance | Highly significant | | Highly significant | | Highly significant | |

OSF - Oral submucous fibrosis

**Table 2: Comparison of malondialdehyde, superoxide dismutase and vitamin A amongst clinical grades of oral submucous fibrosis**

| | | Clinical grades of oral submucous fibrosis | | | | F value |
|---|---|---|---|---|---|---|
| | | Grade I (n = 4) | Grade II (n = 12) | Grade III (n = 20) | Grade IV (n = 4) | |
| Malondialdehyde | Mean±SD | 7.50±1.29 | 9.80±0.76 | 10.33±0.651 | 11.50±0.577 | 19.610 |
| Superoxide dismutase | Mean±SD | 2.82±0.0699 | 2.55±0.1352 | 2.37±0.1384 | 1.91±0.0359 | 41.53 |
| Vitamin A | Mean±SD | 23±2.16 | 18.3±1.41 | 17.2±1.35 | 16±0.816 | 19.74 |

*P* value < 0.001

that the difference in levels of MDA, SOD and vitamin A between the clinical grades of OSF was statistically significant (*P* value < 0.001). Significant difference was found by using one-way ANOVA. It was observed that the difference in levels of MDA, SOD and vitamin A between paired means of grades was statistically significant except MDA level between grade II *vs* III and vitamin A level between grade III *vs* IV.

The levels of MDA, SOD and vitamin A within different histopathological grades of OSF were compared [Table 3]. The statistical evaluation of the comparison was done using one-way ANOVA test. From the statistical analysis it was observed that the difference in levels of MDA, SOD and vitamin A between different histopathological grades of OSF was statistically insignificant (*P* value > 0.05).

## DISCUSSION

In the present study, mean serum levels of MDA, SOD and vitamin A levels were compared between OSF group and control group. These levels were correlated with the clinical and histopathological grades of OSF.

The mean level of MDA was increased in the OSF group (9.9±1.21) compared to control group (5.47±1.19). The statistical evaluation by using Student's independent 't' test, showed that the difference in levels of MDA between the OSF group and the control group was statistically significant (*P*<0.001).

Clinical grade-wise analysis showed that mean MDA level gradually increased from grade I to grade IV (grade I 7.50±1.29, grade II 9.80±1.76, grade III 10.33±0.651, grade IV 11.50±0.577). The difference in levels of MDA was seen to be statistically significant by using one-way ANOVA test (*P*<0.001). These findings were similar to Gupta *et al.*[14] who reported a significant increase in the mean MDA level in all clinical grades of OSF cases as compared to healthy controls and the increase was statistically significant (*P*<0.001). The difference among various grades were statistically significant except among grade II and grade III [Figure 1]. It is established that lipid peroxidation increases with severity of the disease reflecting the extent of tissue injury.

In the present study, mean MDA level in relation with histopathological grade was found to be 9.87±1.05 in grade II, 9.71±1.79 in grade III and 11±1.41 in grade IV. As there were no patients in grade I, the levels could not be estimated in this grade. The statistical analysis using one-way ANOVA showed that the difference in levels of MDA between the histopathological grades of OSF was statistically insignificant (*P*>0.05) [Figure 1]. To the best of our knowledge, reports on MDA level in relation to histopathological grades of OSF were

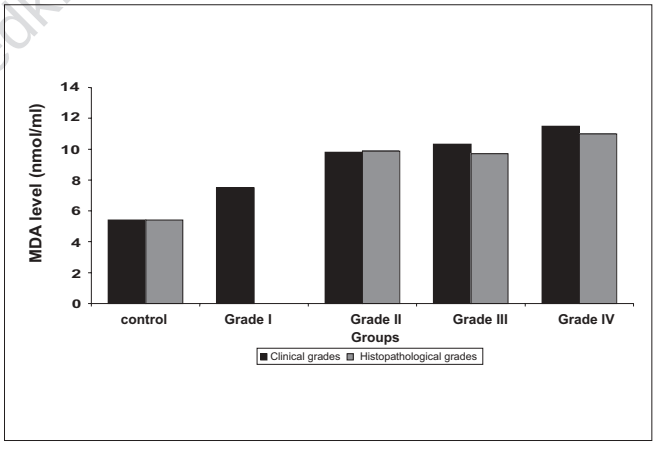


**Figure 1:** Comparison of MDA level between control group and clinical grades and control group and histopathological grades of OSF

**Table 3: Comparison of malondialdehyde, superoxide dismutase and Vitamin A amongst histopathological grades of oral submucous fibrosis**

| | | Histopathological grades of oral submucous fibrosis | | | | F value |
|---|---|---|---|---|---|---|
| | | Grade I (n = 0) | Grade II (n = 31) | Grade III (n = 7) | Grade IV (n = 2) | |
| Malondialdehyde | Mean±SD | - | 9.87±1.05 | 9.71±1.79 | 11±1.41 | 0.905 |
| superoxide dismutase | Mean±SD | - | 2.51±1.99 | 2.34±0.39 | 2.16±0.28 | 2.934 |
| Vitamin A | Mean±SD | - | 18.29±2.036 | 18±3.266 | 18±2.828 | 0.055 |

*P* value > 0.05

not found in the available literature, therefore comparison was not possible.

The increase in lipid peroxidation product in OSF as compared to control group may be due to the poor antioxidant system, excessive free radical formation due to various tissue abuse habits and decomposition of polyunsaturated fatty acids present in membranes.

The mean level of SOD was decreased in the OSF group (2.46±0.256) compared to control group (3.46 ±0.256). The statistical evaluation by using Student's independent 't' test, showed that the difference in levels of SOD between the OSF group and the control group was statistically significant ($p<0.001$), similar to Uikey *et al*.[15]

The mean SOD level gradually decreased in relation with clinical grades of OSF from early to advanced grades (grade I 2.8±20.0699, grade II 2.55±0.1352, grade III 2.37±0.1384, grade IV 1.91±0.0359). The statistical analysis using one-way ANOVA showed that the difference in levels of SOD between clinical grades of OSF was statistically significant ($P<0.001$). The difference among various grades was found to be statistically significant on using Student's independent 't' test ($P<0.001$). Though the SOD level was observed to be decreasing in relation with histopathological grades from grade II to grade IV, the difference was not statistically significant ($P>0.05$) [Figure 2].

Uikey *et al.*[15] demonstrated the decrease in the level of SOD in OSF as compared to control group. Gupta *et al.*[14] assessed enzymatic antioxidant defense by SOD activity, which did not show any significant change in any stage of the OSF. However, available literature did not reveal any histopathological grade wise analysis, hence a comparison was not possible.

Vitamin A level was found to be significantly deceased in OSF group (18.22±2.247) compared to control group (37.85±5.151) in accordance with earlier workers.[16,17]

The mean vitamin A level gradually decreased from clinical grade I to grade IV (grade I 23±2.16, grade II 18.3±1.41, grade III 17.2±1.35, grade IV 16±0.816). The difference in levels of vitamin A was found statistically significant by using one-way ANOVA test ($P<0.001$). The difference among various groups was found to be statistically significant except between grade III and grade IV [Figure 3].

The mean vitamin A levels in relation with histopathological grades were found to be 18.29±2.036 in grade II, 18±3.266 in grade III and 18±2.828 in grade IV. The statistical analysis using one-way ANOVA showed that the difference in levels of vitamin A between the histopathological grades of OSF was statistically insignificant ($P>0.05$) [Figure 3].

Gupta *et al.*,[14] Raina *et al.*[18] had observed that the levels of serum β-carotene were below the normal range for all grades of OSF. Thus, we can conclude indirectly from their study that the OSF subjects have vitamin A deficiency as β-carotene, a provitamin-A carotenoids is efficiently converted to vitamin A than other carotenoids. Byproducts of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes. Enzymatic and non-enzymatic antioxidants scavenge lipid peroxidation byproducts formed under physiological and pathological conditions. Thus, the observed decrease in SOD and vitamin A in OSF can be due to utilization of these antioxidants by affected tissues or in combating the excessive oxidative stress in circulation.

Increased lipid peroxidation product MDA and decreased antioxidant SOD and vitamin A have been reported in various pathological conditions including OSF. Our results support these observations.

In the present study, significant correlation between serum levels of MDA, SOD and vitamin A with histopathological grading was not found. This may be because histopathological examination shows localized tissue changes (buccal mucosa in present study) whereas OSF is a well-recognized precancerous



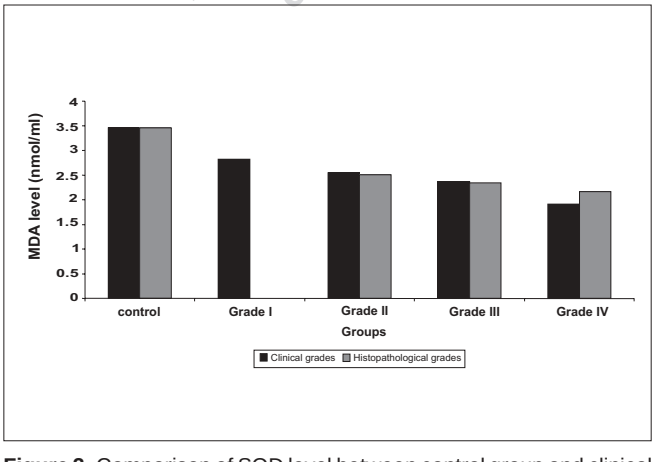


**Figure 2:** Comparison of SOD level between control group and clinical grades and control group and histopathological grades of OSF

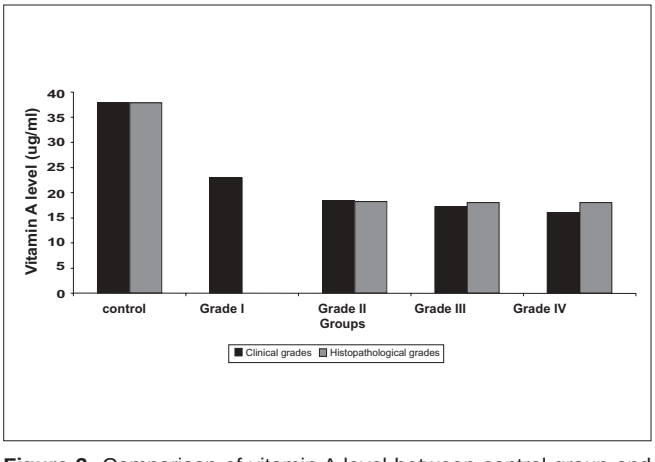


**Figure 3:** Comparison of vitamin A level between control group and clinical grades and control group and histopathological grades of OSF

condition. It may affect various parts of oral cavity to a different extent; sometimes even pharynx and oesophagus may be involved. On the other hand, changes in blood are seen consistently even though they are secondary to the tissue changes taking place anywhere in the body. Furthermore, in histopathologic grading, more weightage is given to the connective tissue changes rather than the epithelial changes since it is primarily affecting the connective tissue. Malignant transformation is related to the epithelial changes prima facie. So there is a need for such a histopathological grading criteria, which includes the epithelial changes (e.g. epithelial dysplasia) along with connective tissue changes. This would also help in correlation of biochemical parameters to the histopathological grade in a better manner.

## SUMMARY AND CONCLUSION

From the present study, it is evident that by estimation of lipid peroxidation and antioxidants in circulation of OSF patients, one can assess the degree of oxidative damage of the disease. Further correcting the underlying deficiency of antioxidants the treatment plan can be improved. This in turn, may be helpful for successful management of this condition, thereby arresting it in early stages and avoiding the possible consequences of OSF (malignancy).

However further elaborate studies with a larger sample size including OSF with coexisting oral cancer along with follow-up are needed to ascertain the actual role of these parameters in the initiation and promotion of carcinogenesis.

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