ORIGINAL ARTICLE

# Comparative Analysis of Oxidative Stress Markers in Smokers and Non-Smokers with Oral Submucous Fibrosis

Mohammed Enamur Rashid


## ABSTRACT

### Background

Oral submucous fibrosis (OSMF) is a chronic, insidious, potentially malignant disorder characterized by juxta-epithelial inflammatory reaction and progressive fibrosis of the lamina propria. Its pathogenesis is multifactorial, with oxidative stress playing a pivotal role. Tobacco smoking, a known independent source of reactive oxygen species, may exacerbate this oxidative burden. Methods: A cross-sectional study was conducted on 100 participants, divided into four groups: Group A (OSMF smokers, n=25), Group B (OSMF non-smokers, n=25), Group C (healthy smokers, n=25), and Group D (healthy non-smokers, n=25). Clinical staging of OSMF was performed. Serum levels of MDA, SOD, GSH, and vitamin C were estimated using standard biochemical assays.

### Results

Group A exhibited the highest mean MDA level (6.18 ± 1.32 nmol/mL), significantly higher than Group B (4.82 ± 1.15 nmol/mL, $p < 0.001$), Group C (3.48 ± 0.81 nmol/mL, $p < 0.001$), and Group D (2.09 ± 0.52 nmol/mL, $p < 0.001$). Conversely, antioxidant levels were lowest in Group A (SOD: 1.85 ± 0.48 U/mL; GSH: 3.21 ± 0.88 mg/dL; Vit C: 0.72 ± 0.25 mg/dL) and progressively increased through Groups B, C, and D. Within the OSMF cohort (A+B), a significant positive correlation was found between MDA levels and clinical stage of OSMF ($r = 0.71$, $p < 0.001$), while SOD, GSH, and vitamin C showed a significant negative correlation.

### Conclusion

The study demonstrates that smoking significantly exacerbates oxidative stress in patients with OSMF, as evidenced by higher MDA and depleted antioxidant levels compared to non-smokers with OSMF. This heightened oxidative state may contribute to accelerated disease progression and increased malignant transformation risk, underscoring the critical importance of smoking cessation in the management of OSMF.



## Keywords

Oral submucous fibrosis, Oxidative stress, Smoking, Malondialdehyde, Antioxidants, Potentially malignant disorder


## INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, insidious, and debilitating disease of the oral cavity, recognized as a potentially malignant disorder (OPMD) with a documented malignant transformation rate ranging from 2% to 8% [1]. The disease is characterized by inflammation and progressive fibro-elastosis of the lamina propria, leading to stiffness of the oral mucosa, trismus, and difficulty in eating and speaking [2]. The etiopathogenesis of OSMF is multifactorial, with the chewing of areca nut being the most significant causative factor. Arecoline, the primary alkaloid in areca nut, is known to stimulate fibroblast proliferation and collagen synthesis, while also generating reactive oxygen species (ROS) [3].

Oxidative stress, defined as an imbalance between the production of ROS and the capacity of the antioxidant defense system, has been increasingly implicated in the pathogenesis of OSMF [4]. ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, can cause lipid peroxidation, protein damage, and DNA alterations, leading to cellular dysfunction and carcinogenesis. Several studies have reported elevated levels of lipid peroxidation byproducts like malondialdehyde (MDA) and depleted levels of antioxidant enzymes such as superoxide dismutase (SOD) and non-enzymatic antioxidants like reduced glutathione (GSH) and vitamin C in patients with OSMF [5, 6].

Tobacco smoking is another well-established etiological factor for oral precancers and cancers.


### Correspondence:

**Mohammed Enamur Rashid**

Department of Oral & Maxillofacial Diagnostic Sciences, College of Dentistry, Taibah University, Al Madinah Al Munawara, Kingdom of Saudi Arabia. erashid2002@gmail.com


Bangladesh Journal of Medical Science Vol. 25. Supplementary Issue 2026

DOI: https://doi.org/10.3329/bjms.v25i10.86630

Cigarette smoke contains over 4,000 chemicals, including a high concentration of free radicals and ROS, which overwhelm the body's antioxidant defenses [7]. Smoking independently induces systemic oxidative stress, leading to elevated MDA levels and reduced antioxidant capacity in otherwise healthy individuals [8].

Given that both OSMF and smoking are potent inducers of oxidative stress, it is logical to hypothesize that their combination could have a synergistic or additive effect, leading to a more severe oxidative burden. This heightened oxidative state could potentially accelerate the progression of fibrosis, increase the severity of clinical symptoms, and elevate the risk of malignant transformation in OSMF patients who smoke. However, there is a paucity of research that directly compares the quantitative levels of oxidative stress markers between smokers and non-smokers within the OSMF patient population.

Recent studies have focused on individual markers but have often lacked a comprehensive comparative analysis between these distinct patient cohorts [9, 10]. A clear understanding of how smoking modulates the oxidative stress profile in OSMF is crucial for risk stratification, patient counseling, and developing targeted therapeutic strategies, such as aggressive antioxidant supplementation and smoking cessation programs.

Therefore, the present study was designed to conduct a comparative analysis of key oxidative stress markers—MDA (a marker of lipid peroxidation) and SOD, GSH, and vitamin C (markers of antioxidant defense)—in smokers and non-smokers with OSMF, against appropriate healthy control groups, to elucidate the additive effect of smoking on the oxidative stress status in this potentially malignant disorder.

## MATERIALS AND METHODS

### Study Design and Setting

This cross-sectional, case-control study was conducted in the Department of Oral Medicine and Radiology at a tertiary care teaching hospital. The study duration was 18 months, from January 2022 to June 2023.

### Sample Size Calculation

Based on a pilot study and previous literature, the mean difference in serum MDA levels between OSMF patients and healthy controls was estimated to be 2.5 nmol/mL with a standard deviation of 2.0 nmol/mL. Using a two-tailed test, an alpha error of 0.05, a power (1-β) of 90%, and a sample size ratio of 1:1:1:1, the minimum required sample size was calculated to be 22 per group. To account for potential dropouts and increase the statistical power, 25 participants were recruited for each of the four groups, resulting in a total sample size of 100.

### Study Groups

The participants were divided into four groups:

- **Group A (OSMF Smokers):** 25 clinically and histopathologically diagnosed OSMF patients with a history of smoking >10 cigarettes/day for >5 years.
- **Group B (OSMF Non-Smokers):** 25 clinically and histopathologically diagnosed OSMF patients with no history of smoking or other tobacco use.
- **Group C (Healthy Smokers):** 25 age- and gender-matched healthy individuals with a history of smoking >10 cigarettes/day for >5 years, without any oral mucosal lesions.
- **Group D (Healthy Non-Smokers):** 25 age- and gender-matched healthy individuals with no history of any tobacco use or oral mucosal lesions, serving as the baseline control.

### Inclusion and Exclusion Criteria

#### Inclusion Criteria:

- Age between 20-60 years.
- For OSMF groups: Clinical diagnosis of OSMF confirmed by incisional biopsy.
- For smoker groups: History of smoking as defined above.
- Willingness to provide blood samples.

#### Exclusion Criteria:

- History of any systemic disease (e.g., diabetes mellitus, cardiovascular diseases, autoimmune disorders).
- Current use of antioxidant supplements or medications that could affect oxidative status (e.g., steroids, NSAIDs) within the last month.
- Presence of any other oral mucosal disease or active infection.

- History of alcohol consumption or other substance abuse.
- For OSMF groups: Patients who had received any prior treatment for OSMF.

### Clinical Assessment and Sample Collection

All participants underwent a thorough clinical examination. For OSMF patients, the disease was staged clinically according to the mouth opening and functional impairment, as classified by Haider et al. [11].

Approximately 5 mL of fasting venous blood was drawn from the antecubital vein between 8:00 AM and 9:00 AM to minimize diurnal variation. The blood was collected in plain vacutainers, allowed to clot for 30 minutes at room temperature, and then centrifuged at 3000 rpm for 15 minutes. The separated serum was aliquoted into sterile Eppendorf tubes and stored at -80°C until biochemical analysis.

### Biochemical Assays

All biochemical estimations were performed by a biochemist blinded to the clinical groups.

- **Malondialdehyde (MDA):** Serum MDA level, an indicator of lipid peroxidation, was estimated using the thiobarbituric acid reactive substances (TBARS) assay. The results were expressed as nmol/mL.
- **Superoxide Dismutase (SOD):** SOD activity was measured based on its ability to inhibit the auto-oxidation of pyrogallol. The activity was expressed as U/mL.
- **Reduced Glutathione (GSH):** Serum GSH level was determined using the DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) method, where the color formed was read spectrophotometrically. The results were expressed as mg/dL.
- **Vitamin C:** Serum ascorbic acid (vitamin C) level was estimated using the 2,4-dinitrophenylhydrazine method. The results were expressed as mg/dL.

### Statistical Analysis

The collected data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation (SD) for quantitative variables and frequency (percentage) for categorical variables. The normality of the data was assessed using the Shapiro-Wilk test.

One-way Analysis of Variance (ANOVA) was used to compare the mean values of oxidative stress markers among the four groups. For pairwise comparisons, Tukey's post-hoc test was applied. For the combined OSMF cohort (Groups A and B), Pearson's correlation coefficient was used to assess the relationship between oxidative stress markers and the clinical stage of OSMF. A p-value of less than 0.05 was considered statistically significant.

## Results

### Demographic and Clinical Data

The study comprised 100 participants, with 25 in each group. The demographic and clinical characteristics of the participants are summarized in Table 1. There were no statistically significant differences in age ($p = 0.812$) or gender distribution ($p = 0.918$) among the four groups, ensuring comparability. Within the OSMF groups, the mean duration of symptoms was comparable between smokers and non-smokers. However, Group A (OSMF smokers) had a higher proportion of patients in advanced clinical stages (Stage III and IV) compared to Group B (OSMF non-smokers).

**Table 1:** *Demographic and clinical characteristics of the study participants*

| Parameter | Group A (OSMF Smokers) | Group B (OSMF Non-Smokers) | Group C (Healthy Smokers) | Group D (Healthy Non-Smokers) | p-value |
|---|---|---|---|---|---|
| Age (years, mean ± SD) | 38.2 ± 9.5 | 36.8 ± 10.1 | 37.5 ± 8.9 | 37.1 ± 9.2 | 0.812 |
| Gender (Male/ Female, n) | 21/4 | 20/5 | 22/3 | 21/4 | 0.918 |
| Duration of OSMF symptoms (years, mean ± SD) | 4.8 ± 2.1 | 4.5 ± 1.9 | - | - | 0.623 |
| OSMF Clinical Stage (n, %) | | | - | - | 0.034* |
| Stage I & II | 8 (32%) | 15 (60%) | | | |
| Stage III & IV | 17 (68%) | 10 (40%) | | | |

*p-value from chi-square test comparing clinical stages between Group A and B; OSMF: Oral submucous fibrosis

### Comparison of Oxidative Stress Markers

The comparative analysis of serum oxidative stress

markers among the four groups is presented in Table 2. Serum MDA levels were significantly elevated in Group A (OSMF smokers) compared to all other groups ($p < 0.001$). Group B (OSMF non-smokers) also had significantly higher MDA levels than Group C (healthy smokers) and Group D (healthy non-smokers) ($p < 0.001$).

Conversely, the antioxidant markers (SOD, GSH, and vitamin C) showed a reverse trend. The levels were significantly depleted in Group A compared to all other groups ($p < 0.001$). Group B had significantly lower antioxidant levels than both healthy control groups ($p < 0.01$). Furthermore, Group C (healthy smokers) also showed significantly lower SOD and GSH levels compared to Group D (healthy non-smokers) ($p < 0.05$), indicating the oxidative effect of smoking even in a healthy population.

**Table 2:** *Comparison of serum oxidative stress markers among the study groups*

| Marker | Group A (OSMF Smokers) | Group B (OSMF Non-Smokers) | Group C (Healthy Smokers) | Group D (Healthy Non-Smokers) | p-value (ANOVA) |
|---|---|---|---|---|---|
| MDA (nmol/mL) | 6.18 ± 1.32 | 4.82 ± 1.15 | 3.48 ± 0.81 | 2.09 ± 0.52 | <0.001* |
| SOD (U/mL) | 1.85 ± 0.48 | 2.64 ± 0.61 | 3.12 ± 0.55 | 3.78 ± 0.63 | <0.001* |
| GSH (mg/dL) | 3.21 ± 0.88 | 4.35 ± 0.92 | 5.18 ± 0.96 | 6.25 ± 1.05 | <0.001* |
| Vitamin C (mg/dL) | 0.72 ± 0.25 | 1.05 ± 0.31 | 1.28 ± 0.34 | 1.52 ± 0.38 | <0.001* |

*All pairwise comparisons between groups were statistically significant ($p < 0.05$) except where noted; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Reduced glutathione

### Correlation with Clinical Stage of OSMF

For the combined OSMF cohort (Groups A and B, n=50), a correlation analysis was performed between the oxidative stress markers and the clinical stage of the disease. As shown in Table 3, a strong, statistically significant positive correlation was observed between serum MDA levels and the clinical stage of OSMF ($r = 0.71$, $p < 0.001$). In contrast, the antioxidant markers SOD, GSH, and vitamin C demonstrated a significant negative correlation with the clinical stage, indicating that as the disease severity increased, the antioxidant defense system became more depleted.

**Table 3:** *Correlation of oxidative stress markers with clinical stage of OSMF (n=50)*

| Marker | Correlation Coefficient (r) | p-value |
|---|---|---|
| MDA | 0.71 | <0.001 |
| SOD | -0.63 | <0.001 |
| GSH | -0.58 | <0.001 |
| Vitamin C | -0.52 | <0.001 |

MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Reduced glutathione

## DISCUSSION

The present study was designed to investigate the additive effect of smoking on oxidative stress in patients with OSMF. The results unequivocally demonstrate that smokers with OSMF experience a significantly greater degree of oxidative stress compared to their non-smoking counterparts, as evidenced by the highest levels of the lipid peroxidation marker MDA and the most profound depletion of key antioxidants (SOD, GSH, and vitamin C).

The elevated MDA levels in OSMF patients (both smokers and non-smokers) compared to healthy controls corroborate the findings of numerous previous studies that have implicated oxidative stress as a central mechanism in the pathogenesis of OSMF [12, 13]. Arecoline, the principal alkaloid in areca nut, is known to generate ROS during its metabolic activation, leading to lipid peroxidation and cellular damage [14]. The observed depletion of antioxidants like SOD, GSH, and vitamin C in OSMF patients further confirms that the disease process overwhelms the endogenous antioxidant defense system, as these molecules are consumed in the process of neutralizing the excess ROS [15].

The critical finding of our study is the significant exacerbation of this oxidative imbalance in OSMF patients who smoke. Group A (OSMF smokers) had significantly higher MDA levels and significantly lower antioxidant levels than Group B (OSMF non-smokers). This suggests that the ROS generated from

tobacco smoke act synergistically with those produced by areca nut metabolism, creating a state of heightened oxidative stress. Cigarette smoke contains billions of free radicals per puff, including superoxide and nitric oxide, which directly initiate lipid peroxidation and deplete antioxidant reserves [16]. This additive oxidative burden likely contributes to the more severe clinical presentation observed in our OSMF smoker group, which had a higher proportion of patients in advanced stages of the disease.

The correlation analysis strengthens this argument. The strong positive correlation between MDA levels and the clinical stage of OSMF indicates that the degree of lipid peroxidation is directly proportional to the severity of the disease. This aligns with the pathophysiological model where persistent oxidative damage leads to increased fibroblast activity, excessive collagen deposition, and progressive fibrosis [17]. The concurrent negative correlation of antioxidant levels with disease stage further reinforces that the body's defense mechanisms are progressively compromised as OSMF advances.

The findings also have significant implications for the malignant potential of OSMF. Oxidative stress is a well-known carcinogenic process. ROS can cause DNA damage, including strand breaks and base modifications (e.g., 8-hydroxy-2'-deoxyguanosine), which, if unrepaired, can lead to mutations in oncogenes and tumor suppressor genes, initiating carcinogenesis [18]. By significantly amplifying the oxidative environment, smoking may thus accelerate the timeline for malignant transformation in OSMF patients. This provides a strong biochemical rationale for the clinically observed increased cancer risk in patients with multiple deleterious habits [19].

From a clinical management perspective, our results underscore the non-negotiable importance of smoking cessation as a primary intervention in OSMF. Counseling patients to quit smoking is not merely a general health recommendation but a critical step to reduce the oxidative burden and potentially slow disease progression. Furthermore, the findings suggest that OSMF patients who smoke may benefit from more aggressive antioxidant therapy. While the efficacy of antioxidant supplementation in OSMF is still under investigation, our study identifies a specific subgroup (OSMF smokers) that is most deficient and may stand to gain the most from such therapeutic interventions [20].

The study is not without limitations. Its cross-sectional design provides a snapshot in time and cannot establish a causal relationship. A longitudinal study tracking oxidative markers and disease progression in OSMF patients who quit smoking versus those who continue would provide more definitive evidence. Additionally, we relied on self-reported smoking history, which is subject to recall bias. Future studies could incorporate biochemical markers of smoking exposure, such as cotinine levels, for more accurate categorization.

## CONCLUSION

This study provides compelling evidence that smoking significantly exacerbates oxidative stress in patients with oral submucous fibrosis. The combination of OSMF and smoking leads to a profound pro-oxidant state, marked by elevated lipid peroxidation and severe depletion of antioxidant defenses. This heightened oxidative stress correlates with more advanced clinical stages of the disease, suggesting a role in accelerated progression and potentially increased malignant transformation risk. The findings highlight the critical need for intensive smoking cessation counseling and suggest that OSMF patients who smoke represent a high-risk group that may warrant more aggressive antioxidant-based therapeutic strategies.

## REFERENCES


1. Sarode SC, Sarode GS, Karmarkar S, Tupkari JV. A new classification for oral submucous fibrosis. J Oral Maxillofac Pathol. 2011;15(3):279-284. doi: 10.4103/0973-029X.86668. PMID: 22022049.
2. Aziz SR. Update on oral submucous fibrosis: a comprehensive review. J Oral Maxillofac Surg. 2020;78(9):1534-1545. doi: 10.1016/j.joms.2020.04.018. PMID: 32560715.
3. Jeng JH, Chang MC, Hahn LJ. Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. J Oral Pathol Med. 2001;30(8):449-464. doi: 10.1034/j.1600-0714.2001.300801.x. PMID: 11508886.
4. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis--a collagen metabolic disorder. J Oral Pathol Med. 2005;34(6):321-328. doi: 10.1111/j.1600-0714.2005.00307.x. PMID: 15910387.
5. Chaudhary M, Singh M, Kumar M, Singh N. Oxidative stress in oral submucous fibrosis: a preliminary study. J Oral Biol Craniofac Res. 2019;9(2):120-123. doi: 10.1016/j.jobcr.2018.09.003. PMID: 31104954.
6. Gupta S, Chaudhary K, Komal G, Singh K. Evaluation of oxidative stress and antioxidant status in patients with oral submucous fibrosis: a case-control study. Eur J Dent. 2020;14(2):266-271. doi: 10.1055/s-0039-3402762. PMID: 32291528.
7. Valavanidis A, Vlachogianni T, Fiotakis K. Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. Int J Environ Res Public Health. 2009;6(2):445-462. doi: 10.3390/ijerph6020445. PMID: 19440393.
8. Dietrich M, Block G, Norkus EP, Hudes M, Traber MG, Cross CE, Packer L. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intake. Am J Clin Nutr. 2003;77(1):160-166. doi: 10.1093/ajcn/77.1.160. PMID: 12499332.
9. Patil S, Rao RS, Majumdar B, Sankar K. Oxidative stress markers in oral submucous fibrosis: a pilot study. Quintessence Int. 2011;42(9):e100-e106. PMID: 21992156.
10. Khan M, Siddiqui FA, Yousuf R, Ali A. Estimation of serum malondialdehyde level in oral submucous fibrosis. J Ayub Med Coll Abbottabad. 2012;24(3-4):67-70. PMID: 25252528.
11. Haider SM, Mehrotra R, Singh A, Singh M. Clinical staging of oral submucous fibrosis: a new proposal. J Maxillofac Oral Surg. 2010;9(3):238-243. doi: 10.1007/s12663-010-0005-9. PMID: 21499557.
12. Babu S, Bhat KM. Oxidative stress and its implications in oral submucous fibrosis: a review. J Oral Maxillofac Pathol. 2013;17(3):411-415. doi: 10.4103/0973-029X.125208. PMID: 24255554.
13. Pillai R, Balachandran R, Elango V, Srinivasan M. Lipid peroxidation and antioxidant status in patients with oral submucous fibrosis. Indian J Dent Res. 2011;22(4):621-624. doi: 10.4103/0970-9290.90323. PMID: 22144813.
14. Chang MC, Ho YS, Lee PH, Chan CP, Wang YJ, Chen YJ, Hahn LJ, Jeng JH. Areca nut extract induces oxidative stress and upregulated hypoxia-inducing factor leading to autophagy in oral fibroblasts. J Oral Pathol Med. 2013;42(8):642-650. doi: 10.1111/jop.12074. PMID: 23651184.
15. Halliwell B. Oxidative stress and neurodegeneration: where are we now? J Neurochem. 2006;97(6):1634-1658. doi: 10.1111/j.1471-4159.2006.04107.x. PMID: 16873923.
16. Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann N Y Acad Sci. 1993;686:12-27. doi: 10.1111/j.1749-6632.1993.tb39143.x. PMID: 8240309.
17. Garewal HS, Katz J, Boren H, Pandya R, Sampliner RE, Prasad M, Naik R. Antioxidants in oral submucous fibrosis. A preliminary report. Indian J Cancer. 1993;30(4):168-171. PMID: 8159056.
18. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog. 2006;5:14. doi: 10.1186/1477-3163-5-14. PMID: 16722545.
19. Lee CH, Ko YC, Huang HL, Chao YY, Tsai CC, Shieh TY, Lin LM. The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. Br J Cancer. 2003;88(3):366-372. doi: 10.1038/sj.bjc.6600729. PMID: 12556963.
20. Kumar A, Ramesh V, Shukla D. Role of antioxidants in generalised oral submucous fibrosis: evaluation of clinical improvement and oxidative stress status. J Maxillofac Oral Surg. 2017;16(4):511-516. doi: 10.1007/s12663-017-0998-6. PMID: 28875068.