



## Original Article

## The Role of Lipid Peroxidation Products in the Development of Oral Sub Mucous Fibrosis- A Cross-Sectional Survey

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## ABSTRACT

Oral sub mucous fibrosis (OSMF) is a premalignant condition. Factors that cause OSMF are areca nut, nutritional deficiency and genetics. These factors produce free radicals which generate oxidative stress by damaging DNA, lipids and proteins. Levels of 4 hydroxynonenal (4HNE), 8-hydroxy-2-deoxy guanosine (8-OHdG), C reactive protein (CRP), and malondialdehyde (MDA) were raised due to oxidative DNA damage and lipid damage. **Objective:** To determine the levels of lipid peroxidation by-products such as MDA, 4HNE, 8-OHdG, and CRP in patients with OSMF. **Methods:** A cross-sectional comparative study was conducted at The University of Lahore from January 2020 to August 2021. Fifty OSMF cases and fifty healthy controls were recruited by using non-probability convenient sampling technique. Serum of participants was analyzed for lipid peroxidation byproducts by using ELISA kits. The results were analyzed by applying independent t-test in SPSS version 21.0. **Results:** The mean MDA levels in OSMF patients were  $(3.22 \pm 1.265 \text{ nmol/ml})$  as compared to those of control group  $(1.26 \pm 0.568 \text{ nmol/ml})$ . Serum CRP levels were significantly raised  $(1.23 \pm 0.124 \text{ mg/l})$  in patients of OSMF as compared to those of control group  $(1.04 \pm 0.0324 \text{ mg/l})$ . The levels of 4-HNE, 8-OHdG were recorded as significantly increased in OSMF  $(1.5 \pm 0.965 \text{ pg/ml})$  and  $(1.9 \pm 0.265 \text{ pg/ml})$  as compared to the those of control group  $(0.72 \pm 0.065 \text{ pg/ml})$  and  $(0.09564 \pm 0.00058 \text{ pg/ml})$ . **Conclusions:** It was concluded that the levels of MDA, 4HNE, 8-OHdG, and CRP were increased significantly in patients with OSMF compared to healthy controls.

## INTRODUCTION

Oral sub mucous fibrosis (OSMF) is a progressive and precancerous disease that involves the whole oral cavity and sometimes extends to different parts of neck [1, 2]. Patients with oral sub mucous fibrosis often present with discoloration of teeth and gums, limited mouth opening and burning sensation on taking spicy food. There are many factors which are associated with oral sub-mucosal fibrosis like excessive chewing of areca nut (Betel nut), nutritional deficiency and some genetic factors. There is a high tendency 7% to 30% of OSMF to be converted into malignant disorders such as oral squamous cell carcinoma [3]. One of the early clinical signs of OSMF is inflammation. Inflammation and decrease in vascularity ultimately lead to thick bands of fibrous tissue which are white in color called

“fibrosis in diseased area”. There are different stages of oral sub mucous fibrosis and severity increases as the stage increases. In last stage of OSMF, the mouth opening is limited which causes poor oral hygiene, difficulty in eating food and problems with speech. In stage-I, mouth opening is  $(>3 \text{ cm})$ , stage-II  $(2-3 \text{ cm})$  and in stage-III  $(<2 \text{ cm})$ . Fibrosis involves lips (thick and rubber like appearance), cheeks which are puffed out, tongue (unable to move due to fibrosis), soft palate, uvula and gums. Fibrosis spreads to different parts of throat such as pharynx, esophagus, eustachian tube that blocks these regions [4]. Worldwide, oral cancer is 6th most common cancer. Its occurrence rate is high in India and Southeast Asia and also in some of the Western countries. OSMF is a precancerous condition

which is converted to oral squamous cell carcinoma [5]. The chewing habit of areca nut is the most common and potent endogenous factor leading to OSMF that ultimately transforms into cancerous lesion [6, 7]. More than 80% of cases are transformed into cancer [8]. Epithelium becomes hyperplastic with the use of Betel nut. There are two mechanisms running side by side in human cancerous cells, one is the formation of free radicals such as reactive oxygen species (ROS) which generate oxidative stress and other is antioxidant defense system. Presence of an unpaired electron on a fragmented molecule is known as free radical. Superoxide anions and hydroxyl radicals are the most frequently formed free radicals [9]. ROS changes the macromolecules such as carbohydrates, lipids and proteins. ROS are produced due to the formation of byproducts and intermediate molecules in normal cellular processes. There is oxidative damage to nucleic acids such as 8-hydroxydeoxyguanosine (8-OHdG) and proteins (Protein carbonyl) [10]. Lipid peroxidation produces ROS which damage cell products and its membrane. Malondialdehyde (MDA) is a poisonous aldehyde and is a potent marker of oxidative stress produced by lipid peroxidation. Free radical formation such as 8-OHdG is one of the most commonly used biomarker for oxidative stress conditions and cancerous diseases [11]. 8-OHdG was found in oral leukoplakia and lichen planus [12]. Areca nut contains some chemicals which increase the production of ROS and DNA adducts. 4-hydroxynonenal (4HNE) is also a byproduct of lipid peroxidation is activated and causes DNA mutation [10]. Several genes are responsible for OSMF. Cytochrome P450 subtype gene is decreased in OSMF [3]. There are also some undergoing immunological processes in OSMF patient such as increased expression of HLA-A10, HLA-B7, and HLA-DR3 and CD4 cells. This leads to an increase in Langerhans cell. These cells imbalance the immune regulation by increasing the number of CD4 in OSMF leading to atrophic changes in oral epithelium. These processes are activated either due to ingestion of areca nut or autoimmune responses as a result of tissue alteration [13]. There was limited data available on the role of lipid peroxidation by products (MDA, 4-HNE and 8-OHdG) in oral sub mucous fibrosis.

The study was conducted to compare lipid peroxidation byproducts levels in patients of oral sub mucous fibrosis and control group.

## METHODS

This cross-sectional comparative study was conducted at the institute of Molecular Biology and Biotechnology, The University of Lahore, from January 2020 to August 2021 after obtaining approval from Institutional Review Board of The University of Lahore, IMBB/BBBC/24/324 and permission letter, RS-FAHS-24921/1. Sample size was

calculated using "Open Epi calculator" by keeping mean levels of MDA in OSMF group as 3.3nmol and in healthy control as 2.4nmol. Mean S.D was 0.45 and variance was 0.2025. Confidence interval was taken as 5. Power was taken as 80 [14]. The estimated sample size was 5 for each group but for better power of study was took 50 subjects in both control group and case group. Fifty dental patients (male or female above 18 years of age) visiting ULTH dental OPD from March 2020 to August 2020 having pain and burning sensation in mouth, limited mouth opening, oral ulcers, white and red patches in oral mucosa with or without depigmentation were recruited in the study after taking informed consent by using non-probability convenient sampling technique. Study participants with suspicious clinical signs of oral carcinoma were excluded. Healthy dental patients (male or female above 18 years) with no clinical features of OSMF were taken as controls. 5ml blood was taken from both cases and controls after clinical assessment. Blood was centrifuged at 4000 rpm for 10 min for serum separation. Serum was analyzed for 4HNE, 8-OHdG, 8-isoprostane and MDA by using Enzyme-linked immunosorbent assay (ELISA) (OxiSelect™) kits as per Manufacturers protocol. CRP levels were assessed by using Latex kit (Bioscien). The results were analyzed by applying independent t-test in SPSS version 21.0. Mean values and standard deviations for quantitative variables were calculated. Independent t-test was applied to determine the statistical significance of various parameters amongst two groups. Results were presented as mean  $\pm$  SD. p value  $\leq$  0.005 was considered significant and p value  $\leq$  0.001 was considered highly significant.

## RESULTS

A total of 50 participants were enrolled in each group, out of which 44 (88%) were males and 6 (12%) females in control group, whereas 46 (92%) males and 4 (8%) females were in OSMF group. The mean age of the OSMF participants was 34.6 years, with a standard deviation of 7 years. In the control group, the mean age was 33.4 years, with a standard deviation of 7 years (Table 1).

**Table 1:** Distribution and mean age and standard deviation of males and females in both groups (n=50)

Gender	Control (%)	Mean $\pm$ SD*	OSMF* (%)	Mean $\pm$ SD
Male	44 (88)	34.1 $\pm$ 8	46 (92%)	33.8 $\pm$ 7
Female	6 (12)	26.6 $\pm$ 0	4 (8%)	44.5 $\pm$ 4
Total	50 (100)	33.4 $\pm$ 7	50 (100)	34.6 $\pm$ 7

\*SD= Standard deviation \*\*OSMF= Oral sub mucous fibrosis

The data assembled in table 2 shows that serum CRP levels were significantly raised in the OSMF patients (1.23  $\pm$  0.124mg/l) as compared to those of control group (1.04  $\pm$  0.0324mg/l). The levels of 4-HNE in OSMF were also significantly raised (1.5  $\pm$  0.965pg/ml vs 2.6  $\pm$  0.845pg/ml) as compared to the healthy group. Levels of 8-OHdG were also

significantly raised in OSMF patients ( $0.09564 \pm 0.001$ pg/ml) as compared to those of control groups ( $1.9 \pm 0.27$ pg/ml). The MDA levels were significantly raised in patients suffering from oral sub mucous fibrosis ( $3.22 \pm 1.265$ nmol/ml) as compared to controls ( $1.26 \pm 0.568$ nmol/ml).

**Table 2:** Lipid Peroxidation Products in Patients of Oral Sub Mucous Fibrosis

Variables	Control (n= 50)	OSMF* (n=50)	p-Value
4-HNE (pg/ml)	$0.72 \pm 0.065$	$1.5 \pm 0.97$	0.02
8-OHdG (pg/ml)	$0.09 \pm 0.001$	$1.9 \pm 0.27$	0.02
CRP (mg/l)	$1.04 \pm 0.03$	$1.2 \pm 0.12$	0.01
MDA (nmol/ml)	$1.26 \pm 0.95$	$3.22 \pm 1.26$	0.002

\*OSMF= Oral sub mucous fibrosis

## DISCUSSION

The levels of lipid peroxidation by-products that is MDA, 4HNE, 8-OHdG, and CRP were raised in the patients having oral sub mucous fibrosis. In a scoping review conducted by Saso *et al.*, various articles were evaluated for the levels of different oxidative stress markers and antioxidants in patients with oral sub mucous fibrosis. All studies whether in vitro or human revealed that levels of oxidative stress markers increased as the disease progressed. Furthermore, the use of antioxidants decreased the levels of oxidative stress markers and improved the symptoms of patients. We checked the levels of lipid peroxidation byproducts in patients of OSMF, but did not administer any antioxidants to the patients. Future studies may be planned to see the baseline levels of antioxidants as well as the effects of administration of antioxidants on prognosis of OSMF [15]. In a study conducted by Shirzaiy *et al.*, 34 patients of OSMF of different grades were studied for association of ROS and OSMF. Plasma MDA levels were found to be significantly higher in OSMF patients. Vitamin E and beta carotenes levels were significantly reduced in these patients and oral administration of these 2 antioxidants increased their level and decreased the levels of MDA in plasma [9]. Our study had the same results regarding levels of stress markers. In a cross-sectional survey conducted by Sumithrarachchi *et al.*, 368 patients attending the dental clinic were assessed for the use of tobacco, areca nut and presence of oral lesions. This study revealed that more than 90% of tobacco users and areca nut had oral lesions. OSMF had a prevalence of 2.4%. This study highlighted the importance of awareness programs for the patients visiting dental clinics [16]. In this study, we took samples from the patients of OSMF consuming areca nut and assessed the levels of various oxidative biomarkers. Mobeen *et al.*, administered a herbal paste to 80 patients with OSMF for 3 months and assessed various subjective and objective symptoms of the patients like tongue protrusion, blanching, mouth opening, intolerance

to spicy food and burning sensation. This study showed that Gutka was being used by most of the patients with OSMF. Use of herbal paste containing turmeric, tulsi and honey significantly improved various parameters like mouth opening, tongue protrusion, burning sensations, mucosal bands and oral blanching [17]. The limitation of our study is that it did not include the symptoms and various pathological parameters of OSMF or use of any antioxidant remedy. Singh *et al.*, studied the prevalence of various oral lesions among tobacco users. The most common lesions observed were pouch keratosis in chewers and leukoplakia amongst chronic smokers [18]. In our study, all the OSMF patients were consumers of areca nut. Levels of oxidative markers like MDA, 4HNE, 8-OHdG and CRP were raised in all the patients because areca nut causes oxidation in the oral mucosa. Yashveer *et al.*, demonstrated that patients of OSMF have lower levels of serum HDL, LDL, cholesterol, VLDL, TG probably because these lipids were utilized for the synthesis of new cell membranes. They proposed that serum lipid levels may be used for the diagnosis of OSMF [19]. However, we did not measure serum lipid levels. Pant *et al.*, demonstrated that areca nut induced TGF- $\beta$  (transforming growth factor-beta) pathway to induce fibrosis in OSMF. TGF- $\beta$  is an inflammatory biomarker. This finding would help in defining the treatment strategies for OSMF [20]. We determined the levels of oxidative biomarkers that might have similar mechanistic induction of fibrosis, but we did not measure inflammatory biomarkers. Rai *et al.*, studied various intermediate products of metabolism and products of oxidative stress in 20 patients of OSMF. They demonstrated how the metabolic pathways are switched to adapt to malignant changes in epithelial cells and how these pathways are reprogrammed to fulfill the nutritional demands of malignant cells [5]. Our study could also be extended to find out the biochemical mechanisms responsible for oxidative stress and metabolic reprogramming as a result of malignant changes.

## CONCLUSIONS

This study showed that levels of MDA, 4HNE, 8-OHdG, and CRP were increased significantly in patients with oral sub mucous fibrosis as compared to healthy controls. Lipid peroxidation byproducts such as MDA, 4HNE, CRP and 8-OHdG are biomarkers of oxidative stress. Elevated levels of these byproducts in OSMF may help in the early detection of disease. These may serve as a tool to assess the severity of the disease and may be used as prognostic markers. These markers may guide the clinician for therapeutic interventions in the patient of OSMF to reduce the oxidative stress generated by these byproducts. Our study may improve patient outcome by designing a non-invasive diagnostic and prognostic tool for oral sub mucous fibrosis.

## Authors Contribution

Conceptualization: NN

Methodology: NN, MS

Formal analysis: CN

Writing-review and editing: ZH, SM, MN

All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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