

# Oxidative Stress in Oral Submucous Fibrosis- A Clinical and Biochemical Study

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## Abstract

**Background and Objectives:** A Biochemical study was undertaken to determine the effect of betel quid chewing on serum and salivary total antioxidant capacity (TAC) in Oral sub mucous fibrosis(OSMF) patients and its association with duration and frequency of habit.

**Methodology:** The study consisted of four groups, one control and three study groups, each with 15 subjects. Thorough intraoral examination was done for all the groups. Salivary and serum samples were collected from all the groups, which were further subjected for biochemical analysis. The observations of the study were subjected to statistical analysis and the results were tabulated.

**Results and Conclusion:** The level of salivary and serum TAC decreases secondary to betel quid chewing in OSMF patients. TAC values, both in saliva and serum showed negative correlation with duration and frequency of habit in OSMF patients. As the severity of OSMF increases, the level of TAC decreases.

*Key words: Oral Submucous Fibrosis, Total antioxidant capacity, Reactive oxygen species, Free radicals*

## Introduction

OSMF is a high risk precancerous condition characterized by changes in the connective tissue fibres of the lamina propria and deep parts leading to stiffness of the mucosa and restricted mouth opening. OSMF has been reported almost exclusively among Indians living in India and among other Asiatics, with a reported prevalence ranging up to 0.4% in Indian rural population. Epidemiological and in vitro experimental studies have shown that chewing areca nut (Areca catechu) is the major etiological factor for OSMF [1].

Betel quid chewing is a popular oral habit with potential links to the occurrence of oral cancer. Reactive oxygen species (ROS) produced during auto oxidation of areca nut (AN) polyphenols in the betel quid chewer's saliva, are crucial in the initiation and promotion of oral cancer [2]. Saliva is a unique fluid and interest in it as a diagnostic medium has advanced exponentially in the last 10 years. The major advantage for using saliva in diagnosis rather than blood is easy access & non-invasive collection [3]. Saliva possesses a variety of defense mechanisms responsible for the protection of the oral cavity from oxidative attacks, including uric acid, vitamin c, glutathione and others. It also inhibits the production of ROS, the superoxide free radical ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) from betel quid tobacco, the most potent inducer of oral cancer. However, as antioxidants work in concert, total antioxidant capacity (TAC) is the most relevant parameter indicating oxidative stress [4].

OSMF being a premalignant condition and associated with carcinogens is thought to be associated with ROS. Salivary antioxidant parameters are altered in OSMF. The profound effect of betel quid chewing on salivary antioxidant parameters may be of great importance in respect to the diagnosis, prognosis and evaluation of disease.

Since saliva is a non invasive, easily available diagnostic medium and the biochemical estimation is an affordable parameter, this study was undertaken to determine the use

of salivary antioxidants levels as predictors of the disease progress.

## Materials and Methods

A total no of 60 subjects reporting to the Department of Oral Medicine and Radiology were enrolled in the study. Study Group comprised of 45 individuals with the clinical diagnosis of OSMF. Study group was further divided into 3 groups of 15 each, according to the clinical grading of OSMF using validated criteria modified from different literature reviews [5,6] (Table 1).

Group S1 – 15 OSMF patients (mild).

Group S2 – 15 OSMF patients (moderate).

Group S3 – 15 OSMF patients (severe).

Control Group was comprised of 15 healthy individuals. Subjects with any other systemic diseases or taking medications or antioxidants supplements were excluded.

Subjects who were not on any medications or antioxidants supplements. Informed consent was obtained from the patients included in the study. The subjects were asked not to indulge in habitual chewing and consume any food 2 hours prior to the collection of saliva. Following a thorough mouth rinse using distilled water, saliva was allowed to accumulate in his or her

*Table 1. Literature reviews.*

Mild	Moderate	Severe
Burning sensation, vesicles ulcerations.	Burning sensation, Dryness of mouth +/-	Burning sensation, Dryness of mouth +/-
Mild blanching	Oral mucosa is blanched. Loss of mucosal elasticity.	Blanched opaque leather like oral mucosa.
Mouth opening - normal. > 40mm	Mouth opening- Slightly restricted. 21-40mm	Mouth opening- severely restricted. Mouth opening <20mm
Tongue movements - normal	Tongue movements - normal or slightly restricted	Tongue movements - severely restricted
Palpable bands absent	Palpable buccal bands	Palpable buccal and circumoral bands

mouth for 5 minutes. Accumulated saliva was collected by spit method. Intra oral examination was done & findings were recorded. Blood sample were drawn and were subjected for analysis of TAC. 2 ml of collected saliva and serum were stored at a temperature of +4°C in glass vials, which was then subjected to analysis by Phosphomolybdenum method using Spectrophotometer [7].

Data obtained were analyzed using ANOVA, Tukey’s honestly significance difference test, & Fisher’s Test using SPSS version 12 software.

**Results**

The mean salivary and serum TAC of saliva of all 4 groups represented in Tables 2 and 3, Charts 1 and 2 respectively. Values were very highly significant.

Comparison of salivary and serum TAC Group I (control) with all the three study groups (II, III, IV) was very highly statistically significant (Tables 4 and 5, Chart 3) which is suggestive of significant effect of betel quid chewing on serum and saliva. Correlation of salivary and serum TAC with duration and frequency of habit, revealed negative correlation in all the 3 study groups (Tables 6 and 7). The negative correlation of salivary and serum TAC with the duration and frequency of betel quid chewing suggest that, with the increase in duration of chewing habit, there is an increase in the production of free radicals which would probably deplete the TAC.

**Discussion**

In the field of free radical and antioxidants research, most of the studies were carried out to estimate individual antioxidant components in either serum or saliva samples and more so often in serum. But, it has been suggested that antioxidant systems act in concert rather than alone and investigation of individual antioxidant may be misleading & measurement of individual antioxidant may be less representative of the whole antioxidant status [4].

In the present study, the mean age of the OSMF was found to be 31.6 years which is in accordance with previous studies [8-11]. Majority of the patients were in the age group of 25-35 years. The prevalence of OSMF in the younger age group may be attributed to various other factors like genetic predisposition, nutrition, etc other than areca nut chewing.

It was also seen that the habit of areca nut chewing was very much prevalent in even the younger age group like students due to easy availability or peer pressure.

Inability to open mouth was the most common presenting symptom by the patients followed by the complaint of burning sensation of mouth.

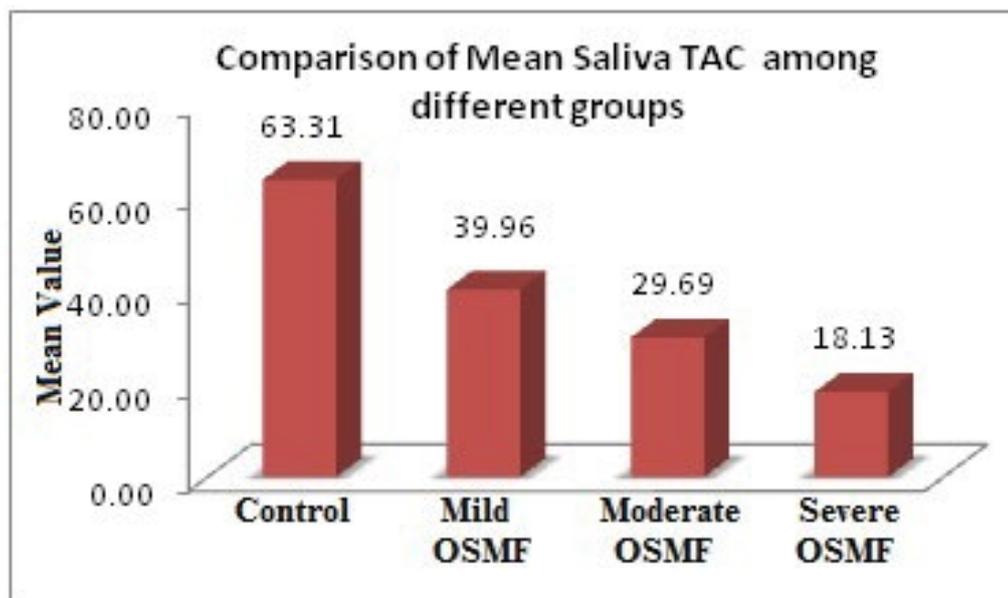
In the present study, mean salivary TAC of all study groups was significantly less than that of control subjects, suggestive of effect of areca nut chewing on salivary TAC. This could probably result from constant exposure of saliva to free radicals in OSMF patients/areca nut habitues which lead to a depletion in the TAC of saliva. This finding is in

*Table 2. Mean TAC of saliva of subjects in different groups.*

Group	N	Mean	Std deviation	Minimum	Maximum
Control	15	63.309	15.72064	42.32	90.3
Mild	15	39.958	9.14256	26.68	57.9
Moderate	15	29.69	11.64532	11.04	56.4
Severe	15	18.127	8.11797	3.68	31.2

*Table 3. Mean TAC of serum of subjects in different groups [Serum TAC] (a. F= 64.786 p< 0.001 vhs).*

Group	N	Mean	Std deviation	Minimum	Maximum
Control	15	298.947	43.23747	223.5	392.8
Mild OSMF	15	236.52	31.14249	203.6	328.4
Moderate OSMF	15	179.887	13.96285	159.1	198.5
Severe OSMF	15	169.773	15.73247	140.7	207.8



*Chart 1. Mean TAC of saliva of subjects in different groups.*

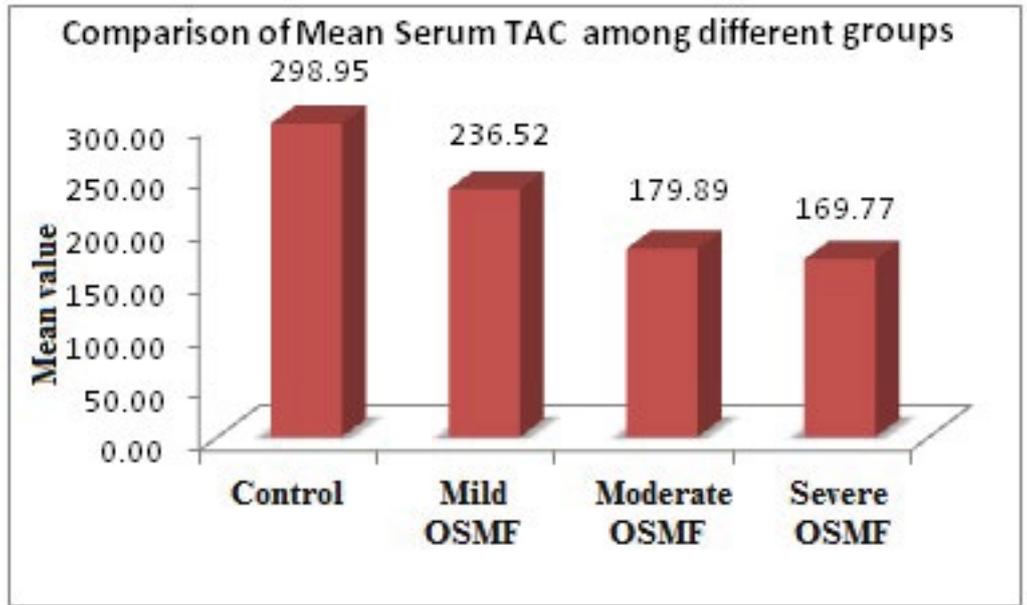


Chart 2. Mean TAC of serum of subjects in different groups.

Table 4. Multiple Comparisons of Saliva TAC among different groups ( Dependent variable: Saliva TAC, Turkey HSD).

(I) Group	(J) Group	Mean Difference (I-J)	p
Control	Mild OSMF	23.3513	0.001 vhs
	Moderate OSMF	33.6193	0.001 vhs
	Severe OSMF	45.1827	0.001 vhs
Mild OSMF	Moderate OSMF	10.268	0.082
	Severe OSMF	21.8313	0.001 vhs
Moderate OSMF	Severe OSMF	11.5633	0.039 sig

Table 5. Multiple Comparisons of Serum TAC among different groups ( Dependent variable: Saliva TAC, Turkey HSD).

(I) Group	(J) Group	Mean Difference (I-J)	p
Control	Mild OSMF	62.4267	0.001 vhs
	Moderate OSMF	119.06	0.001 vhs
	Severe OSMF	129.1733	0.001 vhs
Mild OSMF	Moderate OSMF	56.6333	0.001 vhs
	Severe OSMF	66.7467	0.001 vhs
Moderate OSMF	Severe OSMF	10.1133	0.769

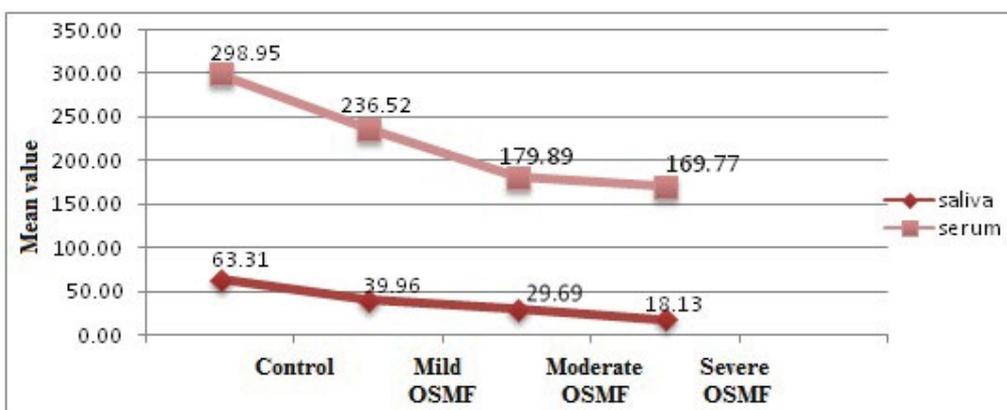


Chart 3. Mean TAC of saliva and serum.

agreement with the previous study done by Nada M et al who investigated whether a relationship possibly exists between salivary antioxidants and the development of OSMF in betel quid (BQ) chewers. Significantly decreased salivary glutathione levels were noted in OSMF patients as compared to non-BQ chewers. The findings also suggest that alterations of the salivary antioxidant system, particularly the glutathione pathway, might be of biological and diagnostic significance as related to the pathogenesis of OSMF and decrease in salivary

glutathione level might be an early event preceding and predisposing to the development of OSMF [12].

Another study was done by Rai B et al, who investigated the salivary lipid peroxidation products in different oral diseases such as OSMF, leukoplakia, candidiasis etc. It was concluded that the malonaldehyde (MDA) levels were significantly higher in OSMF, leukoplakia, cancer, periodontitis as compared to healthy subjects which is a predictor of increased oxidative stress and saliva being non-

**Table 6.** Correlation of salivary TAC with duration and frequency of habit.

Group (Saliva TAC)	Duration of habit	Frequency of habit
Mild OSMF		
r	-0.157	-0.296
p	0.576	0.283
N	15	15
Moderate OSMF		
r	-0.154	-0.052
p	0.584	0.855
N	15	15
Severe OSMF		
R	-0.066	-0.168
p	0.816	0.549
N	15	15

invasive and easy to collect, can be used to assess MDA and antioxidant status of the patients with an oral lesion. It also emphasized protective role of antioxidant supplementation in prevention of precancerous lesions and periodontal diseases [13].

In the present study, mean serum TAC of all study groups was significantly less than that of control subjects, with least in the severe form of OSMF patients reflecting the increase in free radical damage with the progression of the disease. The decrease in the TAC may be due to poor antioxidant system and excessive free radical formation leading to the utilization of antioxidants by the affected tissues or in combating the oxidative stress in circulation.

This finding is in agreement with the previous study done by Gupta S. et al who evaluated lipid peroxidation product MDA and antioxidants in plasma and erythrocytes in different grades of OSMF with equal number of healthy controls to evaluate the association of ROS and OSMF. The plasma MDA was found to be higher and plasma beta carotene and vitamin E levels were found to be decreased in patients with respect to healthy controls. It was also observed that administration of nutrient antioxidants may have protecting effect with clinical improvement [14].

A study was done by Uikey et al. to estimate the serum status of antioxidants, superoxide dismutase and glutathione peroxidase in OSMF patients. The results suggested that low values of superoxide dismutase and glutathione peroxidase may be associated with development of carcinoma in OSMF patients and its estimation may be used for prediction of disease progress [15].

Another study was done by Metkeri et al. by enrolling 40 OSMF patients and 40 healthy subjects to evaluate serum malondialdehyde, superoxide dismutase and Vitamin A. It was found that there was increase in level of malondialdehyde and decrease in superoxide dismutase and vitamin A levels among different grades of OSMF patient's and they concluded that lipid peroxidation increases with severity of diseases reflecting the extent of tissue damage [7].

A similar study was done by Raina et al. who estimated levels of beta carotene in 100 patients with OSMF. It was

**Table 7.** Correlation of serum TAC with duration and frequency of habit.

Group (Serum TAC)	Duration of habit	Frequency of habit
Mild OSMF		
r	-0.144	-0.072
p	0.608	0.799
N	15	15
Moderate OSMF		
r	-0.287	-0.139
p	0.299	0.62
N	15	15
Severe OSMF		
R	-0.022	-0.233
p	0.937	0.404
N	15	15

found that Serum beta carotene levels were below the normal in all the three grades of OSMF lowest being in grade 3 [16].

Hence, the present study shows that both saliva and serum can be used to monitor the total antioxidant level in OSMF patients.

The present study showed negative correlation of salivary and serum TAC with duration and frequency of areca nut chewing. However, it was not statistically significant probably because of smaller sample size.

Negative correlation might have caused by the fact that, with the duration of OSMF, there is an increase in the production of free radicals which would probably deplete the TAC as has been reported in previous literature [14-16].

The present study adds to the existing literature as it is the first to examine salivary and serum total antioxidant capacity in OSMF patients based on their clinical grading and also correlating the salivary and serum TAC in the same. It also helps in estimating the extent of the free radical damage with duration and frequency of areca nut chewing.

From the clinical standpoint, it can be stated that salivary TAC can be a useful marker to assess the oxidative stress, as an indicator of progression of OSMF. Further, it can be used to motivate the OSMF patients to refrain from the habit.

## Conclusion

In conclusion, the findings of this study strengthen the need for extensive research to identify the potential relationship between depletion of TAC in saliva and serum of OSMF patients. As most of the studies in the field of free radical research have been done in serum, more attention should be given to salivary antioxidant studies in OSMF patients. Since Antioxidant systems appear to act in concert rather than alone, measurement of individual antioxidant may be less representative of the whole antioxidant status. More emphasis should be given for studies which estimate salivary TAC in OSMF patients. Biochemical investigations are of prime importance because of their advantages such as simplicity, less invasiveness, less time consuming and comparatively economical. So more emphasis should be given for biochemical investigations in OSMF patients.

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