

## Estimation Of Salivary Superoxide Dismutase Level In Oral Submucous Fibrosis: A Clinical And Biochemical Study

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### ABSTRACT:

**Objective:** The objective of the study was to compare the salivary Superoxide dismutase (mU/L) level among stage one patients of Oral sub mucous fibrosis and healthy controls.

**Study Design and Setting:** It was a comparative cross sectional study design conducted at Outpatient clinic of Dental Department at Ziauddin University Hospital from January 2012 till December 2013.

**Methodology:** Eighty histo-pathologically confirmed patients of clinical stage one Oral Sub mucosal fibrosis (OSMF) and eighty healthy controls were matched for age and gender to recruit in the study. Socio demographic information (i.e. age, gender, ethnicity, education and occupation), oral health status, measurement of mouth opening (mm) and Superoxide dismutase (mU/L) were recorded. The study was conducted after the approval granted from the ethical review committee of Ziauddin University Hospital. Data was entered and analyzed using SPSS version 21 (IBM).

**Result:** The standard mean (+/-SD) of Superoxide dismutase (mU/L) in both groups was 4.99 (+/-2.18). The mean of Superoxide dismutase (mU/L) in confirmed cases of oral sub mucosal fibrosis was 3.02 (+/-0.44) which was significantly lower as compared to the control group which was 6.96(+/-1.24) and the calculated p value was 0.001. The strong positive correlation was identified in mouth opening (mm) and superoxide dismutase (0.842).

**Conclusion:** It was concluded that the levels of salivary superoxide dismutase was significantly lower in the sample of OSMF and lower levels of superoxide dismutase (mU/L) was the indication of decrease in mouth opening among the cases of OSMF. Therefore, salivary superoxide dismutase can be used as a biomarker for the early detection as well as successful treatment of OSMF.

**Keywords:** Free radicals, Lipid peroxidation, mouth opening, oral submucosal fibrosis (OSMF), salivary, Superoxide dismutase (SOD).

### INTRODUCTION:

Oral sub mucosal fibrosis (OSMF) being a chronic, progressive and irreversible disease.<sup>1</sup>The common features associated with OSMF are blanching (marble like appearance) due to impairment in local blood vessels along with the stiffening and fibrosis of any part of the oral cavity.<sup>2</sup> Oral sub mucosal fibrosis is characterized by progressive fibrosis of oral mucous membrane involving soft palate, buccal and lips mucosa, and anterior pillar of fauces.<sup>3</sup> Decrease mouth opening is an adverse clinical outcome associated with OSMF.<sup>3</sup>

Oral cancer is a significant public health concern and account for 2-4% of all malignant tumors worldwide.<sup>4</sup>It is a significant

wide reaching health concern in Asia as well. In Southeast Asia, oral cancer accounts for 40% of all cancers.<sup>5</sup>The epidemiological findings indicated as this disease is a concern in South Asia. The inhabitants of the urban population of India reported the prevalence of 0.2% to 1.2% at the dental practices.<sup>6-7</sup>Multiple cases have also been reported among Pakistani population. A retrospective analysis of Karachi population evidenced that oral cancer is the second most common cancer in both genders.<sup>8</sup>

Tobacco intake induces generation of free radicals and reactive oxygen species (ROS).<sup>9</sup>Lipid peroxidation is produced by free radicals and responsible for oxidative degradation of lipids, the end product of lipid peroxidation can be carcinogenic or mutagenic.<sup>10</sup>Superoxide dismutase (SOD) is an antioxidant acts as a defense mechanism by restricting the initiation of free radical chain reactions.<sup>11</sup> At a very initial stage the SOD inactivates the superoxide ion by transforming it into the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) which is being catabolized into dioxygen (O<sub>2</sub>) and water (H<sub>2</sub>O) by the action of catalase and peroxidase.<sup>11</sup>The production of hydrogen peroxide by the action of SOD triggers the antioxidant defence mechanisms; thereby SOD acting as a key enzyme of natural defense against free radicals.<sup>12-15</sup>

Despite the high prevalence of OSMF in Pakistan, with a rising trend and potential to undergo malignant transformation, OSMF has not been widely investigated

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with respect to lipid per oxidation and antioxidants. Moreover, previous studies have estimated the level of superoxide dismutase (mU/L) from the blood samples. Considering, saliva sample as a cost effective, non-invasive and associated with low discomfort and fear; the present study was conducted to compare the Superoxide dismutase (mU/L) levels in the cases of OSMF stage 1 and the healthy controls.

#### **METHODOLOGY:**

A comparative cross sectional study was conducted and the participants were recruited from the outpatient clinic of Dental department, Ziauddin University Hospital Karachi from January 2012 till December, 2013. Eighty cases and eighty controls were matched by following similar criteria of age and gender. OSMF cases were histo-pathologically confirmed as a clinical stage 1 Oral Sub mucosal fibrosis (OSMF). The study was conducted after the approval from the ethical review committee of Ziauddin University Hospital. Written informed consent was obtained from all participants prior to execute the study. The participants were completely briefed about the purpose of the research and procedures involved. The study was conducted according to the ethical guidelines of Helenski declaration and Pakistan Medical research Council (PMRC). Anonymity and confidentiality of the study participants were maintained throughout the research.

The subjects with age greater than 18 years, either gender and had addiction of chewing habits (i.e. pan, ghutka and betel nuts) were recruited in this study. Pregnant women and patients with inflammatory conditions i.e. arthritis and periodontal inflammation or with any systemic illness and the subjects which received any prior therapy for OSMF were also excluded. The data was recorded on a pre-designed proforma. Socio demographic information included (i.e. age, gender, ethnicity, education and occupation) were recorded. In addition, oral health status (i.e. burning sensation, addiction or chewing habits, duration and frequency of addiction and habits, brushing frequency) and mouth opening (mm) and Superoxide dismutase (mU/L) were recorded for cases and controls. Early morning saliva samples were obtained from the study participants before that mouth was rinsed with water thoroughly, and this same water was collected in the sterilized container in which PBS solution was dropped for the maintenance of PH. All these samples were kept in storage at temperature of minimal 80°C. Finally using the Superoxide dismutase level Kit assay, salivary superoxide dismutase (mU/L) level was estimated.

Data was entered and analyzed using SPSS version 21 (IBM). Once the data was entered in the analytical software it was weighted twice for incorrect entries. Qualitative or categorical data was presented as frequency and percentage while quantitative data was presented as mean  $\pm$  standard deviation. Qualitative variables were compared between cases and controls using chi square statistics. If the

assumptions of chi square statistics were not satisfied Fisher exact test was used. Independent t test was used to compare the quantitative variables between cases and controls. Correlation of addiction duration, habit frequency and mouth opening with Superoxide dismutase (mU/L) were performed and correlation co-efficient were reported. For inferential statistics p-value  $< 0.05$  was considered significant.

#### **RESULTS:**

The table 1 gives details of the comparison of socio demographic characteristics of cases and controls enrolled in this study. There was no significant difference in mean age in years, gender, ethnicity, and occupation between cases and controls. However, significant difference was found in mean education years, with controls having higher mean years of education (10.65 years) as compared to cases (9.25). Similarly, significant difference was also found in education year categories with greater proportion of controls (45%) attained thirteen or more years of education as compared to cases (15%). The table 2 gives details of the comparison of burning sensation, addiction (chewing habits), duration of addiction in years, habits frequency, brushing frequency and mouth opening (mm) between cases and controls. Significant difference was found in burning sensation, addiction, duration of addiction in years, habits frequency, brushing frequency and mouth opening between cases and controls. Greater proportion cases (67.5%) had burning sensation compared to controls (2.5%) with p-value = 0.001. Moreover, among eighty cases, around sixty three percent were addicted to Ghutka compared to only around thirty three percent among controls. The mean addiction duration in cases and controls were (6.55 Vs. 3.80; p-value = 0.001) with mean frequency habit significantly higher among cases (5.43) compared to controls (3.38). The brushing frequency was significantly lower among cases compared to controls (1.75 Vs. 1.98; p-value = 0.001). Importantly, the overall mean mouth opening among participants enrolled was 36.66 mm, however cases had significantly lower mouth opening (31.10) compared to controls (42.23) with p-value = 0.001. No cases had mouth opening greater than 45 mm compared to around eighteen percent controls in the similar category. The Figure 1 showed comparison of mean Superoxide dismutase (mU/L) between cases and controls. The overall mean (SD) of Superoxide dismutase (mU/L) for participants enrolled were 4.99 (2.18). Cases with confirmed oral sub mucosal fibrosis had mean/SD of Superoxide dismutase (mU/L) as 3.02(0.44) which was significantly lower compared to controls 6.96 (1.24); the difference was significant with p-value = 0.001. The table 3 give details of correlation of addiction duration (years) and habit frequency with Superoxide dismutase (mU/L). For the participants enrolled in this study moderate negative correlation existed between addiction duration in years with Superoxide dismutase (- 0.303); moderate negative correlation existed between habits frequency with Superoxide dismutase (-0.460); and strong positive correlation between mouth

opening (mm) with Superoxide dismutase (0.842). Significant weak positive correlation also existed between addiction duration (years) with Superoxide dismutase both among cases (0.274) and controls (0.333).

**DISCUSSION:**

The present study findings highlighted that cases with confirmed oral submucosal fibrosis (OSMF) had lower mean Superoxide dismutase(mU/L) levels as compared to controls.

Socio demographic Characteristics	Cases (n = 80)	Controls (n = 80)	Total (n* = 160)	P-value
Age in years	22.53 ± 2.76	23.03 ± 3.67	22.78 ± 3.25	0.332
Age Categories				
< 20 years	8 (10)	8 (10)	16 (10)	0.016
20-25 years	64 (80)	50 (62.5)	114 (71.2)	
>25 years	8 (10)	22 (27.5)	30 (18.8)	
Gender				
Male	46 (57.5)	54 (67.5)	100 (62.5)	0.253
Females	34 (42.5)	26 (32.5)	60 (37.5)	
Ethnicity				
Urdu speaking	20 (25)	26 (32.5)	46 (28.8)	0.157
Sindhi	22 (27.5)	14 (17.5)	36 (22.5)	
Punjabi	18 (22.5)	20 (25)	38 (23.8)	
Balochi	16 (20)	10 (12.5)	26 (16.2)	
Pathan	4 (5)	10 (12.5)	14 (8.8)	
Education years	9.25 ± 3.50	10.65 ± 3.78	9.95 ± 3.70	0.016
Education Categories				
= 5 years	24 (30)	12 (15)	36 (22.5)	0.001
6-12 years	44 (55)	32 (40)	76 (47.5)	
= 13 years	12 (15)	36 (45)	48 (30)	
Occupation				
Skilled	48 (60)	50 (62.5)	98 (61.2)	0.871
Unskilled	32 (40)	30 (37.5)	62 (38.8)	

Table 1: Comparison of socio demographic characteristics of Cases and Controls

Oral health status	Cases (n = 80)	Controls (n = 80)	Total (n = 160)	P-value
Burning Sensation				
Yes	54 (67.5)	2 (2.5)	56 (35)	0.001
No	26 (32.5)	78 (97.5)	104 (65)	
Addiction (Chewing habits)				
Pan	10 (12.5)	8 (10)	18 (11.2)	0.001
Ghutka	50 (62.5)	26 (32.5)	76 (47.5)	
Betel nuts	20 (25)	46 (57.5)	66 (41.2)	
Addiction duration (years)	6.55 ± 4.20	3.80 ± 1.17	5.18 ± 3.37	0.001
Habits frequency (packets/ day)	5.43 ± 2.26	3.38 ± 1.17	4.40 ± 2.16	0.001
Brushing frequency	1.75 ± 0.44	1.98 ± 0.27	1.86 ± 0.38	0.001
Brushing frequency Categories				
Once a Day	20 (25)	4 (5.1)	24 (15.2)	0.001
Twice a Day	60 (75)	74 (94.9)	134 (84.8)	
Mouth opening (mm)	31.10 ± 2.48	42.23 ± 3.48	36.66 ± 6.34	0.001
Mouth opening (mm) Categories				
26-35 mm	76 (95)	2 (2.5)	78 (48.8)	0.001
36-45 mm	4 (5)	64 (80)	68 (42.5)	
> 45 mm	0 (0)	14 (17.5)	14 (8.8)	

Table 2: Comparison of Oral health status of Cases and Controls

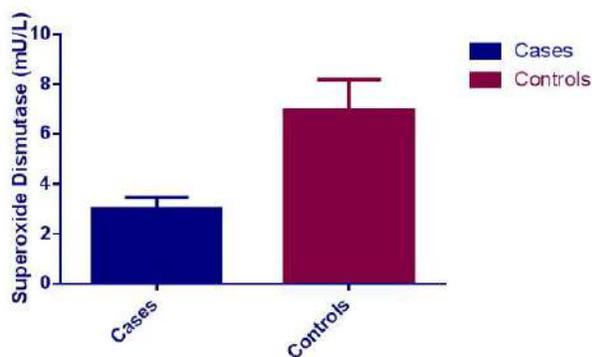


Figure 1: Comparison of Superoxide dismutase (mU/L) between Cases and Controls

Oral health status	Cases (n = 80)	Controls (n = 80)	Total (n = 160)
Addiction duration (years)	0.274*	0.333**	- 0.303**
Habits frequency (packets/day)	-0.034	0.015	- 0.460**
Mouth opening (mm)	0.096	0.024	0.842**

Table 3: Correlation of Addiction duration (years) and Mouth Opening (mm) with Superoxide dismutase (mU/L) among Cases and Controls

Moreover, strong negative correlation between addiction duration in years and habits frequency with SOD was found, thereby indicating increase in addiction duration as well as habit frequency leads to decrease in superoxide dismutase (mU/L) levels. Moreover, strong positive correlation was found between mouth opening and SOD, thereby indicating that increase in Superoxide dismutase (mU/L) levels leads to increase in mouth opening.

In the present study; it was identified that cases with OSMF had mean (SD) Superoxide dismutase (mU/L) as 3.02 which was significantly lower compared to controls 6.96 with the difference being highly significant. The results are consistent with the evidence in the literature. A case control study conducted in a tertiary care setting of India that recruited forty cases (patients with OSF) and forty controls reported that SOD (mU/L) levels were significantly lower among cases compared to controls.<sup>16</sup> The study reported that patients with OSF had mean SOD of 2.46 mU/L while controls had significantly higher mean SOD of 3.46 mU/L.<sup>16</sup> Another clinical study reported a significant decrease in SOD (mU/L) levels in OSF, oral leukoplakia and oral cancer group as compared to the control group.<sup>17</sup> Another clinical and biochemical study reported that SOD (mU/L) levels were significantly lower in cases as compared to controls.<sup>18</sup> A recent study in which venous blood sample was collected to estimate the SOD levels using an ultraviolet spectrophotometer and revealed 204.2 nmol/dl mean serum SOD levels in healthy volunteers whereas in OSMF group

mean serum SOD was 82.7 nmol/dl; and the estimated difference was highly significant.<sup>19</sup> The similar study also reported that increase in staging of OSF the mean serum SOD levels significantly decreases thus exhibiting disease progression being associated with decrease in serum SOD levels. The mean serum SOD level for OSF stage 1 (123.4 n/ mol), for OSF stage 2 (88.9 n/ mol) and least for OSF stage 3 (67.7 n/ mol).<sup>19</sup>

The enzymatic as well as non enzymatic antioxidants scavenge lipid peroxidation byproducts formed both under physiological as well as pathological conditions.<sup>20-21</sup> The decrease in SOD levels in OSF can be accounted due to the utilization of these antioxidants by tissues being affected or combating excessive oxidative stress in circulation.<sup>21-24</sup> Thereby, SOD can be a potential biochemical index for evaluating the disease progress.<sup>25-27</sup>

The study had certain limitations. Firstly, cases i.e. of stage one oral sub mucosal fibrosis were recruited only. Secondly, the sample size was limited and was a single centered study. participants were recruited from only Therefore, it is recommended that comparative cross sectional study with greater number of cases and controls should be conducted in future being from multiple clinical sites.

**CONCLUSION:**

From the present study it was evident that Superoxide dismutase (mU/L) levels were significantly lower among patients with oral submucosal fibrosis. Moreover, progressively decrease in Superoxide dismutase (mU/L) levels leads to decrease in mouth opening among patients with OSMF. Thus, salivary Superoxide dismutase can be used as a biomarker for the early detection as well as successful treatment and management of OSMF, thereby arresting it at an early stage and reducing the possible consequences of malignant transformation.

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