International Journal of Innovative Research in Medical Science (IJIRMS)
Volume 02 Issue 07 July 2017, ISSN No. – 2455-8737
Available online at - www.ijirms.in

Open Access Journal | Research Article | DOI: 10.23958/ijirms/vol02-i07/01

Evaluation of Antioxidant status in Oral Submucous Fibrosis: A Hospital Based study

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Abstract:

Purpose: *- Measurement of activity of Glutathione peroxidase (Gpx) and Superoxide dismutase (SOD) in oral submucous fibrosis.*

Methods: *- For the study comprising total 128 cases suffering from oral submucous fibrosis were selected. All patients were clinically and histopathologically diagnosed. A total of 100 age and sex matched healthy subjects taken as control. The circulating levels of SOD and GPx activity were assayed in the in the serum of control group and in patients with oral submucous fibrosis.*

Results: *- Mean SOD and Gpx activity in serum were significantly decreased in oral submucous fibrosis patients as compared to control ($p<0.001$).*

Conclusion: *- **In** the present study I concluded that the activity of SOD and Gpx were significantly decreased found in oral submucous fibrosis patients because reactive oxygen species (ROS) are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) control their accumulation. Oxidative stress caused by increased free radical generation and decreased antioxidant level in the target cells and tissues has been suggested to play an important role in oral submucous fibrosis. In this study antioxidant enzyme SOD with and GPx shows positive correlation.*

Keywords: *- **oral sub mucous fibrosis, superoxide dismutase, antioxidant, reactive oxygen species, Glutathione peroxidase Stomatitis, Fibrosis and Sequelae of oral sub mucous fibrosis***

Introduction:

Oral sub mucous fibrosis (OSMF) is a chronic debilitating disease of the oral cavity characterized by inflammation and progressive fibrosis of the sub mucosal tissues. OSMF results in marked rigidity and an eventual inability to open the mouth. OSMF is a chronic and progressive disease and potentially malignant condition involving oral mucosa. It is characterized by fibrosis of the soft-tissue resulting in rigidity and progressive inability to open the mouth. The condition has been shown to be precancerous and carries a high relative risk for malignant conversion even after the control of tobacco use, which is known to play a major role in the development of the disease.[1] It may be genetic, viral infection and may be due to by environmental or social effects.

In India, the incidence rate of OSMF is 4 per 1000 adults were found. About 5 million young Indians suffer from OSMF as a result of increasing tobacco as well as tobacco containing pan chewing is main cause; this disease is increasing rapidly over a period of time. The OSMF begins with a burning sensation or intolerance to spicy foods.[2] In most OSMF patients, areca nut were chewed alone more frequently than it was chewed in combination with pan i.e. betel leaf plus lime plus betel catechu, with or without tobacco or had higher areca nut content.[3]

Symptoms of OSMF

a. Xerostomia
b. Recurrent ulceration
c. Pain in the ear
d. Nasal intonation of voice
e. Thinning and stiffening of the lips
f. Pigmentation of the oral mucosa
g. Dryness of the mouth and burning sensation
h. Decreased mouth opening and tongue protrusion

Clinically OSMF mainly divided into three stages[4]

1. Stage 1: Stomatitis
2. Stage 2: Fibrosis
3. Stage 3: Sequelae of oral sub mucous fibrosis

Epidemiological studies have shown that the process of carcinogenesis occurs by generation of Reactive Oxygen

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Species (ROS); which act by initiating lipid peroxidation (LPO). Prevention against LPO mediated damage is done by antioxidants like β-carotene, Vit E, Superoxide dismutase (SOD) glutathione peroxidase (GPx).[5] Antioxidants means a substance which is present at very low concentration inhibits the oxidation of any molecule. Oxidative stress caused by increased free radical and decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis. The harmful effects of free radicals obtained in metabolism are inactivated by antioxidants.[6] Free radicals are molecules that are extremely reactive and either donate or extract electrons from neighboring molecules.[7] Aerobic organisms have an antioxidant defense system that neutralizes these free radicals. This system includes both enzymes and non-enzymatic antioxidants that play an important role in scavenging these free radicals. Antioxidants present in cells, function to prevent the damage done by oxidative stress. SOD and GPx are the two major enzymatic antioxidant defense systems responsible for scavenging free radicals and nascent oxygen. Antioxidant enzymes catalyze decomposition of ROS. Redox modulation is seen by distinctive changes in the activities of these enzyme systems in oxidative stress. Thus, an overall balance between production and removal of ROS may be more important in OSMF and various cancers including OSCC.[8] Therefore the present study was carried out to evaluate the status of SOD and GPx in OSMF patients.

MATERIAL AND METHODS

I. Selection of Patients

Present study comprising total 128 cases (89 males and 39 females) of OSMF. All patients were clinically and histopathologically diagnosed. For control total 100 normal healthy age and sex matched persons were selected.

II. Collection of samples

Overnight fasting venous 5ml blood samples were collected in plain bulb. Serum was separated and used to estimate SOD and GPx in OSMF and control subjects. Serum GPx activity measured by, using Hafeman D G, et al. method[9] and For Estimation of Superoxide dismutase use Mishra H. P., Fridovich I, et al., method.[10]

Inclusion criteria

- Patients clinically and histopathologically diagnosed with oral sub mucous fibrosis.
- Patients not on any treatment for the same
- Patients agreed for the hematological examination
- Normal subjects without any oral lesions and systemic diseases.

Exclusion criteria

- Patients below the age of 18 and above 55 years
- Patients suffering from any systemic diseases like diabetes, hypertension, cardiovascular diseases, renal dysfunction, or liver disorders

III. Data Analysis

Data were expressed as mean ±SD. Mean values were assessed for significance by unpaired student – t test. A statistical analysis was performed using the Statically Package for the Social Science program (SPSS, 21.0). Frequencies and percentages were used for the categorical measures. Probability values $p < 0.05$ were considered statistically significant.

TABLE1: Distribution for control and OSMF patients on the basis of Characteristics

Characteristics		Control group	OSMF Patients
Sex	Male	**73**	94
	Female	**27**	34
Age Range		34.61± 8.49	37.48± 11.53
Habits	Tobacco Chewers	63	**72**
	Pan Chewers	13	**17**
	Both Chewers	24	**39**
Affected Site	Tongue	-	**57**
	Soft palate and uvula	-	**38**
	labial mucosa	-	**24**
	Buccal mucosa	-	**09**
Stage	Stage I	**Nil**	**86**
	Stage II	**Nil**	42

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Observation and Results

The mean age of control group and OSMF patients was 34.61± 8.49 and 37.48± 11.53 Years respectively, this indicates that the subject population mostly being affected. Control group consist 73 (73%) males and 27 (27%) females and in OSMF patients group consisted of 94 (73.43%) males and 34 (26.56%) females, shown in above table no1.

We observed gender distribution in study patients both stomach and esophagus cancer cases among control group as shown in below table no 2.

Table 2: Distribution of genders in study subjects and control group

Sex	Control (n=42)		OSMF patients Stage I		OSMF patients Stage II	
	Frequency	%	Frequency	%	Frequency	%
Male	73	73	65	75.58	29	69.04
Female	27	27	21	24.41	13	30.95
Total	100	100	86	100	42	100

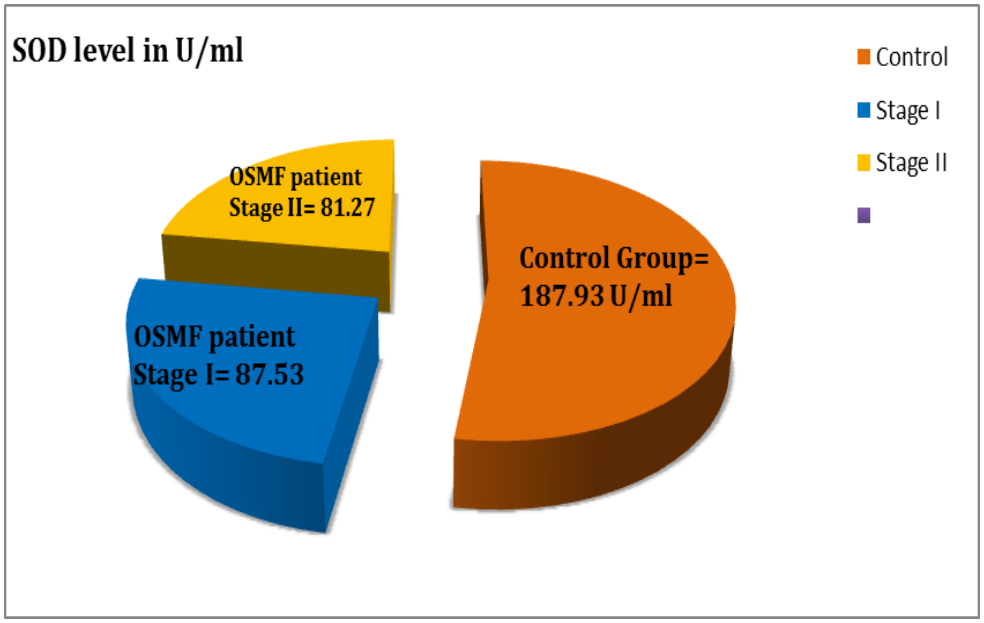
The mean± SD level of SOD in control, OSMF stage I and OSMF stage II group was 187.93 ± 31.27 U/ml, 87.53 ± 22.48 U/ml and 81.27 ± 18.93 respectively. This study shows that the similar trend of antioxidant enzyme deprivation a marker of failing defense oxidative stress A highly significantly decreased (P<0.001) levels of SOD (87.53 ± 22.48 U/ml and 81.27 ± 18.93 U/ml) were found in OSMF patients in both stages than control group (187.93 ± 31.27 U/ml). The mean± SD GPx level in control group was 7.81 ± 0.45 U/mg of Hb, in stage I of OSMF patients was 3.89 ± 0.68 U/mg of Hb and in stage II of OSMF patients was 3.18 ± 0.91 U/mg of Hb respectively. Tables 2 show a statistically significant (P < 0.001) decreased level of SOD and GPx in OSMF patients (stage I and Stage II) shown in table no 3.

Table 3: Comparison of mean SOD and GPx levels between OSMF patients and control groups

Biomedical Parameters	Control Group		OSMF patient Stage I		OSMF patient Stage II		"P" value	Significance
	Mean	SD	Mean	SD	Mean	SD		
SOD U/ml	187.93	31.27	87.53	22.48	81.27	18.93	< 0.001	Highly
GPx U/ mg of Hb	7.81	0.45	3.89	0.68	3.18	0.91	< 0.001	Highly

Table 3- Shows the SOD and GPx activity was statistically significantly lower in patients with OSMF than control group. The 128 of 128 patients of OSMF had lowered value of serum SOD and GPx.

Graph I: Comparative level of SOD in Control group and OSMF patient group (Different stages).



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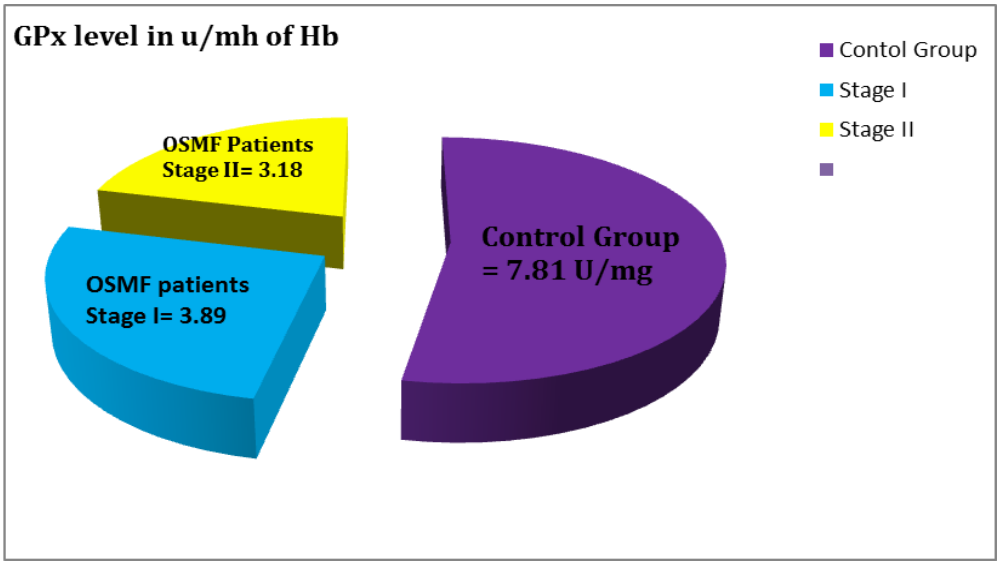
Graph II: Comparative level of GPx in Control group and OSMF patient group (Different stages).



Discussion

The present study was carried out in the Dept. of Biochemistry in collaboration with Dept. of ENT Chandulal Chandrakar Memorial Medical College and Hospital Kachandur, Durg. Serum sample obtained from 128 OSMF patients (94 male and 34 female) admitted for evaluation & treatment were analyzed for the assay of superoxide dismutase (SOD), Glutathione peroxidase (GPx), and routine investigation.

The present study groups consisting 94 male and 34 females; who had tobacco, areca nut, betel quid chewing, alcohol consumption, and other habits. Earlier studies have shown that these habits have clastogenic and carcinogenic effects.[11] The fundamental hypothesis is, free radicals damage the cellular materials, which would result in triggering or transforming normal cells into malignant ones. But, the magnitude of such damage is dependent on the body's defense mechanism, which is mediated by various cellular antioxidants. The two verified mechanisms favoring radical alteration of ROS metabolism in cancer cells are production of huge amounts of ROS compared with non-neoplastic cells and suppression of antioxidant system.[12] Antioxidant enzymes such as SOD and GPx can directly counterbalance the oxidant attack and protect the cells against DNA damage. Superoxide dismutase is a decisive antioxidant enzyme in aerobic cells, which is responsible for the elimination of superoxide radicals. SOD converts two toxic species: Superoxide and hydrogen peroxide (H2O2) into water. This diminishes the toxic effects of superoxide radical and other radicals formed by secondary reactions. GPx is a selenocysteine – dependent enzyme. GPx in cells is the most important hydrogen peroxide (H2O2) scavenging enzyme.[13]

The present study shows, a statistically significant decrease in SOD and GPx levels were observed in OSMF patients, in comparison with control group ($P < 0.001$). This finding was in accordance with studies done by Uikey et al.[14] and Gupta et. al.[15]. Thus, this study forms an archetype; for it correlates the antioxidant enzyme status between patients with a pre-malignant condition and lesion. Oral leukoplakia is caused due to tobacco; mainly by smoking. The sustained inhalation of ROS for a prolonged duration in the gas and tar phases of tobacco imposes an oxidative stress. Hemalatha et al.[16] and Khanna et al.[17] have clearly showed the use of tobacco suppressed the production of the antioxidant enzymes like SOD and GPx, which was evident among the smokers than the non-smokers. Patel et al.[18] showed risk of oral cancer development in habitual controls with lower antioxidant enzymes, lower oxidative stress markers, and higher lifetime tobacco exposure. Therefore, in patients having tobacco, betel quid, pan and other addictive habits, the equilibrium between oxidative stress and antioxidant enzyme is adversely affected. A close inter-networking between genetic susceptibility, tobacco usage, and oxidative stress can synergistically induced carcinogenesis in such patients.

Conclusion

In the present study I concluded that the activity of SOD and Gpx were significantly decreased found in oral sub mucous fibrosis patients because reactive oxygen species (ROS) are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) control their accumulation. Oxidative stress caused by increased free radical generation and decreased antioxidant level in the target cells and tissues has been suggested to play an important role in oral sub mucous fibrosis. In this study

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antioxidant enzyme SOD with and GPx shows positive correlation. These antioxidant enzymes might also serve as a therapeutic targets and a guide for prognosis in patients suffering from OSMF. Furthermore studies with larger number of sample size of OSMF patients with different clinical stages and follow-up are needed to ascertain the actual role of these biochemical parameters in the initiation and promotion of OSMF.

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DOI: 10.23958/ijirms/vol02-i07/01