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Original Article

# Assessment of Serum Antioxidant Enzymes Superoxide Dismutase (SOD) and Glutathione Peroxidase in Oral Submucous Fibrosis

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#### ABSTRACT:

**Background:** OSMF is a potentially malignant disease that results in alteration in Superoxide dismutase (SOD) and glutathione peroxidase levels. The present study was conducted to determine Superoxide dismutase (SOD) and glutathione peroxidase level in patients with OSMF. **Materials & Methods:** The present study was conducted on 60 subjects with OSMF of both genders. Equal number of controls was also included. In all subjects, Superoxide dismutase level and Glutathione peroxidase assay level was measured **Results:** Both group I and II comprised of 60 subjects each. The mean level of Superoxide dismutase (SOD) in group I was 102.3 U/ml and in group II was 204.7 U/ml. The mean glutathione peroxidase (GPx) level in group I was 24.6 U/g Hb in group I and 62.5 U/g Hb in group II. **Conclusion:** There was reduction in both Superoxide dismutase & glutathione peroxidase as compared to control subjects. **Key words:** Glutathione peroxidase, OSMF, Superoxide dismutase.

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#### INTRODUCTION

Oral submucous fibrosis (OSMF) is a potentially malignant disease of the oral cavity. It is defined as insidious, chronic disease that affects any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by, or associated with, formation of vesicles, it is always associated with a juxtaepithelial inflammatory reaction followed by fibroelastic change of the lamina propria and epithelial atrophy that leads to stiffness of the oral mucosa and causes trismus and an inability to eat.<sup>1</sup>

In normal cells there is an intricate balance between prooxidant and antioxidant states but in oxidative stress this balance shifts towards pro-oxidants. The imbalance between pro-oxidants and antioxidants linked to decreased smoke related antioxidant capacity and increased free radical generation especially in arterial tissue might render smokers more prone to peroxidative stress.<sup>2</sup> A number of compounds and enzymes may function to protect cellular components from oxidative damages. The major antioxidant defense system consisting of Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) appears to be responsible for scavenging free radicals and nascent oxygen.<sup>3</sup>

Reactive oxygen metabolites (ROMs) such as superoxide anion (O2), hydrogen peroxide (H2O2) and hydroxyl radical (OH), malondialdehyde (MDA) and nitric oxide (NO) are directly involved in multi stage process of carcinogenesis by bringing out a continuous endogenous damage to cellular DNA. Despite therapeutic and diagnostic advances, the rate at which oral pre-cancerous and cancerous lesions are spreading is dangerous. This highlights the need for continued efforts to discover suitable biomarkers for early diagnosis.<sup>4</sup> The present study was conducted to determine Superoxide dismutase (SOD) and glutathione peroxidase level in patients with OSMF.

### **MATERIALS & METHODS**

The present study was conducted in the Department of Dentistry, Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar. It comprised of 60 subjects with OSMF of both genders. Equal number of controls was also included. The study was approved from institutional ethical committee before commencement of the study. All participants were informed prior and written consent was obtained.

Inclusion criteria included clinically and histopathologically diagnosed cases of oral submucous fibrosis and patients not taking any medicine for the same condition. Exclusion criteria included patients suffering from any systemic diseases like diabetes, hypertension, cardiovascular diseases, renal dysfunction, or liver disorders.

General information such as name, age, gender etc. was recorded. In all patients, 5 ml overnight fasting venous blood was obtained from the antecubital vein using sterile disposable syringe and was stored in heparinized vacutainer tubes. Serum was separated in 2.5 ml of blood by centrifugation at 3000 rpm for 15 mins.

Superoxide dismutase level was measured based on the inhibition of a superoxide-induced NADH oxidation. The decrease in the rate of NADH oxidation is dependent on the enzyme concentration, and saturation levels were attainable by recording the corresponding readings, spectrophotometrically (520 nm).

Glutathione peroxidase assay level was measured by estimating GPx activity in cytosol and hemolysate was based on the method of Paglia and Valentine using hydrogen peroxide and the rate of disappearance of NADPH at  $37^{\circ}$ C and was recorded spectrophotometrically 340 nm. Results thus obtained were subjected to statistical analysis using chi- square test. P value less than 0.05 was considered significant.

## RESULTS

#### **Table I Distribution of patients**

Total-120				
Groups	Group I (Study)	Group II (Control)		
Number	60	60		

Table I shows that both group I and II comprised of 60 subjects each.

#### Table II Estimation of level of Superoxide dismutase (SOD) in both groups

 Groups	Group I	Group II	P value
Mean (U/ml)	102.3	204.7	0.01

Table II, graph I shows that mean level of Superoxide dismutase (SOD) in group I was 102.3 U/ml and in group II was 204.7 U/ml. The difference was significant (P < 0.05).

#### Graph I Level of Superoxide dismutase (SOD) in both groups



 Table III Estimation of level of glutathione peroxidase (GPx) in both groups

Groups	Group I	Group II	P value
Mean (U/g Hb)	24.6	62.5	0.05

Table III, graph II shows that mean glutathione peroxidase (GPx) level in group I was 24.6 U/g Hb in group I and 62.5 U/g Hb in group II. The difference was significant (P < 0.05).

Graph II Glutathione peroxidase (GPx) in both groups



#### DISCUSSION

OSMF is multifactorial in origin. Various epidemiological studies have provided irresistible evidence that areca-nut is the main etiological factor in OSMF. It is also considered

as a disorder of collagen metabolism and is characterized by increased production and decreased degradation of collagen fibers.<sup>5</sup>

Superoxide dismutase is an enzyme that alternately catalyzes the dismutation of the superoxide (O2-) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide  $(H_2O_2)$ . Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging and is degraded by other enzymes such as catalase. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. Glutathione peroxidase (GPx) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.<sup>6</sup>

Free radical associated damages leads to an imbalance between pro-oxidant and anti-oxidant states. This imbalance plays an important causative role in carcinogenesis. Anti-oxidants have a shielding role by scavenging the free radicals and SOD, GPx form the first line defense anti-oxidants.<sup>7</sup> The present study was conducted to determine Superoxide dismutase (SOD) and glutathione peroxidase level in patients with OSMF.

In present study, we included 60 patients of OSMF who were diagnosed clinically and histopathologically. Equal number of age and sex matched controls were included. We

Source of support: Nil

found that mean level of Superoxide dismutase (SOD) in group I was 102.3 U/ml and in group II was 204.7 U/ml.

OSMF is one of the potentially malignant disorders. The use of arecanut leads to the formation of vertical fibrotic bands in various parts of oral cavity such as buccal mucosa, lips, soft palate. The characteristic feature is hockey stick like shrunken uvula. Patient complains of limited mouth opening, difficulty in speaking, eating and swallowing. Blanching of the oral mucosa is caused by impairment of local vascularity because of increasing fibrosis and results in a marble-like appearance. Blanching may be localized, diffuse or reticular. In some cases, blanching may be associated with small vesicles that rupture to form erosions.<sup>8</sup>

Naga et al<sup>9</sup> found that SOD and GPx was decreased in cases compared to control group. Gurudath et al<sup>10</sup> found statistically significant decrease in E-SOD and GPx levels in OSMF, oral leukoplakia, and oral cancer groups as compared to the control group. Oral leukoplakia group showed lower levels in comparison with OSMF. Oral cancer group had the lowest levels amongst the study groups.

We found that mean glutathione peroxidase (GPx) level in group I was 24.6 U/g Hb in group I and 62.5 U/g Hb in group II. Beena P Patil and Yildi<sup>11</sup> showed that erythrocyte SOD was lowered and GPx was elevated in tobacco users compared to non tobacco users. The limitation of the study in small sample size and comparison of different treatment modalities for the condition could provide additional weightage to the study.

#### CONCLUSION

OSMF is a premalignant condition strongly associated with arecanut chewing habit. There was reduction in both Superoxide dismutase & glutathione peroxidase as compared to control subjects.

Conflict of interest: None declared

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