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Research Article

"ANTIOXIDANT-OXIDANTS TO ASSESS THE OXIDATIVE STRESS IN ORAL SUBMUCOUS FIBROSIS"- AN ORIGINAL STUDY

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A B S T R A C T

Background: Oral submucous fibrosis (OSMF) is a highrisk precancerous condition characterized by changes in the connective tissue fibers of the lamina propria and deep parts leading to stiffness of the mucosa and restricted mouth opening. Worldwide estimates of OSMF shows a confinement to Indians and Southeast Asians, with overall prevalence rate in India is 0.2% to 0.5 %.

Areca nut induces generation of free radicals promoting lipid peroxidation. Malondialdehyde is the most widely used marker for lipid peroxidation. Antioxidant enzymes Catalase can form conjugate and neutralize Reactive Oxygen Species (ROS) protecting the cellular macromolecules. Repeated exposure of oral mucosa to arecanut ingredients will reduce antioxidant status, due to utilization of antioxidants by body's defense to neutralize free radicals.

Methods

This study involves 30 OSMF patients as study group and 30 healthy individuals as control group. Blood withdrawn, centrifuged, serum separated and biochemically evaluated for Malondialdehyde (MDA) and Catalase (CAT). Results obtained were statistically analyzed using student't' test.

Results: The study revealed increased serum MDA and decreased Catalase levels in OSMF patients compared to healthy patients.

Conclusion: The study showed impairment in oxidant-antioxidant status in the body due to excess consumption of areca nut and tobacco. Increased malondialdehyde levels in the serum indicates increased lipid peroxidation and decreased catalase levels in the serum indicates consumption of antioxidant enzymes in neutralizing free radicals.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a high risk precancerous condition characterized by changes in the connective tissue fibers of the lamina propria and deeper parts leading to stiffness of the mucosa and restricted mouth opening. It is seen predominantly in people of Asian descent. The disease is predominantly seen in India, Bangladesh, Sri Lanka, Pakistan, Taiwan, and China with overall prevalence rate in India being about 0.2% to 0.5 % and prevalence by gender varying from 0.2-2.3% in males and 1.2- 4.57% in females.(1)

OSMF is a precancerous condition with multifactorial etiology.

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Arecanut is the chief causative agent for the pathological changes seen in this condition. Free radicals released from arecanut causes imbalance in the bodies oxidant-antioxidant status thereby disturbing the cellular homeostasis. Such an imbalance is directly related to frequency duration and type of arecanut consumed. Hence the body's antioxidant-oxidant status can be used as a reliable maker to assess damage that tissues sustain in OSMF.(2,3) Antioxidant –oxidant assay levels also determine the efficacy of the treatment and prognosis.

Arecanut induces generation of free radicals, which are responsible for high rate of lipid peroxidation. Malondialdehyde (MDA) is the most widely used marker of lipid peroxidation. Reactive oxygen species produced during auto oxidation of Arecanut polyphenols in the betel quid chewer's saliva are crucial in the initiation and promotion of oral cancer. Antioxidants such as cellular Glutathione (GSH), N-acetyl-L-cysteine (NAC) and enzymes such as Glutathione

Peroxidase, Catalase (CAT), and Superoxide Dismutase can form conjugates with Reactive Oxygen Species (ROS) and reactive intermediates, thereby degrading reactive toxic species and protecting the critical cellular macromolecules. Repeated and continuous exposure of oral mucosal cells to betel quid ingredients, however will lead to the decrease in antioxidant status, due to utilization of antioxidants by body's defense to neutralize free radicals.(2, 3)

The present study was undertaken to assess serum levels of lipid peroxidation product Malondialdehyde and antioxidant enzyme Catalase in OSMF cases. These biochemicals could prove to be potential prognostic markers for further studies.

MATERIALS AND METHODS

Selection of case

This study involved total of 60 cases, among which 30 clinically and histologically diagnosed cases of OSMF with a age range between 20 to 45 years and 30 control cases of same age range attending the outpatient in the Department of Oral Medicine and Radiology, A.J.Institute of Dental Sciences, Mangalore. All the subjects were explained about the objectives of the study. Written consent from every participant was obtained; Institutional ethical committee approval was taken for the study.

Oral submucous fibrosis in association with any other malignancies and systemic diseases of renal and cardiac were not included in the study.

Sample collection and storage

Under all aseptic precautions, about 5 ml of fasting venous blood was collected from antecubital vein of study and control group into plain sterile bulb. The sample was allowed to clot at room temperature for about two hours and then centrifuged at 3000 rpm for 10min; serum was separated and stored at -20ºC. Serum obtained was then biochemically evaluated for levels of Malondialdehyde and Catalase.

Biochemical estimation of Malondialdehyde (MDA) and Catalase (CAT)

MDA was estimated by Thiobarbituric acid method- *MDA* reacts with thiobarbituric acid at 1000c in acidic medium to form pink coloured complex. The colour intensity of MDA-TBA complex is measured at 535 nm against a reagent blank using spectrophotometer. MDA concentration is calculated using the molar extinction coefficient of MDA-TBA complex. [1.56X105L mol-1.cm -1].

Catalase assay was performed by Continuous Spectrophotometric rate determination method using Hydrogen peroxide- The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance, the enzyme activity can be calculated. The difference in absorbance of Hydrogen peroxide per unit time is a measure of catalase activity.

$$2\,H_2O_2 \longrightarrow O_2 + 2\,H_2O$$

The disappearance of hydrogen peroxide (H_2O_2) is measured spectrophotometrically at 240 nm.

Mean serum levels of MDA, and Catalase levels were compared between OSMF and control groups.

Statistics

Obtained values was statistically analyzed in SPSS software using Student't' test, *P* value <0.05 was considered for statistical significance.

RESULTS

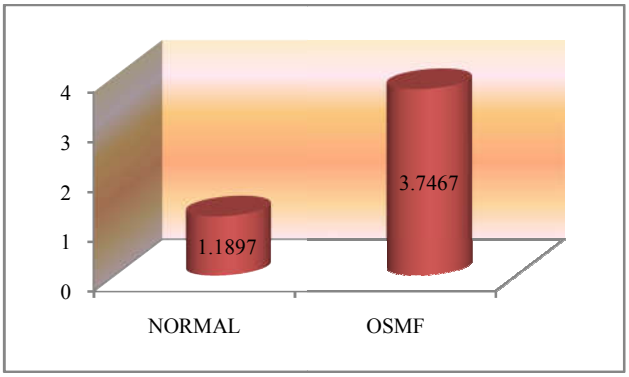
Our study showed decrease in the antioxidant enzyme Catalase levels in OSMF patients as compared to control group *(P= 0.00)* which signifies its role in neutralizing free radicals released from arecanut products and its importance in OSMF condition. The lipid peroxide MDA levels were significantly increased in OSMF patients when compared to Control group *(P< 0.01).*In accordance with the many other studies the present study, also concludes that imbalance in oxidant-antioxidant levels play a major role in carcinogenesis.

On comparing mean MDA, and CAT levels between control group & OSMF patients highly significant difference value *(P = 0.00)* was obtained.

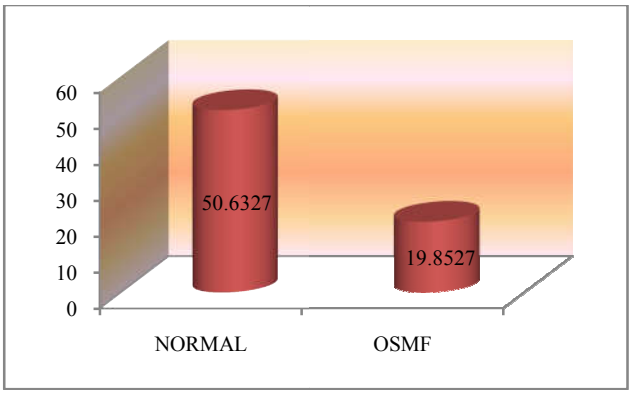
Results of our study are depicted in **Table.1** and presented graphically.

Table 1 Estimation of oxidant-antioxidant levels in OSMF patients and their comparison with healthy control subjects

Parameters	Control n=30 (mean±SD)	OSMF n=30 (mean±SD)	*P* value
Malondialdehyde(MDA) (nmol/ml)	1.1897±.36547	3.7467±.76365	*>0.01*
Catalase(CAT) (kU/l)	50.6327±2.81877	19.8527±1.15687	*0.00*



Graph 1 MDA levels in nmol/ml



Graph 2 Catalase levels in kU/l

DISCUSSION

Oral submucous fibrosis was first described by Schwartz in 1952 among five Indian females living in Kenya and he coined the term atrophiaidiopathica (trophica) mucosae oris. Even though the etiology of OSMF is multifactorial, arecanut plays

the main role in the pathogenesis of OSMF. Arecanut contains several alkaloids like arecoline, arecaidine, arecolidine, guvacoline and guvacine. Arecoline is the most abundant alkaloid product of arecanut.

Arecadine and other tobacco products contain quinine/hydroquinone from which hydrogen peroxide and hydroxyl free radicals are released. Such hydroxyl radicals in turn attack lipids containing carbon-carbon double bond, especially polyunsaturated fatty acids (PUFAs).**(4,5)** It involves the abstraction of hydrogen from a carbon, with insertion of oxygen resulting in lipid peroxyl radicals and hydroperoxides to form stable adducts which weakens and alters the permeability of cell membrane causing disturbance in normal homeostasis of the cell resulting in cell damage. Due to lipid peroxidation, many damaging aldehydes are formed particularly malondialdehyde (MDA), propanedial, 4-hydroxynonenal (4-HNE).(6)

MDA is one of the major aldehyde products of lipid peroxidation and is highly reactive. It helps in cross linking of collagen by providing its aldehyde group to lysine and helps in lysine to lysine bridging in the presence of enzyme lysyl oxidase. The MDA-collagen cross-link complex will still contain a free reactive aldehyde group capable of reacting to form different intermolecular cross-links. Hence MDA facilitates in forming inter and intra molecular crosslinking of collagen thereby stiffening of tissues and reducing their function. Such an action of MDA on collagen is also showed in studies related to aorta and atheroma which also contains types I and III fibrous collagens.(7)

MDA is one of the stable adduct product of lipid peroxidation responsible for cytopathological effects seen due to oxidative stress and also its reaction with thiobarbituric acid (TBA) makes it possible for early and accurate measurement of cellular damage caused by the free radicals. Therefore, measurement of MDA is widely used in determining the lipid peroxidation of cell membrane by free radicals.

Tar phase of tobacco contains quinine and hydroquinone complex which is the main free radical present in it. These complexes are able to reduce molecular oxygen to superoxide which is rapidly converted to form hydrogen peroxide that eventually causes cell damage. In the presence of catalase, hydrogen peroxide will be neutralized; but as more H_2O_2 is released due to more of areca nut ingestion, catalase will be utilized more, thus serum catalase level is reduced in OSMF.(8, 9, 10)

Thus more ingestion of tobacco and areca nut causes more release of free radicals that in turn causes increased MDA levels. So in order to neutralize such free radicals, antioxidant enzymes like Glutathione Peroxidase, Catalase, and Superoxide Dismutase were consumed by the body which in turn results in the decrease in antioxidant enzyme levels in OSMF patients as observed by many researchers.(8) High levels of lipid peroxides causes DNA mutation or mispairing and progress to oral cancer.(11)

Biochemically assessing these markers pose great advantage in the tests being simple, less invasive, less time consuming, ease of interpretation, economical and yet quite confirmatory for its diagnostic and prognostic values compared to biopsy which is more time consuming and invasive. Future studies regarding assessment of oxidant-antioxidant status in OSMF patients in view of selecting appropriate mode of therapy and the effectiveness of such therapy in limiting the progression and recurrence of this condition has needed to be carried out.

From the present study, it is evident that by estimation of lipid peroxidation and antioxidants in circulation of OSMF patients, one can assess the degree of oxidative damage of the disease. Further correcting the underlying deficiency of antioxidants the treatment plan can be improved. This in turn, may be helpful for successful management of this condition, thereby arresting it in early stages and avoiding the possible consequences of malignancy. A therapeutic strategy to increase the antioxidant capacity of cells may be used to fortify the long term effective treatment in future.

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