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Original Research

Biochemical Estimation of Serum Malondialdehyde and Superoxide Dismutase Levels in Oral Submucous Fibrosis Patients

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ABSTRACT:
Introduction: The most expected culprit of dysplastic changes in these lesions include the free radicals and reactive oxygen species that lead to lipid peroxidation. Early detection and knowledge of the dysplastic changes occurring in OSMF are helpful in prevention or progress of such lesions turning into oral cancer. Hence the aim was to estimate the serum superoxide dismutase (SOD) and serum Malondialdehyde (MDA) levels in patients with OSMF. **Method**: A total of 40 patients were included in the present study ranging between 18 to 75 years. Twenty OSMF cases which were clinically diagnosed were divided into different stages and grades respectively. Twenty healthy subjects without any habits and clinically normal appearing oral mucosa were included as controls. Serum MDA will be measured using the method of Ohkawa et al. and Serum Superoxide will be measured using the method of Fridvich and Mishra. **Result**: Serum MDA and serum SOD were compared within the clinical stages of OSMF patients. Comparison of MDA, SOD among the OSF group and control group showed statistically significant increased levels of malondialdehyde and decreased levels of superoxide dismutase among the OSF groups. Significant changes were not seen in MDA and SOD levels between the different grades of OSF. **Conclusion**: The present study showed significant increase in the serum MDA levels in all the clinical stage and histopathological grades, the maximum being in stage IV and Grade III. It was also observed a significant decrease in SOD levels in all the clinical stages of OSMF patients, the maximum being in stage IV and grade III.
Key words: Malondialdehyde, Oral Cancer, Superoxide Dismutase, OSMF.

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INTRODUCTION

Oral cancer is the eighth most common form of cancer in the world with a 5- year's survival rate of less than 50%. In India oral cancer is the most common cancer in males and 3rd most common cancer in females.[1] In India, the incidence rate of oral cancer is 12.6 per 100,000 populations. In India oral cancer is the most common cancer in males and 3rd most common cancer in females. An Indian survey showed that about 80% of oral cancers were preceded by oral potentially malignant lesions or conditions.[2]

Early detection and proper knowledge of the dysplastic changes occurring in OSMF are helpful in prevention or progression of such lesions turning into oral cancer. The most expected culprit of dysplastic changes in these lesions include the free radicals and reactive oxygen species that lead to lipid peroxidation.[3] These reactive free radicals may cause profound alteration in the structural integrity and the function of cell membrane resulting in damage to the cell. It is known that free oxygen radicals are probably mediators for tissue damage in neoplastic disease.[4]

The enzyme Superoxide dismutase (SOD) has many variants. The predominant among these are copper-zinc containing enzymes found in the cytoplasm, and manganese SOD located in the

mitochondria. Dismutation reaction, hydrogen peroxide, which helps to conduit in transmission of the injury caused by free radicals.[5] The human body produces an incredible number of reactive oxidants such as hydrogen peroxide, superoxide and hydroxyl radicals. The hydroxyl radical is the most catastrophic to the tissue causing destruction of the adjacent cells.[6]

Although the histopathological findings are the mainstay of dysplasia, some of the biochemical alterations in serum have found to be indicative of such changes. Therefore identification of suitable biomarkers which are simple, rapid, convenient, inexpensive and reliable is need of the time. Such biomarkers include serum superoxide dismutase and serum malondialdehyde.[7]

Free radicals, primarily the reactive oxygen species are highly reactive. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids. Superoxide dismutase, primarily an antioxidant present in the body, acts in the defense mechanism mainly by blocking the initiation of free radical chain reactions.[8, 9]

Malondialdehyde is the organic compound with the formula CH2 (CHO)2. The structure of this species is more complex than this formula suggests. This reactive species occurs naturally and is a marker for oxidative stress. Three forms of superoxide dismutase are present in humans, in all other mammals, and most chordates. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular.[10]

There are some exist studies which indicate the excessive production of ROS and thereby causing increased levels of MDA and reduced the antioxidant enzyme levels such as SOD in OSMF. However, to our present knowledge, there exist few studies in which they compare the levels of serum MDA and SOD in each of the clinical stages of OSMF. The clinically diagnosed confirmed OSMF cases were involved in the study with various clinical stages and histological grades and their outcome on prognosis of individuals. Hence the present study aims to evaluate the serum levels of SOD and MDA and the correlation of these parameters in OSMF Patients.

MATERIALS & METHODS

The patients diagnosed with oral submucous fibrosis based upon the history, clinical examination and histopathological examination were selected. The ethical clearance was obtained from the ethical committee of the institute. The signed consent forms were obtained from the patients who were included in the study. Total of 40 patients were included in the study as the study group.

Inclusion criteria

Patients above 15 years of age, who were diagnosed as oral submucous fibrous based upon thorough history, clinical examination and histopathological examination without any underlying systemic disease, were included in the present study.

Equal numbers of healthy subjects without any deleterious habits and without any clinically obvious oral lesions or systemic diseases were selected as the control group.The subjects for the study were grouped as follows:

Group 1 (OSF): 20 patients having OSF

Group 2 (Control): 20 healthy subjects.

Exclusion criteria

Patients with any underlying systemic diseases and patients with previous history oftreatment for OSF were excluded from the study

Collection of sample

The included patients were explained in detail about the procedure of the study. After obtaining an informed consent, 5 ml of venous blood was collected from the antecubital vein of each individual and was then centrifuged at 3000 rpm for 10 minutes, to separate the serum.Without delay this serum was used for estimation of MDA, SOD.The estimation of MDA in the serum was done by thiobarbituric acid reactive species method. SOD was assayed by inhibition and auto oxidation of adrenaline method.

Statistical analysis

The data were analyzed with Student's t-test. All statistical analyses were performed with the program Statistical Package for the Social Science and $P < 0.05$ was accepted as statistically significant.

RESULTS

A total of 20 patients were included in the study and 20 healthy individuals were taken as control group. The age range was between 20 to 50 years. Twenty OSMF cases which were clinically diagnosed were divided into different stages based on clinical parameters.

In our study maximum numbers of cases belong to the age group of 21-30 years, followed by cases in the age group of 31-40 years. We found no statistical significant difference among the OSMF and control group with respect to the age (P>0.05).

As per the clinical classification of OSMF in our study group of 20 cases, 8 cases belonged to stage II followed by 5 cases belonging to stage I and 4 cases to stage IV and 3 cases to stage III. There was no statistical significant difference of clinical stages in OSMF group (P>0.05) analyzed by chi-square test.

In the present study when serum MDA and serum SOD were compared between OSMF cases and control group which were statistically analyzed by student ‘t’ test, we found statistically very highly significant difference (P<0.001) The mean MDA was significantly higher in the OSMF group as compared to control group. The mean SOD was significantly lower in the OSMF group as compared to control group.

We compared the serum MDA and serum SOD in OSMF patients within the clinical stages. It was statistically analyzed by ANOVA-test. There was very high significant difference (P<0.001). The mean MDA was significantly increased as clinical stages were increased. This was statistically significant (P = 0.001).

Table 1: Comparison of MDA and SOD among OSMF and Control groups

Serum variables	Study group	Control group	T test value	P value
MDA	310.58 ± 12.82	289.32 ± 0.76	128.89	0.001
SOD	84.98 ± 0.34	165.32 ± 8.37	56.23	0.002

Table 2: Comparison of clinical stages within the stages of MDA and SOD in OSMF group

Clinical stages	MDA	SOD
Stage I	312.65 ± 24.56	74.30 ± 0.72
Stage II	326.34 ± 12.45	70.60 ± 0.22
Stage III	379.38 ± 26.78	68.63 ± 0.52
Stage IV	393.32 ± 44.21	64.65 ± 0.83
P value	0.003	0.002

DISCUSSION

Oral submucous fibrosis is the most common precancerous condition affecting the oral cavity with various etiological factors. Oral submucous fibrosis has a prevalence of 2.01%. It has been shown that 1% to 18% of oral potentially malignant lesions turn into malignant lesions,4 and OSMF has a malignant transformation rate of 2.3 to 7.6%. In India oral cancer is the most common cancer in males and 3rd most common cancer in females. The incidence rate of oral cancer is 12.6 per 100,000 populations.

Superoxide dismutase is an enzyme with a generalized presence in the body which catalyzes the dismutation of superoxide. As a by-product of this reaction, hydrogen peroxide is produced which helps to conduit in transmission of the injury caused by free radicals. The human body produces an incredible number of reactive oxidants such as hydrogen peroxide, superoxide and hydroxyl radicals. The hydroxyl radical is the most catastrophic to the tissue causing destruction of the adjacent cells.

Superoxide dismutase (SOD), primarily an antioxidant present in the body, acts in the defense mechanism mainly by blocking the initiation of free radical chain reactions.

Antioxidant enzymes SOD can directly counterbalance the oxidant attack and protect the cells against DNA damage. Superoxide dismutase is a decisive antioxidant enzyme in the aerobic cells, which is responsible for the elimination of superoxide radicals. Therefore, SOD seems to be the key enzyme in the natural defense against free radicals. In the present study, a statistically significant decrease in the mean level of SOD in the OSF patients in comparison with the control groups. As the reported cases of OSF were only Grade II and Grade III, only these groups were considered in the study. The mean SOD level in different grades of OSF did not show any significant changes.

The mean level of MDA was increased in the OSF group compared to the control group. Clinical grade-wise analysis of MDA did not show any significant changes. The results of present study and the findings by Alexander *et al.* in their study on tumor cells indicate low SOD activity. The decreased levels of SOD in oral submucous fibrosis patients as observed by us may be correlated with the precancerous potential of oral submucous fibrosis. It is established that the lipid peroxidation increases with severity of the disease reflecting the extent of tissue injury. However, in the present study it did not show any significant variation between grade II and grade III which was similar to the study conducted by Gupta *et al*.

Mean MDA levels were increased with the clinical stages, where as SOD levels decreased with increased progressive stage of OSMF in our study. But our study with similar analysis of parameters on statistical analysis by ANOVA test, reveal a significant difference (p<0.001), we are of the view that, different types of processed areca nut, betel nut, tobacco consumed with slacked lime, and other additive substance, the duration of the individuals chewing these substances and also the intensity and interval or frequency of use of these products may have had an additive influence synergistically on cellular damage leading to the observation of significant difference of MDA and SOD levels in our present study.

CONCLUSION

By this the underlying deficiency of antioxidants can be corrected and the treatment plan can be improved. Further long term studies are required with larger sample size in order to assess the pre-treatment and post treatment effect on serum levels of SOD and MDA which includes different grades with varying age groups and duration of OSF. This in turn, may be helpful for successful management of this condition, thereby arresting it in early stages and avoiding the possible consequences of OSF

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