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**Research Article**

# Estimation of Serum Lipid Peroxides Before and After Radiotherapy in Oral Squamous Cell Carcinoma Patients

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## Abstract

**Background:** Oral Cancer is a term used to identify those malignant tumors which originate in the mucous membrane lining the oral cavity. Many researchers have shown that lipid peroxides (Malondialdehyde) are the products of the chemical damage done by oxygen free radicals, to the lipid components of cell membrane and showed discrepancies in its serum values in radiation treated malignant cases. **Aims and Objectives:** 1. To estimate and compare serum Malondialdehyde level of normal healthy individuals with that of oral Squamous cell carcinoma patients. 2. To estimate and compare serum Malondialdehyde level in the oral Squamous cell carcinoma patients before and after radiotherapy. **Materials and Methods:** In this study serum malondialdehyde was measured according to Kei Satosh Method in 20 normal individuals and 20 patients each with histopathologically diagnosed oral cancer. **Results:** The mean serum MDA level in the pre-radiotherapy study group was 0.598 ± 0.1609 which was significantly higher than control group 0.3084 ± 0.1016 and the mean serum MDA level in the study group after radiotherapy was 0.792 ± 0.1157 which was higher as compared to pre-radiotherapy mean serum level of 0.598 ± 0.1609. **Conclusion:** Thus it can be concluded that the mean serum MDA level increases in the oral squamous cell carcinoma patients as compared to the healthy individuals and this level further increase after the radiotherapy which indicates more damage to the cellular structure from free radicals leading to oxidative stress. This increase in oxidative stress in serum MDA level again suggests the requirement of an adjunct therapy to treat the oral squamous cell carcinoma along with radiotherapy.

**Keywords:** Malondialdehyde; Lipid Peroxidation; Oral Cancer; Blood Serum; Plasma; Squamous Cell Carcinoma; Radiotherapy.

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## Introduction

Neoplasm represents a pathological disturbance of growth characterized by an excessive and increasing proliferation of cells. The etiology of oral cancer is voluminous but few firm conclusions can be drawn except for the role of tobacco usage. The role of factors such as immunological susceptibility and enzymatic induction in promoting the cancer remains unclear. The incidence of oral Squamous cell carcinoma appears to be directly related to the use of tobacco in any form i.e. smokeless tobacco and smoking tobacco.[1] The relative risk of oral cancer in both sexes increases with the quantity smoked and the duration of the exposures.[2] Radiotherapy has been used successfully in the treatment of malignancy for many years, due to rapid development in medical radiotherapy instrumentation and technology resulting from recent research in application of nuclear energy.[3] Radiotherapy is usually prescribed either as a radical or a palliative measure according to the intentions. Radiotherapy is being used with increasing frequency as a primary treatment modality in the management of oral cancers. Small, exophytic, well vascularized and well oxygenated tumors respond best to the radiation therapy. The mode of radiotherapy has generated additional free radicals during the irradiation process, and less defended by poor antioxidant system in these individuals.[3]

### The mechanism of free radicals chain reaction (leading to formation of unsaturated fatty acid hydroxide)

The lipid peroxidation is a chain reaction in which there is a continuous supply of radicals that is used for further peroxidation. Lipid peroxidation may be initiated by any primary free radical with sufficient reactivity to extract a hydrogen atom from a reactive methylene group; a polyunsaturated fatty acid.[4] Propagation is the iron catalyzed breakdown of lipid hydroperoxides formed during initiation, generating reactive intermediates and products, which are characteristic of lipid peroxidation. Propagation results in the rapid formation of

thiobarbaturic acid reactive substance (TBARS) and lipid hydroperoxides.[5]

Malondialdehyde (MDA) is formed only by fatty acids with three or more double bonds and is used as a measure of lipid peroxidation. MDA can cause cross linking and polymerization of membrane components.[6,7] This can alter intrinsic membrane properties such as deformability, iron transport, enzyme activity and the aggregation state of cell surface determinants. Because MDA is diffusible, it will also react with nitrogenous bases of DNA.[8] All of these effects may explain why MDA is mutagenic[9], genotoxic to cultured cells[10] and carcinogenic.[11] Hence present study was conducted with the aim to estimate and compare the serum Malondialdehyde level in normal individuals, in oral cancer patients before and after radiotherapy.

**Materials & Methods**

For the present study, 40 patients were selected at random from Out Patient Department of Oral Medicine and Radiology, and were divided into two groups. Group I: Control Group consists of 20 healthy, not having any oral lesions or known systemic diseases in the age group of 40-75 years. Group II: Study group consisted of 20 patients in the age group of 40-75 years, who had histopathologically confirmed oral Squamous cell carcinoma.

The inclusion criteria for selection of study group included patients receiving only radical radiotherapy with a total 50 Gy radiation dose given over a period of 5 weeks with a daily dose of 2 Gy using cobalt – 60 unit. The Exclusion criteria for the present study was that the patients receiving any other mode of treatment and with any known systemic diseases or disorders.

The relative history of each patient from both the groups was recorded. Intraoral and extra oral examination was carried out with the help of mouth mirror, probe / explorer in good light and Squamous cell carcinoma was clinically classified on the basis of American Joint Commission of Cancer (1997) tumor staging system with TNM grading. They were also classified on the basis of histopathology and divided into three groups; poorly differentiated, moderately differentiated and well differentiated. The complete data of the patients were filled in the case history Performa. Under aseptic conditions, 5 ml of blood was drawn from each patient using sterile disposable 22 gauge needle and 5 ml syringe. In patients with oral Squamous cell carcinoma it was drawn twice, i.e. before onset of radiotherapy and after completion of radiotherapy.

**Determination of Lipid Peroxides In Serum - (Kei Satosh Method)[12]**

The principle of this method is to determine the level of serum lipid peroxides using Malondialdehyde as a marker, thiobarbaturic acid as a main reagent and measuring these values on photoelectric calorimeter at 530 nm of light under green filter.

**Experimental Procedure**

Blood was collected in a sterile plain bulb and kept standing for 20 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm for 15 minutes. The 0.5 ml serum was mixed thoroughly with 2.5 ml of Tri-chloroacetic acid and mixture was allowed to stand for 10 minutes at room temperature. This was further centrifuged at 3000 rpm for 10 minutes and the supernatant was discarded. The precipitate collected was washed twice with dilute sulphuric acid. About 2.5 ml of dilute sulphuric acid was added to the tube containing precipitate and was mixed well with 3 ml of thiobarbaturic acid reagent. Reaction mixture was heated in boiling water bath for 30 minutes and allowed to cool to room temperature. Then 4 ml of n-butyl alcohol was added and with vigorous shaking chromogen was extracted in organic phase. Color intensity of the chromogen was measured at 530 nm in photoelectric colorimeter. Results were compared using standard solution of 1,1,3,3-tetramethoxypropane and expressed as µ mol per liter.

The student paired t-test was used for comparing the sample from the two groups i.e. pre-radiotherapy & post-radiotherapy. However student unpaired t-test was used for comparing the samples from control Vs pre-radiotherapy.

**Observations and Results**

There were 8 cases of carcinoma of tongue comprising 40% of all cases. Similarly there were 4 cases of buccal mucosa, 1 cases of labial mucosa, 2 cases of mouth, 4 cases of alveolus and 1 case involving palate comprising of 20%, 5%, 10%, 20% and 5% of total cases respectively.

The mean level of serum malondialdehyde in control group was found to be 0.3084 µmol/ltr with standard deviation of ± 0.10167 µmol/lit and the mean level of serum malondialdehyde in study group A1 was 0.598 µmol/ltr with standard deviation of ± 0.1609 µmol/ltr. Also it was observed the mean serum MDA level in group A1 was 0.598 µ mol/ltr with standard deviation of ± 0.1609 and in group A2 it was 0.792 µmol/ltr with standard deviation of ± 0.1157. The mean increase in serum MDA of group A2 was statistically highly significant (p<0.001) as compared to group A1 (Table 3).

When the study group was divided into well, moderate and poorly differentiated squamous cell carcinoma and findings were correlated with MDA levels, (Table 4), no correlation in MDA level was observed between degrees of differentiation of malignant lesions.

| Age (years) | No. of cases (%) | Male | Female | Mean age ± Standard deviation |
|---|---|---|---|---|
| 45-54<br>55-64<br>65-70 | 6 (30)<br>13 (65)<br>1 (5) | 4<br>8<br>1 | 2<br>5<br>0 | 54.1 ± 6.7 years |
| Total | 20 (100) | 13 (65) | 7 (35) | |

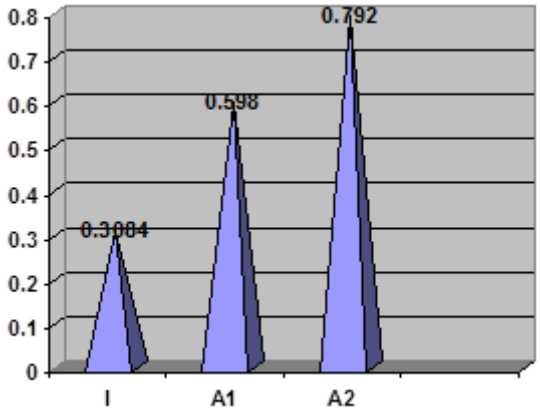
Table 1: Age and sex distribution in group I (Control Group)

| Age (year) | No. of cases (%) | male | female | Mean age ± Standard deviation |
|---|---|---|---|---|
| 45-54<br>55-64<br>65-70 | 4 (20)<br>13 (65)<br>3 (15) | 4<br>11<br>3 | 0<br>2<br>0 | 58.05 ± 6.4 years |
| Total | 20 (100) | 18 (90) | 2 (10) | |

Table 2: Age and sex distribution in group II (Study Group)

| Group | No. of cases | Mean ± Standard deviation | P value |
|---|---|---|---|
| A- Control | 20 | 0.3084 ± 0.1016 | P < 0.001 |
| A1- OSCC patients before radiotherapy | 20 | 0.598 ± 0.1609 | |
| A2- OSCC patients after radiotherapy | 20 | 0.792 ± 0.1157 | |

Table 3: The mean and standard deviations of Serum malondialdehyde levels in control and oral Squamous cell carcinoma patients.




Graph 1: Serum malondialdehyde level in control group and oral squamous cell carcinoma patients.

| Squamous cell carcinoma | Before radiotherapy | After radiotherapy |
|---|---|---|
| Well Differentiated | 0.57 ± 0.09 | 0.72 ± 0.09 |
| Moderately Differentiated | 0.590 ± 0.10 | 0.77 ± 0.11 |
| Poorly Differentiated | 0.84 ± 0.08 | 0.80 ± 0.05 |

Table 4: The effect of radiotherapy and degree of differentiation on mean serum MDA level in study group.

## Discussion

Oral cancer is a multifocal disease and experimental studies have shown that such lesions develop in several steps. According to Goldhaber (1957) the phases in the development of malignant neoplasm of the oral cavity are – initiation and promotion. The increasing incidence of oral cancer is clearly age related, which may reflect the declining immune surveillance with age and duration of exposure to initiators and promoters. These include exposure to chemical irritants, physical irritants, viral infections, hormonal effects, cellular ageing and immunological surveillance[13,14]

Radiation therapy is one of the clinical means by which oral cancer can be treated. Many biochemical complications such as damage to cellular DNA[15] and membrane structure and alterations in the immune system,[16] arise as a result of a radiation treatment. The biological effects they produce are thought mainly to be caused by the production of free radicals from the interaction with the cell constituents, especially water.[17] Oxygen, mainly present in most biological systems, aggravates the damage done by radiation. Much of the initial damage done is due to the formation

of hydroxyl radical, which can react with other cellular components to produce organic radicals.[18] One of the important underlying phenomena is an oxidative / change in the lipid membranes, which may be triggered by free radicals. Lipid peroxidation is initiated by very potent free radicals, which include super peroxide $(O_2)^-$ and hydroxyl radical $(OH)^-$ and the reactive molecule hydrogen peroxide ($H_2O_2$). Hydrogen peroxide and $O_2$ may be directly damaging or more often interact to form a highly reactive species that can attack almost every molecule in the living cells.[19] The well characterized product of the lipid peroxidation is malondialdehyde, a three carbon dialdehyde (OCH-CH2-CHO) produced during the oxidative decomposition of polyunsaturated fatty acids (PUFA) and is formed during the metabolism of certain hydrocarbon carcinogens.[20] It is also reported to initiate skin carcinogenesis.[21]

The mean level of serum MDA in control group was found to be higher when compared to the study group carcinoma patients. This increase in level of MDA was statistically highly significant ($p<0.001$). The mean level of serum MDA in study group patients after radiotherapy was higher as compared to pre radiotherapy level. This increase in level was statistically highly significant ($p<0.001$) (Table 3). The above findings are similar to the study done by Sabitha KE et al (1993)[22] on MDA levels in pre and post radiotherapy oral cancer patients. Balasubramanyan N et al (1994)[23] estimated the circulating lipid peroxide, antioxidant component and the activities of defense enzymes in uterine cervical carcinoma patients before and after radiotherapy and radiotherapy combined with chemotherapy and compared it with control. They found that lipid peroxides levels increased in all the stages of uterine cervical carcinoma compared to that of normal. Bhuvarahamurthy V et al (1999)[24] studied tissue lipid peroxides in patients who had carcinoma of uterine cervix and the value were compared with those of normal. The tissue level of lipid peroxides and activity of glutathione s-transferase were found to be significantly higher than that of normal from stage II onwards. Huang et al (1999)[25] determined the serum MDA level in breast cancer patients (n=35) and controls (n=35). They found that there was significant increased lipid peroxidation in the serum of the breast cancer patients as compared with the control group. Almost similar results were obtained in two separate studies by Arivashagan S et al (1997)[26] and Ahmed MI et al (1999)[27]. Punnonen R et al (1993)[28] also analyzed lipid peroxidation in normal as well as endometrial cancer patients from Finnish and Japanese patients and found that lipid peroxidation was slightly higher in endometrial cancer patients as compared with normal and these findings were consistent with the findings of Chiou JF et al (1999)[29] and with the findings of Ray S et al (1999)[30] who studied the lipid peroxidation in cervicitis patients and in human uterine tumors. In the present study when the study group was divided into well, moderate and poorly differentiated squamous cell carcinoma and findings were correlated with MDA levels, no correlation in MDA level was observed between degrees of differentiation of malignant lesions.

## Summary and Conclusion

Thus it can be concluded that the mean serum MDA level increases in the oral squamous cell carcinoma patients as compared to the healthy individuals. This level further increase after the radiotherapy which indicates more damage to the cellular structure from free radicals leading to oxidative stress. This oxidative stress with increase in serum MDA level again suggests the requirement of an adjunct therapy[31] to treat the oral squamous cell carcinoma along with radiotherapy.

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