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Lipid peroxidation and Vitamin C levels in saliva of oral precancerous patients- an *In-vitro* study

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ABSTRACT

Free radical is any atom (E.g.: oxygen) with at least one unpaired electron pair in the outermost shell and is capable of independent existence. They are highly reactive due to the presence of unpaired electrons. They may react with cellular molecules such as DNA, protein and carbohydrate to initiate or promote inflammatory, toxic or carcinogenic process. Lipid peroxidation is the free radical mediated oxidative degradation of the lipids resulting in cell damage. Precancer is a neoplasm that is not invasive but has the potential to progress to cancer and become invasive. Our study was aimed to find whether lipid peroxidation and vitamin C level in saliva can be used as marker for cancer. This study was carried out in A.B.Shetty Memorial Institutes of Dental Sciences. The saliva was collected from the consented subjects. A total of 20 people were selected in which 10 normal subjects and 10 oral precancerous patients. Saliva was tested for Lipid Peroxidation (MDA) and Vitamin C levels by TBA (Thiobarbituric acid) method and DNPH (dinitrophenyl hydrazine) method respectively. Our results indicates the significant increase in lipid peroxidation in oral precancerous stages and also slight increase in vitamin C level, this suggests that there is increased oxidative stress in oral precancerous and may increase further in cancerous stage. Further study on this may lead to development of new types of cancer markers in saliva.

Key words: Lipid peroxidation, Vitamin c level, Oral precancerous condition.

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INTRODUCTION

The term oral cancer encompasses all malignancies that originate in the oral tissues. Usually included are the cancers of lip tongue, pharynx and oral cavity. It spreads quickly, if left untreated can affect the lungs, neck or lymph nodes from the neck area. The World Health Organization (WHO) reported oral cancer as having one of the highest mortality ratios amongst all malignancies. It ranks 12th among all the cancers. The vast majority of malignant neoplasms in the mouth are squamous cell carcinomas (SCC) [1].

A neoplasm that is not invasive but has the potential to progress to cancer and become invasive [2]. Benign morphologically altered tissue which has greater than normal risk of containing a microscopic focus of cancer at diagnosis or of transforming into a malignancy after diagnosis [2]. Leukoplakia and Erythroplakia are precursors to cancer. Often caused by smoking or chewing tobacco, these benign conditions can occur anywhere in mouth. Only a biopsy can determine whether precancerous cells (dysplasia) or cancer cells are present in leukoplakia or Erythroplakia [3].

Lipid peroxidation is the free radical mediated oxidative degradation of the lipids resulting in cell damage. End products of lipid peroxidation may be mutagenic and carcinogenic; one such end product is Malondialdehyde which reacts with deoxyadenosine and deoxyguanosine in DNA, forming adducts which may lead to oxidative stress. . The termination being brought about by antioxidants including Vitamin C by catching free radicals and thereby protecting the membrane from free radical mediated degradation of lipids [4]. To prevent free radical damage the body has a defense system of antioxidants [5]. Vitamin C is one of the Free radical scavenging non enzymatic antioxidant [6]. This interacts with free radicals and terminate chain reaction before vital molecules are damaged [7].

Studies on the role of oxidant-antioxidant system and its potential to undergo malignant transformation in Oral Sub mucous Fibrosis (OSF) correlation between the serum levels of lipid peroxidation product Malondialdehyde (MDA), antioxidants Superoxide dismutase (SOD), and Vitamin A in relation to clinical and histopathological grading of OSF. It is suspected that progressively increased MDA and progressively decreased SOD and Vitamin A levels have positive correlation with clinical grades of OSF. They concluded that by estimation of lipid peroxidation and antioxidants in circulation of OSF patients, one can assess the degree of oxidative damage of the disease [8]. A study on generation and sub cellular distribution of protein adducts with acetaldehyde (AA), the first metabolite of ethanol and end products of lipid peroxidation, malondialdehyde and 4-hydroxynonenal (HNE) in oral biopsy specimens obtained from Japanese and British patients diagnosed with oral Precancer. They found that higher levels of AA, MDA, although not HNE in Japanese subgroup compared with British sample. They concluded that AA and lipid peroxidation derived adducts are found in oral tissues of alcohol misusers with oral leukoplakia and cancer; supporting pathogenic role of AA and excessive oxidative stress in carcinogenesis [9].

Study on analysis of lipid peroxidation product Malondialdehyde in patients of leukoplakia, oral sub mucous fibrosis, Lichen planus, oral cancer and healthy subjects who served as control. Significantly elevated levels of MDA were observed in leukoplakia, oral sub mucous fibrosis, Lichen planus and cancer as compared to controls, indicating a role of free radical in pathogenesis of precancerous condition lesion and cancer [10]. Hence we wanted to estimate the levels of lipid peroxidation product Malondialdehyde in Oral Precancerous conditions, Vitamin C levels in Oral Precancerous condition and their comparison between normal individuals and Oral Precancerous conditioned individuals.

MATERIALS AND METHODS

50 patients from the O.P.D of A.B. Shetty Memorial Institute of Dental Sciences with oral precancerous lesion and 10 healthy age and sex matched individuals were selected for this study. Saliva was tested for Lipid Peroxidation (MDA) and Vitamin C levels by TBA (Thiobarbituric acid) method and DNPH (dinitrophenyl hydrazine) method respectively.

Statistical Analysis: The data obtained is statistically analyzed by student t' test

RESULTS

The present study involves the estimation of the levels of lipid peroxidation product Malondialdehyde in Oral Precancerous conditions, Vitamin C levels in Oral Precancerous condition and their comparison between normal individuals and Oral Precancerous conditioned individuals. The results are expressed in Table-1, Fig-1 and 2. In this study MDA level was found to be increased significantly ($P=0.05$) in oral precancerous patients than compared with the healthy subjects. In this study Vitamin C level in oral precancerous patients and healthy subjects is found to be insignificant ($P=0.95$).

Table: 1. Result table showing levels of Melondialdehyde (MDA) and Vitamin C in saliva of normal and Precancerous patients.

| Parameters | Normal | Precancerous patients | p Value |
|-------------------------|-------------------|-----------------------|---------|
| MDA ($\mu\text{M/L}$) | 0.144 \pm 0.02 | 0.500 \pm 0.02 | 0.05 |
| Vitamin C (mg/dL) | 0.500 \pm 0.026 | 0.475 \pm 0.022 | 0.95 |

Graph showing Levels of MDA in Healthy and Precancerous Conditions

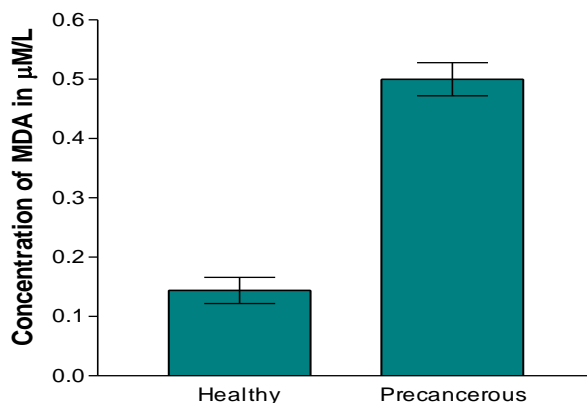


Fig : 1. Graph showing levels of MDA in Healthy and Precancerous conditions

Graph showing Vit C in Healthy and Precancerous Condition

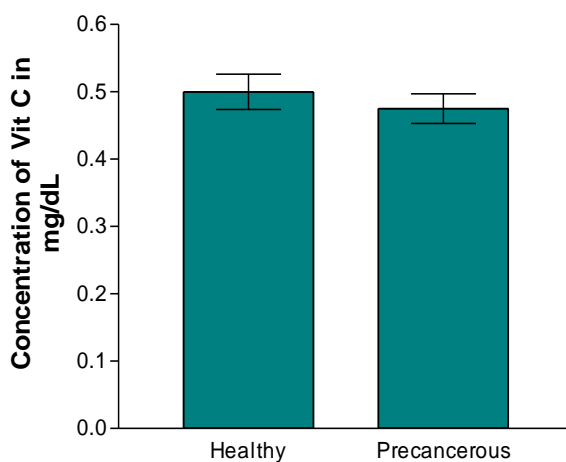


Fig : 2. Graph showing levels of Vit C in Healthy and Precancerous conditions

DISCUSSION

Free radical induced Lipid Peroxidation causes a loss of cell homeostasis by modifying the structure and functions of cell membrane. The most important characteristic of lipid peroxidation is to cause a considerable DNA – MDA adducts by interacting with cellular DNA [8]. Lipid peroxidation byproducts formed under physiological and pathological conditions are scavenged by nonenzymatic and enzymatic antioxidants. Mammalian cells possess elaborate antioxidant defense mechanisms to neutralize the deleterious effects of free radical induced lipid peroxidation. An imbalance between antioxidant defense mechanism and lipid peroxidation processes results in cell and tissue damage [10].

In this study MDA level is found to be increased significantly in oral precancerous patients than compared with the healthy subjects. The high level of MDA in oral precancerous patients directly reflects increased oxidative stress and lipid peroxidation, which might be due to interaction of various carcinogenic agents, generating free radicals to a greater extent in these patients beyond their defending power or may be due to poor antioxidant system existing in these individuals.

Vitamin C is an important non enzymatic antioxidant this free radical scavenger protects the cell against toxic oxygen radicals. In this study Vitamin C level in oral precancerous patients and healthy subjects is found to be insignificant. This insignificant change may be due to intake through diet. Analysis of lipid peroxidation product Malondialdehyde in patients with oral leukoplakia, oral sub mucous fibrosis, and candidiasis, dental carries, oral cancer and healthy subjects who served as control. They observed elevated levels of MDA in periodontitis, leukoplakia, oral sub mucous fibrosis and cancer compare to controls. They concluded that there was role of free radical in the pathogenesis of various dental diseases [9].

CONCLUSION

There is a significant increase in lipid peroxidation in oral precancerous stages than the normal and also slight decrease in vitamin C level, but decrease in vitamin C level is not significant. It shows that for precancerous condition free radical effect is observed, it may be due to smoking, or any other pan chewing habits.

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