

**Research Article**

**An *In-vitro* study of Lipid peroxidation, Vitamin E and Vitamin C levels in saliva of oral precancerous patients in District Hapur of Uttar Pradesh**

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**Abstract**

**Objective:** The objective of our study was to find out whether vitamin E and vitamins C levels in saliva can be used as a marker for cancer.

**Material and methods:** This study was carried out in the dental OPD of Saraswathi Institute of Medical Sciences, Hapur, U.P. A total of 20 people were selected in our study, out of which 10 were normal subjects and 10 were having oral pre cancerous lesion. The saliva was collected from all the subjects. Saliva was estimated for lipid peroxidation (malondialdehyde MDA), Vitamin E and C levels respectively.

**Results :** Our results indicated significant increase in lipid peroxidation (MDA) in oral precancerous stages but there was decrease in vitamin E and C levels. This suggests that there is an increase oxidative stress in oral precancerous lesion and may further increase in cancerous stages.

**Conclusion:** To prevent free radical damage body has a defense system of anti oxidants which are involved in the prevention of cellular damage – the common pathway for cancer ageing and a variety of diseases. From our study it was concluded that lipid peroxidation, vitamin E and C can be used as a marker for oral precancerous lesion.

**Keywords:** Lipid peroxidation, MDA, Vitamin E, Vitamin C, Oral precancerous lesion

**1. Introduction**

The term oral cancer encompasses all malignancies that originate in the oral tissues. The World Health Organization (WHO) reported oral cancer as having one of the highest mortality ratios amongst all malignancies. Epidemiologic studies suggest that low dietary intake and low plasma concentrations of antioxidant vitamins and minerals are associated with increased risk of cancer.<sup>1,2</sup> The vast majority of malignant neoplasms in the mouth are squamous cell carcinomas (SCC).<sup>3</sup> A neoplasm that is not invasive but has the potential to progress to cancer become invasive.<sup>4</sup> Benign morphologically altered tissue, has more risk of containing a microscopic focus of cancer at diagnosis.<sup>3</sup> 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 present in leukoplakia or erythroplakia.<sup>5</sup>

Lipid peroxidation is the free radical mediated oxidative degradation of the lipids resulting in cell damage. End products of lipid peroxidation malondialdehyde (MDA) reacts with deoxyadenosine and deoxyguanosine in DNA, forming

adducts which may lead to oxidative stress and cancer. The termination being brought about by antioxidants including vitamin C by catching free radicles and thereby protecting the membrane from free radical mediated degradation of lipids.<sup>6</sup> To prevent damage by free radicles, the body has defense system of antioxidants.<sup>7</sup> Vitamin C is one of the free radical scavenging non enzymatic antioxidants.<sup>8</sup> This interacts with free radicals and terminate chain reaction before vital molecules are damaged.<sup>9</sup> Since the 1980s  $\alpha$  – tocopherol and  $\beta$  – carotene have been the most studied vitamins in cancer prevention trials because of their antioxidant properties.<sup>10</sup> Many studies have found that progressively increased MDA and progressively decreased superoxide dismutase and vitamin A levels have positive correlation with clinical grades of oral submucous fibrosis (OSF). These studies 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.<sup>11</sup> In an another study it was concluded that acetaldehyde and lipid peroxidation derived adducts are found in oral tissues of alcohol misusers with oral leukoplakia and cancer; supporting pathogenic role of acetaldehyde and excessive oxidative stress in carcinogenesis.<sup>12</sup> In an another study significantly elevated levels of MDA were observed in leukoplakia, oral sub mucous fibrosis, lichen planus and cancer as compared to controls, indicating role of free radicals in pathogenesis of precancerous lesion and cancer.<sup>13</sup> Hence we wanted to estimate the levels of lipid peroxidation product MDA, vitamin E and vitamin C in oral precancerous lesion and compare it with the levels in normal individuals.

## 2. Materials and Methods

The study was carried out in the department of Dentistry in collaboration with department of Biochemistry, Saraswathi Institute of Medical Sciences, Hapur, U.P. The present study was started in 2012 after obtaining ethical clearance from the Institute. Informed consent was obtained from individual patients. Patients having chronic systemic illness like diabetes, hypertension, liver disease, chronic infection and other co existing lesions of mouth were excluded. A total of 20 people were selected out of which 10 were normal subjects and 10 were having oral precancerous lesion. Saliva of all the individuals were estimated for lipid peroxidation (MDA), Vitamin E and Vitamin C. MDA was estimated by thiobarbituric acid (TBA) method<sup>14,15</sup> Vitamin E by Beri *et al*<sup>16</sup> and Vitamin C by 2-4 dinitro phenyl hydrazine (DNPH) method<sup>17</sup> respectively by using colorimeter. Statistical analysis of data was done by Anova.

## 3. Results

The present study involves the estimation of the levels of lipid peroxidation product (MDA), Vitamin E and Vitamin C in oral pre cancerous condition and in normal individuals. In our study we found that MDA levels were increased significantly ( $P < 0.001$ ) in oral precancerous patients when compared with the healthy subjects (Table I). Significant inverse linear correlation ( $P < 0.001$ ) between MDA levels and natural antioxidants levels (Vitamin E and Vitamin C) were found.

**Table I. Comparison of MDA, Vitamin E and Vitamin C in normal and oral pre cancerous patients**

	MDA ( $\mu\text{mol/L}$ )	Vitamin E ( $\mu\text{mol/L}$ )	Vitamin C ( $\mu\text{mol/L}$ )
Normal individuals (n=10)	0.87 $\pm$ 0.03*	38.22 $\pm$ 2.3*	55.31 $\pm$ 2.6*
Oral pre cancerous lesion (n=10)	1.44 $\pm$ 0.04*	18.36 $\pm$ 1.1*	27.49 $\pm$ 1.8*
P value	P<0.001	P<0.001	P<0.001

\*Result expressed in mean  $\pm$  SD

## 4. Discussion

Free radical induced lipid peroxidation causes a loss of cell homeostasis by modifying the structure and functions of the cell membrane. The most important characteristic of the lipid peroxidation is to cause considerable DNA damage by formation of DNA – MDA adducts.<sup>11</sup> Lipid peroxidation formed under physiological and pathological condition are scavenged by non enzymatic and enzymatic antioxidants. Mammalian cell posses elaborate anti oxidant defense mechanism to neutralize deleterious effects of free radical induced lipid peroxidation. An imbalance between anti oxidation defense mechanism and lipid peroxidation process results in cell and tissue damage.<sup>13</sup>

In this study MDA level was found to be increased significantly in patients having oral pre cancerous lesion then compared with healthy subjects. High levels of MDA in patients having oral precancerous lesion directly reflected increased oxidative stress and lipid peroxidation. This could be due to interactions of various carcinogenic agents generating free radicals to a greater extent in these patients beyond their defending power. And this could also 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 free radicals. In this study, vitamin C levels in the patients having pre cancerous lesion were less when compared to healthy subjects. This change may be because of less intake of vitamin C.

In a randomized trial on patients of cancer on  $\alpha$ -tocopherol and  $\beta$ -carotene supplementation, it was found that these antioxidants could reduce the incidence of primary cancers and improve cancer free survival.<sup>18</sup> Use of vitamin E supplementation reduces the risk of mortality (due to any cause) by 34% and risk of heart disease mortality by 47- 63% and cancer risk by 59% as compared to those who are not using vitamin E supplements.<sup>19</sup> Vitamin E supplementation has shown favorable influence on different prostaglandins made by the body. Prostaglandins effects inflammatory response and immune system and also prevent heart disease and cancer.

In relation to cancer protection, vitamin E and vitamin C reduces free radical induced damage to genetic material (DNA) and helps in prevention of the development of mutation and cancer cells.<sup>20, 21</sup> In a study done by Krajcovicova *et al*<sup>22</sup> a significant inverse linear correlation between MDA levels and Vitamin C and Vitamin E levels were observed. Hennekins *et al*<sup>23</sup>, Crick Patrick *et al*<sup>24</sup>, Taylor *et al*<sup>25</sup>, Gridley *et al*<sup>26</sup> have also found similar effect off Vitamin E supplementation on prevention of cancer. Decreased levels of vitamin C were also observed in a study done by Nidarsh *et al*.<sup>27</sup>

## 5. Conclusion

There is significant increase in lipid peroxidation in oral pre cancerous stages as compared to normal. There is also slight decrease in vitamin E and vitamin C levels. In pre cancerous lesion effects of free radical induced damage are observed, which may be due to smoking or any other pan chewing habits (like tobacco, gutka etc). There is a sufficient existing evidence to suggest that patients suffering from pre cancerous lesion would benefit more from antioxidant enriched multivitamin diet along with the treatment.

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