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Estimation of plasma antioxidants beta carotene, vitamin C and vitamin E levels in patients with OSMF and Oral Cancer - Indian population.

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ABSTRACT

Oxidants and antioxidants may play a role in the later stages of cancer development. There is increased evidence that oxidative process contributes to the promotion stages of carcinogenesis, at this stage the level of antioxidants are very crucial in prevention and progression of carcinogenesis. The human body has several in built mechanisms for defence against free radicals and other reactive oxygen species called anti oxidant systems, they act by scavenging them, suppressing their formation or opposing their actions. Epidemiological studies have suggested that high endogenous levels of pro oxidants and deficiencies in antioxidants level are likely to be important risk in the progression of pre cancer to cancer. Free radicals and other reactive oxygen species are difficult to measure quantitatively. Antioxidant nutrients which play a crucial role against defence of pro oxidants can be measured. If they are reduced from the normal level they can be supplemented. This study aims to evaluate the levels of antioxidants â-carotene, Vitamin C, Vitamin E, the most important antioxidant nutrients in Oral sub mucous fibrosis and Oral cancer. This study resulted in significant decrease in the levels of plasma micronutrients, â-carotene, Vitamin C and Vitamin E were observed as the lesion progresses from normal to pre cancer and then to cancer which in turn reflects the significant increase of oxidative stress in the progression of Oral sub mucous fibrosis to Oral cancer.

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1. Introduction

Oxygen is essential for the survival of human life. Yet, paradoxically, Oxygen is also involved in toxic reactions and is therefore a constant threat to the well being of all living things. Most of the potentially harmful effects of oxygen are believed to be due to formation and activity of reactive oxygen species. Reactive oxygen species are either free radicals that are molecules containing at least one unpaired electron or Reactive non radical compounds capable of oxidizing bio molecules. These free radicals and other reactive oxygen species are called prooxidants [1,2]. Reactive oxygen species are produced continuously in the human body as a consequence of normal metabolic process [3]. If they are

The human body has several in built mechanisms for defence against free radicals and other reactive oxygen species called anti oxidant systems, they act by scavenging them, suppressing their formation or opposing their actions. An antioxidant is defined as any substance that when present in low concentration compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate[3]. In a normal cell there is an appropriate pro-oxidant: antioxidant balance. However this balance can be shifted towards the prooxidants when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called oxidative stress and can result in severe cell damage if the stress is massive and prolonged [3].

Oxidative stress is implicated in the causation and progression of different diseases including atherosclerosis, carcinogenesis, neuro degenerative diseases, chronic inflammatory diseases, radiation damage ageing and various other patho biological effects [3]. Oral cancer is the end point of a multi step process involving a

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sequence of events that occur over a period of time. Oxidants and antioxidants may play a role in the later stages of cancer development. There is increased evidence that oxidative process contributes to the promotion stages of carcinogenesis, at this stage the level of antioxidants are very crucial in prevention and progression of carcinogenesis [4].

Various premalignant changes are seen in the oral cavity among them one important precancerous condition which affects the oral cavity is Oral Sub mucous Fibrosis (OSMF), which has shown an increase the prevalence in different states of India [23]. The condition carries an increased potency for malignant conversion even after the control of causative agents, which is known to play a major role in the development of the disease. There is no report suggesting spontaneous regression and there is no effective or widely accepted treatment. As the process of carcinogenesis occurs by the generation of reactive oxygen species, prevention against it can be effectively addressed by administration of non-enzymatic antioxidants especially β -carotene, Vitamin E and Vitamin C [4].

Epidemiological studies have suggested that high endogenous levels of prooxidants and deficiencies in antioxidants level are likely to be important risk in the progression of pre cancer to cancer [4]. Free radicals and other reactive oxygen species are difficult to measure quantitatively [2]. Antioxidant nutrients which play a crucial role against defence of prooxidants can be measured. If they are reduced from the normal level they can be supplemented [5].

This study aims to evaluate the levels of antioxidants β -carotene, Vitamin C, Vitamin E, the most important antioxidant nutrients in Oral sub mucous fibrosis and Oral cancer.

2.Materials and method:

2.1.Sample distribution:

The study group consisted of a total number of 60 patients. Out of the 60 patients, 20 patients were suffering from Oral sub mucous fibrosis, 20 patients suffering from Oral cancer and 20 patients were normal controls. The sample was then divided into three groups.

Group I : 20 patients who visited the Department of Oral Medicine and Radiology. Among these patients 17 were males and 3 females with the age range from 19 – 51 years (mean 36.45 years) having Oral sub mucous fibrosis.

Group II : 20 patients suffering with oral cancer. Among these patients 15 were males and 05 females with the age range from 33 – 66 years (mean 49.5 years).

Group III : The control group comprises of 20 normal. Among them, 16 were males and 04 females with the age range from 25 – 66 years (mean 44.5 years). Thus the control groups were matched with age and sex of the study group. Informed consent was taken from all subjects before including them in study.

2.2.Inclusion and Exclusion criteria's:

Patients clinically [6] diagnosed as oral sub mucous fibrosis and Oral cancer with available histopathological report were included. Patients with history of diabetes, hypertension and any known diseases were excluded from the study.

2.3.Method:

Permission from the ethical committee had been obtained before starting the study. Consent to participate in the study were obtained from the subjects. Subjects were made to sit comfortably on a dental chair. Sterile hand gloves were used during examination of the patients. Patients were examined under artificial illumination and relevant demographic data were collected. Clinical diagnosis was made and patient showed characteristic features of Oral sub mucous fibrosis and Oral Cancer patients with the available histopathological report. The blood samples were collected with a tourniquet applied 1 ½ - 2 inch above the anti cubital fossa. The area was rendered aseptic with 70% alcohol and using 24 gauge needles and vacutainer 6 ml of blood was drawn, then the tourniquet was relieved and the needle was removed, simultaneously, a sterile cotton was placed on the needle puncture site and instructions were given to apply finger pressure for 5 minutes and dispose the cotton. The collected blood was centrifuged and separated plasma was stored in vials at -20 degree Celsius [49,43] until used for biochemical evaluation. The antioxidant nutrients; β -carotene, Vitamin C, Vitamin E analysis were done by standardized protocols using spectrophotometer. The estimation of β -Carotene is done by using the method of Otto A. Bessey et al [39]. The method of Stanley T. Omaye et al [7] was used to estimate the vitamin C content. Vitamin E was estimated according to the method of Indrajit. D. Desai [8].

2.4.Statistical Analysis:

Mean and standard deviation were estimated in the sample for each study group. Mean values were compared by using one-way ANOVA.

3.Result:

The study group consisted of a total number of 60 patients. Out of the 60 patients, 20 patients were suffering from Oral sub mucous fibrosis (Group I) among them 17 (85%) were males and 3 (15%) were females, 20 patients were suffering from Oral cancer (Group II) among them 15 (75%) were males and 5 (25%) females and 20 patients were controls (Group III) among them 16 (80%) were males and 4 (20%) were females. The sample size and sex wise distribution of subjects were found to be statistically non significant. The subjects were divided into five age groups which are as follows: 15-25 years, 26-35 years, 36-45 years, 46-55 years and above 56 years. It was found that the age wise distribution of subjects were found to be statistically not significant, which means that both the experimental and control groups were similar with respect to age distribution.

Mean β -carotene was highest in Controls (Group III) ($161.4 \pm 53.7 \mu\text{g/dl}$) followed by Oral sub mucous fibrosis patients (Group I) ($128.8 \pm 52.4 \mu\text{g/dl}$) and lowest was in Oral cancer patients (Group II) $97.3 \pm 56.6 \mu\text{g/dl}$. Mean Vitamin C was highest in controls (Group III) ($2.93 \pm 1.35 \text{ mg/dl}$) followed by Oral sub mucous fibrosis patients (Group I) ($2.03 \pm 0.94 \text{ mg/dl}$) and the lowest was in Oral cancer patients (Group II) ($1.41 \pm 0.72 \text{ mg/dl}$). Mean Vitamin E was highest in Controls (Group III) ($1.39 \pm 0.85 \text{ mg/dl}$) followed by Oral sub mucous fibrosis patients (Group I) ($1.24 \pm 0.52 \text{ mg/dl}$) and lowest was in Oral cancer patients (Group II) ($0.72 \pm 0.42 \text{ mg/dl}$). (Table- 1)

4. Discussion:

In the study of plasma levels of β -carotene, Vitamin C and Vitamin E were measured quantitatively in OSMF group, Oral Cancer group with the control group. On comparing the β -carotene levels, it was found that the β -carotene levels was lowest in the Oral Cancer group ($97.3 \pm 56.6 \mu\text{g/dl}$) followed by the OSMF group ($128.8 \pm 52.4 \mu\text{g/dl}$) and the control group had the highest value ($161.4 \pm 53.7 \mu\text{g/dl}$) and Vitamin C was highest in controls (Group III) ($2.93 \pm 1.35 \text{ mg/dl}$) followed by Oral sub mucous fibrosis patients (Group I) ($2.03 \pm 0.94 \text{ mg/dl}$) and the lowest was in Oral cancer patients (Group II) ($1.41 \pm 0.72 \text{ mg/dl}$) which is accordance with study conducted by Nicholas Wald [9], which suggested that serum retinol levels in man have predictive value for subsequent cancer, low levels being most clearly associated with an increased risk of lung cancer and gastro intestinal tract cancers. The association was also independent on age and smoking habits, Radhakrishna Pillai [6]. He found a decreased levels of β -Carotene in patients with oral cancer, Geert van Poppel and Alexandra Goldbohm [10] concluded that smoking may reduce β -carotene concentrations. On comparing the vitamin C levels, it was found that the vitamin C levels was lowest in the Oral Cancer group ($1.41 \pm 0.72 \text{ mg/dl}$) followed by the OSMF group ($2.03 \pm 0.94 \text{ mg/dl}$) and the control group had the highest value ($2.93 \pm 1.35 \text{ mg/dl}$), Vishwa N Singh and Suzanne K Gaby [11], Vitamin C and β -Carotene may reduce the risk of aero digestive cancer; World Health Organization World cancer report 2003 [12] stated that low dietary intake of Vitamin C has been found to be associated with increased risk of cancers of the stomach, mouth, pharynx, oesophagus and less consistently with cancers of lung, pancreas and cervix.

On comparing the vitamin E levels, it was found that the vitamin E levels was lowest in the Oral Cancer group ($0.72 \pm 0.42 \text{ mg/dl}$) followed by the OSMF group ($1.24 \pm 0.52 \text{ mg/dl}$) and the control group had the highest value ($1.39 \pm 0.85 \text{ mg/dl}$). This is in accordance with the study conducted by Soma Gupta et al [13] who found that the plasma beta carotene and vitamin E levels were found to be decreased significantly in patients ($81.7 \pm 14.3 \mu\text{g/dl}$ and $9.3 \pm 0.9 \text{ mg/L}$, $p < 0.01$ respectively) with respect to healthy controls ($110 \pm 20.8 \mu\text{g/dl}$ and $10.1 \pm 1.2 \text{ mg/L}$). H.B. Stahelin, et al [14], in their 12-year follow-up study on plasma antioxidant vitamins and subsequent cancer mortality concluded that lower levels of antioxidants such as beta carotene, Vitamin C & Vitamin E are associated with increased mortality rate of cancer; Anthony. T. Diplock et al [1] suggested that the major antioxidant nutrients Vitamin E, Vitamin C and β -carotene play a beneficial role in prevention of several chronic disorders including cancer. Serge Hereberg [15] stated that a combination of antioxidants including β -carotene, vitamin C & vitamin E, at doses achievable through the diet, may have protective effects on mortality rates and on the total number of cancers among apparently healthy men, with no evident increase in cancer risk.

5. Conclusion

In this study a significant decrease in the levels of plasma micronutrients, α -carotene, Vitamin C and Vitamin E as the lesion progresses from normal to pre cancer and then to cancer which in turn reflects the significant increase of oxidative stress in the progression of Oral sub mucous fibrosis to Oral cancer. Further studies with larger samples may be carried out to validate the findings, which may yield definite conclusion in the assessment of oxidative stress in these diseases.

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6. References:

- [1] Anthony. T. Diplock et al, Functional food science and defense against reactive oxidative species. *British journal of Nutrition*: 1995:80 (suppl) (1),s77-s112.
- [2] Barry Halliwell and Mathew Whiteman, Measuring reactive species and oxidative damage in vivo and in cell culture: How should you do it and what do the results mean? *British journal of pharmacology*: 2004:142,231-255.
- [3] Irshad. M and Chaudhari. P. S. Oxidant-Antioxidant system: Role and significance in human body. *Indian journal of experimental biology*: 2002:40,1233-1239.
- [4] Gibanannnda Ray and Syed Akhtar Husain, Oxidants, Antioxidants and Carcinogenesis. *Indian journal of experimental biology*: 2002: 40, 1213-1232.
- [5] Helmuties and Wilhelm Stahl, Vitamins E and C, α -carotene and other carotenoids as antioxidants. *American journal of clinical nutrition*: 1995: 62(suppl), 1315s-1321s.
- [6] Radhakrishna Pillai et al, Pathogenesis of oral submucous fibrosis cancer: 1992:69(8),2011-2020.
- [7] Stanley. T. Omaye et al, Selected methods for the determination of Ascorbic acid in animal cells, tissues and fluids. *Methods in enzymology*: 1979:62,3-24.
- [8] Indrajeet. D. Desai, Vitamin E analysis methods for animal tissues, *Methods in enzymology*: 1984:105:138-147.
- [9] Nicholas Wald et al. The low serum-Vitamin A and subsequent risk of cancer. *The lancet*: 1980:18,813-815.
- [10] Geert Van Poppel et al, Epidemiologic evidence for α -carotene and cancer prevention. *American journal of clinical nutrition*: 1995:62(suppl) 1393s-1402s.
- [11] Vishwa. N. Singh and Suzanne K Gaby, Premalignant lesions: role of antioxidant vitamins and α -carotene in risk reduction and prevention of malignant transformation. *American journal of clinical nutrition*: 1991:53, 386s-390s.
- [12] World Health Organization, World Cancer report: 2003.
- [13] Soma Gupta et al, Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. *Indian journal of Clinical biochemistry*: 2004:19(1), 138-141.
- [14] Stahelin HB, et al. Plasma Antioxidant Vitamins and Subsequent Cancer Mortality in the 12-year Follow-up of the Prospective Basel Study. *American Journal of Epidemiology*, 133(8), April 15, 1991, p. 766-775.
- [15] Shah N, Sharma PP. Role of chewing and smoking habit in the etiology of oral submucous fibrosis: A case control study. *J Oral Pathol Med*. 1998; 27: 475-479.