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Plasma non-enzymatic antioxidants-vitamin C, E, β-carotenes, reduced glutathione levels and total antioxidant activity in oral sub mucous fibrosis

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Abstract. – *Background:* **Oral submucous fibrosis (OSMF) is a crippling slowly progressive disease of oral cavity that predominantly affects people habit of consuming areca nut and its commercial preparations which generates high levels of reactive oxygen species (ROS) during their metabolism.**

Objective: **The objective of this present study is to evaluate the role of oxidative stress in causation and progression of OSMF by measuring the levels of nonenzymatic antioxidants in OSMF patients.**

Materials and Methods: **For this study we selected 27 newly diagnosed OSMF patients of both sex with age group between 23 to 40 years and the same number of age and sex matched healthy individuals were selected as control group. In both the groups we measured plasma non enzymatic antioxidants like vitamin A. E, C and reduced glutathione. Total antioxidant activity was also assessed in both the groups.**

Results and Conclusions: **We observed a very low levels of plasma non-enzymatic antioxidants ($p < 0.001$) and at the same time a very poor antioxidant activity ($p < 0.001$) in OSMF patients when compared to controls. Therefore, consumption of tobacco or areca quid creates an oxidative stress environment which might plays a major role in the causation of OSMF.**

Key Words:

Oral submucous fibrosis, OSMF, Reactive oxygen species, ROS, Non enzymatic antioxidants, Oxidative stress, Total antioxidant activity.

Introduction

Oral sub mucous fibrosis (OSMF) is a crippling disease that impedes the normal functions of the oral cavity. It is a slowly progressive disease characterized by epithelial atrophy, abnormal accumulation of collagen fibers in the sub-epithelial tissues. It predominantly affects people of South-East Asian origin where chewing of areca nut and its commercial preparations is high. The prevalence in India has been reported as being from 0.2 to 0.5%[1]. The malignant transformation rate of OSMF is found to be 7.6%[2], which mades considerable attention to study the etiopathogenesis of OSMF during recent period.

The most important etiological factors for OSMF are tobacco and areca quid chewing[3], which generates high levels of free radicals. Most of the free radicals generated are oxygen derived hence they are called as Reactive Oxygen Species (ROS). Many epidemiological studies have shown that the process of carcinogenesis occurs by generation of ROS, which act by initiating lipid peroxidation[4]. The extent of oxidative damage caused by ROS can be exacerbated by a decreased efficiency of antioxidant defense mechanism of the body. The adverse effects of ROS are inhibited by cellular antioxidant defense system, which is mainly of two types: non-enzymatic antioxidants like Vitamin A, E and C and the other is enzymatic comprising of SOD, CAT and GSH-Px etc[5].

Giving the established precancerous nature of OSMF[6], and role of free radicals in etiology of cancer, the present study was undertaken to estimate the serum levels of non enzymatic antioxidants like Vitamin A, E, C, reduced glutathione and to evaluate the total antioxidant capacity in OSMF so that appropriate modifications can be made to the treatment modalities for patients suffering from OSMF.

Materials and Methods

This study was approved by Institutional Ethical Committee, and written consent was taken from every participant. Study group comprises 27 newly diagnosed both male and female OSMF

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patients of age between 23 to 40 years who have not received any previous treatment, and or on any antioxidant therapy and were conformed after the detailed case history and histopathological confirmation. For the control group same number of age and sex matched healthy individuals who are non tobacco and areca quid consumers, who were not suffering from any systemic illness, were selected.

From both control and study group 5 ml venous blood was taken with heparinised syringe and centrifuged at 4°C and the plasma was stored at –20°C till the biochemical investigations were done. Plasma β-carotene was estimated by Neeld and Pearson method[7]. The plasma Vitamin C was estimated by Roe and Kuether method[8] in which ascorbic acid reacts with dinitrophenylhydrazine to form a colored complex whose absorbance was read at 520 nm by using Systronics UV-VIS double beem spectrophotometer-2201 model (made in India). Plasma Vitamin E was estimated by Fabianek et.al method[9] in which tocopherol oxidized by ferric chloride and reacts with bathophenenthroline to form pink complex whose intensity was read at 536 nm. Reduced glutathione was estimated by Hissin and Milf method[10] in which GSH reacts with di-thionitrobenzoic acid to form yellow colored complex whose intensity was read at 412 nm. Total antioxidant activity (TAA) was measured by Koracevic et al method[11], in which the capacity of plasma to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate was measured.

Statistical Analysis

A Student t test was applied to assess the statistical difference of the above said biological parameters between OSMF patients and control group. *P* value < .001 was considered highly significant and *p* value < .01 was considered significant.

Results

Non-enzymatic antioxidant defense status of the biological system in OSMF was assessed by measuring plasma beta-carotene, Vitamin E, C, reduced glutathione and TAA. The plasma levels of these antioxidants were compared with control group (Table I). We observed a very low levels of antioxidant vitamins, reduced glutathione and impaired antioxidant activity (TAA) in OSMF when compared to control group ($p < 0.001$).

Discussion

In this study we observed plasma -carotene (mean = 110.9 ± 28.4 µg/L) and vitamin E levels (mean = 4.07 ± 0.47 mg/L) in OSMF cases, which were found to be very low in comparison to healthy control group (634.7 ± 45 µg/L and 10.6 ± 1.1 mg/L) $p < 0.001$. The same decrease in their levels in OSMF was observed by Raina, et al[12]. β-carotene is known to act by trapping and quenching ROS, while Vitamin E is known to be the most potent fat-soluble chain breaking antioxidant[13].

In the present study we observed a very low levels of ascorbic acid (mean 0.43 ± 0.23 mg/dl) when compared with the control group (mean 1.08 ± 0.16 mg/dl) $p < 0.001$. As ascorbic acid is potent water soluble antioxidant the biological system[14] might has utilized it in scavenging/neutralizing an array of ROS species which were produced at very high level because of cigarette smoke or areca quid consumption in OSMF patients.

In addition to scavenging ROS, ascorbic acid can regenerate other antioxidants such as α-tocopheroxyl[15,16], and β-carotene radical cation from their radical species. Thus, β-carotene acts as co-antioxidant for α-tocopherol by converting α-tocopheroxyl radical to α-tocopherol. The vitamin C deficiency might be one of the reasons for the low levels of β-

Table I. Comparison of plasma non enzymatic antioxidant levels and total anti oxidant activity between OSMF patients and control group.

Parameters	Control group (n = 27)	OSMF patients group (n = 27)
β-carotene µg/L	634.7 ± 45	110.9 ± 28.4*
Vitamin C mg/dl	1.08 ± 0.16	0.43 ± 0.23*
Vitamin E mg/L	10.6 ± 1.1	4.07 ± 0.47*
Reduced glutathione mg/L	10.07 ± 0.89	5.92 ± 0.93*
Total antioxidant activity mmol/L	2.51 ± 0.43	0.82 ± 0.14*

*p value < 0.001.

carotene and vitamin E levels in OSMF patients which were observed in this investigation.

In this work we observed the significant reduction of plasma reduced glutathione (mean 5.92 ± 0.93 mg/L) when compared to control group (mean 10.07 ± 0.89 mg/L) $p < 0.001$. It is the most essential and powerful antioxidant which enables other antioxidants, like vitamins A and C, to continuously perform their antioxidant activities effectively[17]. As antioxidants neutralize the free radicals, they themselves are consumed. Reduced glutathione allows antioxidants to be restored to their standard electron configuration and become active antioxidants once again[18]. When GSH levels are high, this process takes place almost immediately after an antioxidant donates an electron. As a result, GSH allows the body to maintain the levels of other functional antioxidants. However, reduced glutathione itself is depleted as it performs its various functions. The depletion in plasma GSH levels might be the key point in depletion of other antioxidants and creation of oxidative stress like environment in OSMF patients.

As plasma non enzymatic antioxidant levels are very low OSMF patients has least capacity to quench ROS. Thus, we observed a very low total antioxidant activity (0.82 ± 0.14 mmol/L) in them when compared to control group (2.51 ± 0.43 mmol/L).

Conclusions

We have observed a very low levels of non enzymatic antioxidants, thus creating oxidative stress, which might be playing an important role in progression of OSMF or transforming OSMF into malignant condition. As very few studies are available on the role of vitamin A and E and, as for the best of the knowledge of Authors, no studies are available so far on the role of GSH, vitamin C and levels of TAA in OSMF. Further studies are required to conform the role of ROS and oxidative stress as etiological factors in OSMF and its transformation into malignancy.

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