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Plasma zinc antioxidant vitamins, glutathione levels and total antioxidant activity in oral leukoplakia

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ABSTRACT

Background: Leukoplakia is a common, potentially premalignant lesion with malignant transformation rate from 1 to 17% with highest transformation rate for the lesions on the floor of the mouth, soft palate and tongue. One of the main etiological factors is consuming areca nut and its commercial preparations which generate high levels of reactive oxygen species during their metabolism. So the aim of this present study is to evaluate the plasma levels of antioxidant vitamins, antioxidant mineral zinc, glutathione and total antioxidant status (TAS) in leukoplakia patients.

Materials and Methods: For this cross-sectional study, we selected 23 newly diagnosed oral leukoplakia patients of both sexes within the age group 28–40 years and the same number of age and sex matched healthy individuals without having history of any systemic illness were selected as control group. In both the groups, we measured plasma antioxidant vitamins A, C, E, antioxidant mineral zinc, GSH and TAS. Student's *t* test was applied and the *P* value <0.001 was considered as statistically significant.

Results: We observed very low levels of antioxidant vitamins A, C, E, antioxidant mineral zinc and antioxidant metabolite GSH ($P<0.001$) and at the same time we also observed very poor (TAS) ($P<0.001$) in leukoplakia patients when compared to patients in control group.

Conclusion: The consumption of tobacco or areca quid which contains high copper levels creates an oxidative stress like environment during their metabolism, might play a major role in causation and propagation of oral leukoplakia.

Key Words: Antioxidants, free radicals, leukoplakia, zinc

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INTRODUCTION

Leukoplakia is a common, potentially premalignant lesion described as a predominant white lesion of the oral mucosa which cannot be defined as any other known lesion.[1] The malignant transformation rates of oral leukoplakia have been reported from 1 to 17% with highest transformation rate for the lesions on floor of the mouth, soft palate and tongue.[2,3] Along with HIV and Epstein-Barr viral infections, there are many etiological factors for leukoplakia; risk is much higher in smokers and users of smokeless tobacco than in people who do not use tobacco products of any kind. Betel nut chewers in Asia are also at high risk. Some preliminary studies[4,5] found that people who drink alcohol are more likely to have leukoplakia compared to nondrinkers. Along with all these factors, many epidemiological studies indicate the strong association of low dietary fruits intake with the development of leukoplakia.[6,7] Smoking, consumption of alcohol and betel nut which contains high copper initiates the formation of high concentration of free radicals and along with

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that low dietary intake of carotenoids and other antioxidants and might play an important role in initiation of leukoplakia lesion development. As many epidemiological studies[8,9] have proved the role of free radicals in the initiation, promotion and progression of carcinogenesis and other chronic inflammatory diseases, the aim of the present study is to evaluate the role of oxidative stress in leukoplakia as important etiological factor by estimating the plasma levels of different antioxidants and total antioxidant status (TAS).

MATERIALS AND METHODS

This study was approved by the Institutional Ethical Committee, and written consent was taken from every participant. Study group comprises 23 newly diagnosed both male and female oral leukoplakia patients of age between 23 and 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. All are smokers or alcoholics and or areca quid consumers. 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 a heparinized 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 the method of Neeld and Pearson.[10] The plasma vitamin C was estimated by the method of Roe and Kuether[11] in which ascorbic acid reacts with dinitrophenylhydrazine to form a colored complex whose absorbance was read at 520 nm. Plasma vitamin E was estimated by the method of Fabianek *et al*.[12] in which tocopherol oxidized by ferric chloride and reacts with bathophenenthroline to form pink complex whose intensity was read at 536 nm. Glutathione was estimated by the method of Hissin[13] in which glutathione (GSH) reacts with di-thionitrobenzoic acid to form a yellow colored complex whose intensity was read at 412 nm. And TAS was measured by the method of Koracevic *et al*.,[14] in which the capacity of plasma to inhibit the production of thiobarbituric acid reactive substances from sodium benzoate was measured. Plasma zinc was estimated by readily available commercial colorimetric kit. Student's *t* test was applied to assess the statistical difference of the above said biological parameters between OSMF patients and control group. *P* value <0.001 was considered as statistically highly significant and *P* value <.01 was considered significant.

RESULTS

Plasma levels of antioxidant vitamins, GSH, zinc (Zn) and TAS of leukoplakia patients were compared with normal control group [Table 1], we observed very low plasma levels of vitamin A, C, E, GSH and TAS in leukoplakia group when compared with control (*P*<0.001). At the same time, we also observed statistically significant decreased plasma Zn levels (*P*<0.001) in leukoplakia group in comparison to control group.

DISCUSSION

In the present study by assessing the plasma levels of antioxidant mineral Zn, vitamins A, C, E and very prominent antioxidant metabolite GSH, we observed the prevalence of oxidative stress like environment in leukoplakia because of the release of high levels of reactive oxygen species (ROS) and free radicals from cigarette smoke, alcohol metabolism. The high copper content of areca quid also contributes in generating free radicals. Khanna *et al*. in their study observed significantly higher serum copper levels in areca quid consuming leukoplakia patients.[15] It has been proved by many experimental studies that higher copper levels lead to the release of very high levels of ROS by Haber-Weiss and Fenton reaction.

$$H_2O_2 + Cu\,(I) \rightarrow Cu\,(II) + OH + OH^-\,(I)\ \text{Fenton reaction.} \quad (1)$$

Table 1: Plasma levels of antioxidant vitamins, GSH, zinc and TAS in leukoplakia and control group

Parameters	Control group (*n*=23)	Oral patients group (*n*=23)
β-carotene µg/l	634.97±45	430.47±74*
Vitamin C mg/dl	1.08±0.16	0.57±0.16*
Vitamin E mg/l	10.54±1.1	5.99±0.82*
Reduced glutathione mg/l	10.09±0.89	6.09±0.67*
Total antioxidant status mol/l	2.47±0.43	1.23±0.45*
Zinc µg/dl	91.2±11.8	59.9±6.91*

**P* value <0.001. GSH: glutathione, TAS: Total antioxidant status

$$H_2O_2 + O2^- \rightarrow O2 + OH + OH^- \text{ (II) Haber – Weiss reaction.} \quad (2)$$

There is substantial evidence that the ˙OH radical generated from the above mechanism can destruct tissue by initiation and propagation of lipid peroxidation by abstracting hydrogen from unsaturated fatty acids [Figure 1].

The ˙OH radical can also abstract hydrogen from amino baring carbon, leading to the formation of carbon centered protein radical that undergoes a series of reactions, resulting in the hydrolysis of amino group and formation of aldehyde or a protein carbonyl group which ultimately leads to inactivation of protein.

In this study, we observed significantly lower plasma Zn (P<0.001) in leukoplakia patients (59.9±6.19 µg/dl) when compared to control group (91.2±11.8 µg/dl). This might be because of the consumption of Zn in counter reacting ROS which are generated due to tobacco or high copper of areca quid metabolism. Many experimental studies demonstrated the inhibitory effect of Zn on transition metal mediated site specific oxidative injury by inhibiting metal induced.OH generation.[16]

In this study, we observed low ascorbic acid levels in leukoplakia group (0.5±0.16 mg/dl) when compared with control group (1.08±0.16 mg/dl) P<0.001. As ascorbic acid is a potent and most effective water soluble antioxidant, the biological system might have utilized it in scavenging/neutralizing an array of ROS which were produced at high levels due to tobacco and betel nut consumption in leukoplakia patients. Tuovinen *et al*.[17] in their study observed low serum ascorbic acid levels in leukoplakia.

In this study, we also observed statistically significant low vitamin E (5.99±0.82 mg/l) and β-carotenes (430.47±74 µg/l) levels in leukoplakia group when compared to control group (P<0.001). Barth *et al*.[18] observed increased reversion of oral mucosa in leukoplakia on treating them with vitamin C, E and β-carotenes, which indicates their strong positive role in decreasing oxidative stress like environment in disease.

Drastic reduction in plasma GSH levels were observed in leukoplakia patients (6.09±0.67 mg/l) in comparison to control group (10.07±0.89 mg/l) P<0.001. It is the principal intracellular non protein thiol that plays a major role in the maintenance of the intracellular redox state and is the most essential and powerful antioxidant which enables other antioxidants like vitamins A and C, to continuously perform their antioxidant activities effectively.[19] It is also the cofactor for selenium-containing glutathione peroxidases, which are major antioxidant enzymes. The depletion of GSH indicates the oxidative stress like environment in oral leukoplakia.

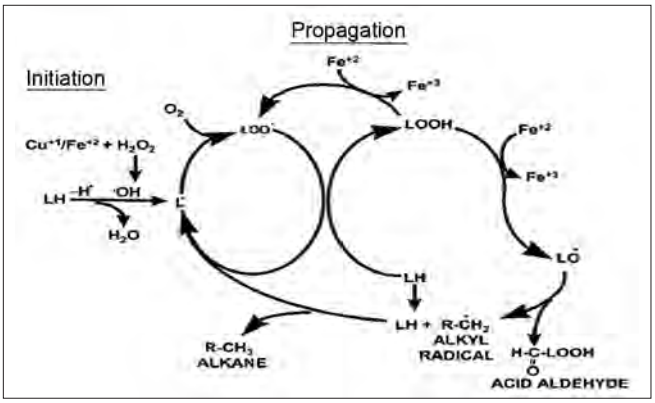

Figure 1: Mechanism of initiation and propagation of lipid peroxidation by copper and other transition metals

TAS parameter summarizes the overall activity of antioxidants and antioxidant enzymes. There is a decrease in TAS in the study group (1.23±0.45) compared with the control group (2.47±0.43) P<0.001, which is mainly due to depletion of antioxidants and antioxidant enzyme system.

CONCLUSION

From this study we observed very low levels of non enzymatic antioxidants and Zn status, thus creating oxidative stress, which might be playing an important role in causation and progression of leukoplakia or transforming leukoplakia into malignant condition. As very few studies are available on the therapeutic role of vitamin A, C, E and no studies are available on the beneficial effect of oral Zn supplementation for leukoplakia treatment. Further studies are required at epidemiological and experimental levels to conform the role of ROS and oxidative stress as etiological factors and beneficial role of oral antioxidant vitamins supplementation in treating leukoplakia and inhibiting its transformation into malignancy.

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