

Evaluation of Antioxidant Supplementation in the Management of Oral Sub mucous Fibrosis and Leukoplakia Patients

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Abstract: The precancerous nature of OSMF and Leukoplakia have been very well established, malignant transformation rates have been reported in both these cases. Tobacco consumption is the most important oral cavity cancer risk factors. Burst of (ROS) have been implicated in the development of oral carcinoma in tobacco and betel quid chewers (1). Epidemiological studies support the protective role of diet rich in antioxidants which could alleviate oxidative stress to some extent. In view of this a preliminary evaluation of chemoprevention with antioxidant supplementation was designed to study the protective effect with clinical response.

Twenty five patients each of OSMF and Leukoplakia were evaluated to study the beneficial role of antioxidant supplementation. Blood samples were collected from patients (Leukoplakia & OSMF), enrolled in Oral Radiology department of Nair college & Hospital. The patients were clinically examined by measuring mouth opening and tongue protrusion in case of OSMF patients and the size of lesion was measured in case of Leukoplakia patients pre and post antioxidant therapy. Blood samples were collected and level of antioxidant enzyme GSH-Px, non enzymatic antioxidant β -carotene and α -tocopherol were determined, plasma levels of TBARS expressed as MDA was determined to find the extent of lipid per oxidation and plasma level of whole blood was used for estimation of Hb.

Enzyme activity was represented as mean \pm SD and the data was analyzed statistically. Student $-t$ test was applied to study the comparison of pretreatment and post treatment therapy. Clinical improvements seen in both the precancerous groups were significant though biochemical parameters such as GSHP-X enzyme and non-enzymatic antioxidant such as β -carotene and α -tocopherol and MDA showed marginal improvement.

Long term supplementation of antioxidant vitamins to the patients is necessary to validate the use of such therapy in precancerous condition and prevent malignant conversion.

Keywords: OSMF – Oral Sub mucous fibrosis, ACD- Acid Citrate, ROS-Reactive Oxygen Species.

1. INTRODUCTION

The bursts of ROS generation occur at different stages of carcinogenesis, and are caused by different mechanisms. In precise, mechanisms by which ROS can directly or indirectly affect neoclassic transformation are still largely unknown. The situation could be due to the ubiquitous presence of ROS, which makes it difficult to attribute one particular role to them. (1) A link between the development of precancerous lesions or oral cancers and the use of particular chewing mixture is well documented. (2). Also the main carcinogenic and / or geotaxis agents released from tobacco or arecanut into the saliva are known.

A Precancerous lesion is a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart, examples are Erythroplakia and Leukoplakia. A precancerous condition is defined as a generalized state associated with a significantly increased risk for cancer; examples are Syphilis and Oral Submucous Fibrosis. (2) Malignant transformation in leukoplakia may occur in the form of a growth or other clinical changes where as OSMF is generally a disease of long duration, the presenaceof oral cancer is a consequence of malignant transformation of the

disease.(2). It has been suggested that a vast majority of OSCC in India arise from preexisting leukoplakias.(4) Leukoplakia is an oral pre-malignant condition with 5-7% malignant transformation rate.(4).Although a number of surgical and medical approaches are tried in the treatment of leukoplakia , there does not appear to be any universally adopted successful treatment .(2) Likewise the incidence of OSMF is increasing like an epidemic , targeting the younger generation . Arecanut is the most important and persistent finding in the history taking of such patients. (5) This precancerous condition is serious and progressive disease involving oral mucosa and carries a high relative risk for malignant conversion.

Observation from animal studies and epidemiologic investigations provide the biologic basis of chemoprevention of cancer. Epidemiologic studies have established the protective role of diets rich in fruits and vegetables in oral carcinogenesis and reduced risk of oral cancer with high intake of vitamins A, C, E and carotenoids .(6,7) It has been reported that leukoplakia can be successfully treated by antioxidant supplementation .(8) Administration of naturally occurring dietary antioxidants in such patients could be one of preventive modality, by conveying their protective effect through its defense system with the aim of preventing further progress of the disease and perhaps reducing the risk of oral cancer. In view of this a supplementation study with antioxidant micronutrient was designed for precancerous (leukoplakia & OSMF) patients and the clinical and biochemical changes were evaluated before and after the treatment.

2. MATERIAL AND METHODS

Twenty five patients each of OSMF & Leukoplakia enrolled in the study were evaluated to study the beneficial role of antioxidant supplementation. Blood samples were collected from patients (Leukoplakia & OSMF) , enrolled in Oral Radiology department of Nair college & Hospital after obtaining their informed consent. A clinical data sheet included history, nutritional status, habits, duration of disease and clinical site in case of leukoplakia patients.

Tobacco Habits in OSMF Patients

Sr. No	Tobacco Habits	No. of Patients
1	Gutkha	8
2	Pan + Supari	5
3	Pan + Pan masala	4
4	Pan masala	2
5	Gutkha + Pan masala	3
6	Supari + Tobacco	3
7	Supari	2
8	Supari + Pan + Gutkha	3

The OSMF cases were divided into Grade II (n= 24) and Grade III (n=6) using the criteria of Bhatt and Dholakia (9).

Tobacco Habits in Leukoplakia Patients

Sr. No	Tobacco Habits	No. of Patients
1	Tobacco Chewing	3
2	Pan + Tobacco	10
3	Beedi + Smoking	6
4	Pan + Supari + Tobacco	2
5	Tobacco + Lime	2
6	Tobacco + Quid	3
7	Pan + Tobacco + Beedi	3
8	Mishri	1

Clinical Site of Leukoplakia Patients

Sr. No	Site	No. of Patients
1	Left Buccal Mucosa	6
2	Right Buccal Mucosa	4
3	Right & Left Buccal Mucosa	8
4	Mandible(Mn) Labial Vestibule	2
5	Mn Labial Vestibule + Labial Vestibule	2
6	Right Buccal Mucosa + Labial Vestibule	2
7	Left angle of mouth	1
8	Right & Left angle of mouth	2
9	Tongue	3

(Antioxidant capsule twice) + Vit E capsule a day was prescribed for 6 weeks. Composition of Antioxidant capsule (manufactured by Dr. Reddys' Lab Ltd)

Beta Caroteen(as mixed carotenoids)	10 mg
Zinc sulfate monohydrate USP	27.5 mg
Selenium Dioxide as selenious acid USP	70 mcg
Magnese (as manganese sulphate monohydrate B.P)	2 mg
Copper (as copper sulphate pentahydrate B.P)	1 mg

Vit E capsule as Tocopheryl Acetate IP-400mg (manufactured by merk Ltd)

The patients put on supplementation were clinically examined by measuring mouth opening (the maximum distance between upper and lower central incisor teeth) and tongue protrusion as described by Kumar & Srivastava (10) pre and post therapy.

The blood was drawn by venipuncture and was collected in ACD bulb. Samples were processed immediately for RBC lysate preparation and the lysate was used for estimation of the enzyme GSH-Px (11). Whole blood was used for the estimation of Hb (12). Plasma was used for MDA (13), β -carotene (14) and α -tocopherol (15).

3. RESULTS

Enzyme activity was represented as mean \pm SD and the data was analysed statistically. Student-t test was applied to study the comparison of pretreatment and post treatment therapy. Following the treatment with Antioxidant capsules after six weeks, OSMF group showed some clinical improvements and amelioration of symptoms in our study. There was an increase of 20% ($P < 0.001$) in mouth opening (**Table No 1**) and increase in tongue protrusion by 14% ($P < 0.01$). Burning sensation in the mouth was reduced by 76% ($P < 0.001$) which proved the efficacy of antioxidant therapy. Antioxidant enzyme GSH-Px activity had exhibited an increase of 11% ($P < 0.01$). Antioxidant β -caroteen expressed an increase of 10% ($P < 0.01$) and α -tocopherol level was raised by 7%. A non-significant drop of 7% was seen in MDA level (**Table No 1**).

In Leukoplakia group clinical improvement shown by the patients following the treatment of antioxidant capsule was encouraging. We found a decrease of 12% ($P < 0.01$) in the size of the lesion as compared to the patients before treatment. The antioxidant enzyme GSH-Px activity was also found to be increased by 10% ($P < 0.01$). Antioxidant vitamin β -carotene

showed a negligible increase of 4% and α -tocopherol with an increase of 11%. Plasma MDA showed a fall of 10% which was not significant (**Table No 2**).

Table No 1 Clinical and Biochemical changes in OSMF patients following Antioxidant Therapy

Variates	Pre – Treatment (n=25)	Post – Treatment(n=25)
Mouth opening(mm)	24.4 \pm 0.64	29.3 \pm 0.71 *
Tongue protrusion(mm)	28.8 \pm 0.65	32.8 \pm 0.63 *
Burning sentation(vas)	6.4 \pm 0.70	1.44 \pm 0.63 **
GSH-Px (IU/gHb)	17.69 \pm 3.0	19.65 \pm 4.8 *
MDA (n mol/ml)	16.92 \pm 2.6	15.68 \pm 2.7 N.S
β -caroteen (μ g%)	83.28 \pm 16.3	91.6 \pm 16.03 *
α -Tocopherol (mg%)	0.97 \pm 0.23	1.04 \pm 0.18 N.S

*P<0.01 as compared to patients before treatment

**P<0.001 as compared to patients before treatment

N.S –Non-significant as compared to patients before treatment

Table No 1 Clinical and Biochemical changes in leukoplakia patients following Antioxidant Therapy

Variates	Pre – Treatment (n=25)	Post – Treatment(n=25)
Size of the Lesion(mm)	37.8 \pm 1.0	33.1 \pm 0.73 *
GSH-Px (IU/gHb)	14.40 \pm 3.09	15.9 \pm 5.08 *
MDA (n mol/ml)	19.27 \pm 4.31	17.40 \pm 3.35 N.S
β -caroteen (μ g%)	76.36 \pm 20.2	78.92 \pm 19.10 N.S
α -Tocopherol (mg%)	0.78 \pm 0.17	0.866 \pm 0.152 *

*P<0.01 as compared to patients before treatment

N.S –Non-significant as compared to patients before treatment

4. DISCUSSION

Epidemiological studies support protective role of diet rich in antioxidants which could alleviate oxidative stress to some extent. Similar to our study **Borle et al**, (16) reported that vitamin A50,000 IU chewable tablets, if given once daily could cause symptomatic improvement. **Rehana Maher et al**, (17) evaluated a combination of micronutrients (vit A,B complex,C and E) and minerals (iron, ca,copper,zinc and magnesium and others) for its efficacy in controlling the symptoms and signs of OSMF patients. Significant improvements in symptoms, notably intolerance to spicy food, burning sentstion and mouth opening was observed at exit. Serum micronutrients at six month showed a significant increase of 87% in retinol level,97% in carotene and 18% in VitE level. **Soma et al**,(18) in their follow up study of OSMF patients with antioxidant tab, 1 tab twice daily for 6 weeks showed improvements in the symptoms. There was an increase in the mouth opening by 50% and tongue protrusion by 26%. As regards antioxidant vitamin they reported 40%, increase in β -caroteen level, 14% in Vit E level and have showed a fall of 23% in MDA level. Another report by **Khanna et al**, (19) evaluated supplementation of Vit A 500IU, Vit C 500 mg and Vit E 400 IU for three months in Leukoplakia patients. Following this treatment a marginal increase in carotene level was observed while a non significant rise was noted in SOD and GSH-Px activity.MDA showed a marginal fall which was no significant.

Against these studies the supplementation tried in the present study was only for six weeks with antioxidant capsule twice daily along with antioxidant capsule twice daily along with Vit E once a day. Therefore the result obtained in this study do not match with any one of these studies, however the clinical improvements seen in the patients was quite encouraging through biochemical parameters such as GSH-Px, GSH, β -caroteen, α -tocopherol and MDA showed marginal

improvements. The reversal or suppression of premalignant lesion is an important strategy against carcinogenesis for the prevention of oral cancer. The fact that a vast majority of Indian population use tobacco, which is a known etiological factor for oral cancer, highlights the need to educate all patients with precancerous condition including common man to discontinue their tobacco use. Cessation of tobacco use should be a primary objective in this endeavor. Administration of naturally occurring antioxidant may have protective effect and prevent the formation of precancerous lesion, induce their remission or inhibit the progression of precancerous lesions into malignant cancer. Long term supplementation of antioxidant vitamins to the patient is therefore necessary to validate the use of such therapy in precancerous condition and prevent malignant conversion. In addition certain tobacco cessation strategies should be employed through appropriate educational and regulatory intervention. Effectiveness of counseling by trained health professionals may improve the awareness about the dreaded effect of tobacco and its consequences.

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