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Research Article

ESTIMATION AND COMPARISION OF SERUM VITAMIN C, VITAMIN E AND REDUCED GLUTATHIONE LEVELS IN ORAL SUBMUCOUS FIBROSIS PATIENTS AND CONTROLS - A STUDY

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ABSTRACT

Background: Oral submucous fibrosis is a common precancerous condition. The primary factor considered in the etiology of OSMF is the habitual use of betel nut and its commercial preparations which generates high levels of reactive oxygen species (ROS). However, several other factors are believed to contribute to the development of OSMF including nutritional and vitamin deficiencies. Aim and objectives: The aim of the study is to evaluate the role of non-enzymatic antioxidants like vitamin C, vitamin E and reduced glutathione in Oral Submucous Fibrosis (OSMF) and thus to evaluate, whether the above antioxidants can be used as a biomarker for the progression of the diseases. Materials and method: For our study 30 newly diagnosed OSMF patients of both sex with age group between 20 to 60 years and the same number of age and sex matched healthy individuals were selected as control group. In both the groups, serum vitamin C, E and reduced glutathione were assessed. Results: The values between serum vitamin C, vitamin E and reduced glutathione in between OSMF patients and healthy groups (n = 50) were statistically significant with P value <0.001. The values of the parameters were decreased with the progression of the duration of habits with the P value of 0.375, 0.081, and 0.204 (not statistically significant) for reduced glutathione, vitamin C and vitamin E. The ROC analysis curve showed 100% diagnostic accuracy for reduced glutathione than vitamin C and E. Conclusion: This study suggests that antioxidants play an important role in OSMF and its progression and very low levels of non-enzymatic antioxidants, thus creating oxidative stress, which might be playing an important role in OSMF and its or transforming OSMF into malignant condition.

Key words:- Serum Vitamin-C, Vitamin -E, oral sub mucous fibrosis patients, reduced glutathione

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INTRODUCTION

Oral sub mucous fibrosis, an insidious chronic precancerous condition of oral cavity which was first described in early 1950s, chiefly occurs in the Indian sub continent [1, 2] It is characterized by blanching, burning. Sensation in the oral cavity and stiffening of the oral mucosa and oro-pharynx, due to excessive collagen formation.

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Its leads to it can occur at any age, but most commonly seen in adolescents and adults especially seen in age between 16 – 35 years [3]

The strongest risk factor for OSF is the chewing of betel quid containing areca nut. The potentially malignant

nature of this condition has been well documented (4). The frequency of malignant transformation reported in the range of 7-13% (Murti et al, Kerala)[5].

Lime which is a major component of betel quid preparations causes changes in oral environment of chewers from neutral to alkaline. Under the alkaline condition areca nut ingredients releases Reactive Oxygen Species (ROS) [6].

The free radicals and other reactive oxygen species are called pro-oxidants. The human body has several in built mechanisms for defense against free radicals and other reactive oxygen species called anti-oxidant systems, they act by scavenging them, suppressing their formation or opposing their action [7].

An antioxidant is a substance significantly delays or inhibits the oxidation of that substrate [7,8]. There is increased evidence that oxidative process contributes to the promotion stages of carcinogenesis, at this stage the level of antioxidants is very crucial in prevention and progression of carcinogenesis[7].

The adverse effects of ROS which were produced at very high level in OSMF patients because of smoking and areca quid consumption were inhibited by cellular antioxidant defense system, which is mainly of two types: non-enzymatic antioxidants like Vitamin A, E and C and the other are enzymatic comprising of SOD (superoxide dismutase), CAT (catalase) and GSH-Px (glutathione peroxidase) etc [9,10]. Giving the established precancerous nature of OSMF 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 E, C and reduced glutathione in OSMF so that nutritional supplements can play a vital role in the treatment modalities for patients suffering from OSMF in addition to steroids[9].

MATERIALS AND METHODS

This study was approved by Institutional Ethical Committee, and written consent was taken from every participant. This study was conducted on 50 subjects. The study group comprises of 30 OSMF patients of both sex between the age group of 20 – 60 years, proven both clinically and histologically. The patients having systemic illness, already treated for OSMF and those who on antioxidant therapy were excluded. For the control group 20 healthy individuals of both age and sex matched, without having any pan chewing habits and systemic illness were selected.

For both study group and controls, around 5ml of venous blood was withdrawn from the vein in anticubital fossa and then was put into a plain heparinised glass test tube. Then the test tube along with the blood was subjected to centrifugation for about 5 - 10 minutes at 2000 rpm. The serum and cells were separated in two vials and stored in the refrigerator.

Vitamin C was determined by Joseph H. Roe and Carl A. Kuether method (12). The solution prepared by using norit filtrate, vitamin C, dinitro phenyl hydrazine, sulphuric acid whose optical density was measured at 520nm using spectrophotometer.

The vitamin E was determined by Baker and Frank method (13). The supernatant was prepared using a serum, xylene and absolute alcohol to which the vitamin E, Dipridyl agent and ferric chloride is added to form a pink colour solution, whose optical density was measured at 520nm using a spectrophotometer.

Reduced glutathione was determined by Beutler et al method (11). The supernatant was mixed with Dinitrobenzoic acid to form a solution whose optical density was read at 412nm by using spectrophotometer.

RESULTS

The independent samples T test is done to compare the mean vitamin C, vitamin E and Reduced Glutathione (mg/L) values between OSMF and control group. Pearson Correlations were done between duration of chewing habits and the above parameters among OSMF cases.

This test was statistically analyzed for both the study and control groups (n = 50) from which the mean vitamin C, vitamin E and reduced glutathione values were compared between both the OSMF and control groups showing the mean of 2.786, 7.869 and 5,131 for OSMF and 4.715, 10.570, 10.500 for controls, which were statistically significant with the P-value of <0.001.

Pearson correlation between the duration of habits and mean reduced glutathione, vitamin C, vitamin E were negative with the P-value of 0.375, 0.081, and 0.204

respectively which is not significant, as the above parameters showed less in their value on the progression of the OSMF. But negative correlation indicates that as the duration of the habits increases the value of the parameters are decreasing.

The ROC curves of vitamin C, vitamin E and reduced glutathione was analyzed statistically which showed the diagnostic accuracy of 92% and 78% for vitamin C and vitamin E. For reduced glutathione the diagnostic accuracy was about 100%.

Table 1:

Variables	Group	N	Mean	Std. Dev	P-Value
Vitamin C (mg/dl)	OSMF	30	2.786	0.818	<0.001
	Controls	20	4.715	0.727	
Vitamin E (mg/L)	OSMF	30	7.869	1.842	<0.001
	Controls	20	10.570	1.590	
Reduced Glutathione (mg/L)	OSMF	30	5.131	0.812	<0.001
	Controls	20	10.500	0.968	

Pearson Correlations between duration of chewing habits and other parameters among OSMF cases

Variables		Duration (Yrs)
Reduced Glutathione (mg/L)	Correlation	-0.168
	P-Value	0.375
	N	30
Vitamin C (mg/dl)	Correlation	-0.324
	P-Value	0.081
	N	30
Vitamin E (mg/L)	Correlation	-0.238
	P-Value	0.204
	N	30

Comparing The Roc Curve Between Vitamin C, Vitamin E And Reduced Glutathione

PARAMETER	DIAGNOSTIC ACCURACY	SENSITIVITY	SPECIFICITY
VITAMIN C	92%	86.6%	100%
VITAMIN E	78%	73.33%	85%
REDUCED GLUTATHIONE	100%	100%	100%

DISSCUSSION

In our study by analyzing the results of the parameters (vitamin C, vitamin E and reduced glutathione) with both the study and control groups (n = 50), which is our aim, showed that mean reduced glutahtione was 5.131 (mg/L) and controls was 10.5 (mg/L) showing the P-value <0.001 which is statistically significant. The mean reduced glutathione was low in OSMF patients when compared to controls. This result was supported by K.S.C. Bose et al (2012) [10]. Reduced glutathione is the most essential and powerful antioxidant which enables other antioxidants, like vitamins A and C, to continuously perform their antioxidant activities effectively. 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 (9). It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the

reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister and Anderson, 1983). Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is the most important cellular antioxidant (16). The mean vitamin C in OSMF patients of our study was 2.786 (mg/dl) and controls was 4.715 (mg/dl) with significant P value < 0.001. Thus, the vitamin C value was low when compared to normal healthy individuals and this result was supported by Aravinth et al (2012) [7]. As ascorbic acid is potent water-soluble antioxidant the biological system 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 (9). Ascorbic acid is a reducing agent which can reduce and thereby neutralize the reactive oxygen species such as hydrogen peroxide (Antioxidants and Cancer prevention, 2007; Ortega, 2006) (16). The mean vitamin E of our study in OSMF patients were 7.869 (mg/L) and controls were 10.570 (mg/L) with significant P value < 0.001. The value of vitamin E was reduced in OSMF patients when compared to controls. This result was supported by Soma Gupta et al (2004). Vitamin E is known to be the most potent fat-soluble chain breaking antioxidant (9). Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties (Herrera and Barbas, 2001). It protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (16). This lipid oxidation gives rise to number of secondary by-products such as Malondialdehyde, which is high in potentially malignant disorders (PMD) and oral cancers [17]. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol. This is in line with findings showing that alpha - tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase (GPX4)-deficient cells from cell death. GPX4 is the only known enzyme that efficiently reduces lipid-hydro peroxides within biological membranes (Herrera and Barbas, 2001; Packer et al., 2001) [16]. On comparing the results of ROC curve of vitamin C, vitamin E and reduced glutathione, the diagnostic accuracy of vitamin C was 92% and vitamin E was 78%, whereas the reduced glutathione showed 100% diagnostic accuracy. This showed that, when compared to vitamin C and vitamin E, the reduced glutathione can be considered as one of the significant biomarker for the progression of the disease.

CONCLUSION:

We have observed 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 C, E and reduced glutathione levels in OSMF. Further studies are required to confirm the role of ROS and oxidative stress (due to low antioxidant capacity) as etiological factors in OSMF and its transformation into malignancy.

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