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Erythrocyte malondialdeyde and antioxidant status in oral squamous cell carcinoma patients and tobacco chewers/smokers

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Abstract

The aim of this study was to evaluate the levels of lipid peroxidation and antioxidants in the erythrocytes of oral cancer patients and tobacco chewers/smokers. 25 patients with biopsy proven oral squamous cell carcinoma(OSCC), 25 cases of tobacco chewers/smokers and 25 age and sex matched healthy control subjects were included in the study. The levels of malondialdehyde (MDA), a lipid peroxidation marker and antioxidants like superoxide dismutase(SOD) and glutathione(G-SH) were determined by colorimetric methods. Erythrocyte MDA levels were significantly increased ($p<0.001$) and erythrocyte SOD and G-SH levels were significantly reduced ($p<0.001$) in OSCC patients compared to control subjects, where as statistically significant increase($p<0.05$) in SOD was observed in tobacco chewers/smokers group. There is a negative correlation between MDA and G-SH in OSCC patients and tobacco chewers/smokers. This study shows that there is higher magnitude of oxidative stress in OSCC patients and a weak antioxidant defense system makes the mucosal cells more vulnerable to the genotoxic effect of reactive oxygen species(ROS). A diet rich in antioxidants may alleviate the oxidative stress to some extent.

Key words: Malondialdehyde, antioxidants, OSCC, smokers

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the head and neck with a worldwide incidence of over 300,000 new cases annually [1]. The major inducer of OSCC is exposure to tobacco, considered to be responsible for 50-90% of cases worldwide [2]. The incidence of OSCC in cigarette smokers is 4 to 7 times higher than in non smokers, when alcohol is also consumed this incidence is even higher. Reactive oxygen species (ROS), have been involved in pathogenesis of several diseases including cancer [3]. Free radical induced lipid peroxidation causes a loss of cell homeostasis by modifying the structure and function of cell membrane. The most important characteristic of lipid peroxidation is to cause a considerable DNA-MDA adducts by interacting with cellular DNA [4]. Though tobacco is considered to be the major inducer of OSCC, there are no reports available on lipid peroxidation in tobacco chewers. Reports on oxidative stress and antioxidant status in OSCC patients are also scanty. Therefore the present study was conducted to evaluate the status of lipid peroxidation and antioxidants in OSCC patients and their comparison with tobacco chewers/smokers and normal healthy control subjects.

Materials and Methods

This study was conducted on patients attending OPD in KMC hospitals and Government wenlock hospital Mangalore. The study was approved by Institutional Ethical committee of KMC Mangalore. Three groups of people were studied after informed consent was obtained from these subjects.

Group I

Included 25 diagnosed cases of oral squamous cell carcinoma (OSCC) with TNM classification between stage 2 and stage 4 .Care was taken in choosing patients who had not received chemotherapy, radiotherapy or both. The age group was between 30 to 60 years of both sexes.

Group II

Included 25 people with 10 years or more history of smoking or chewing tobacco or both, between the age group 30 -60 years of both sexes.

Group III

The control group, included age and sex matched 25 healthy individuals.

Exclusion criteria

Patients with head and neck malignancies, cases with salivary gland, nasopharyngeal malignancies, and cases with a history of chronic inflammatory diseases like tuberculosis, rheumatoid arthritis and diabetes mellitus, cases with history of alcohol consumption were excluded from the study.

Sample collection

5ml of venous blood was collected in EDTA containers from each study subjects under strict aseptic precautions.0.2 ml of whole blood was used for glutathione estimation, rest of the sample was centrifuged at 3000g for 10 minutes. Plasma was discarded. The cells were washed three times in cold saline. The RBC's were then suspended in an equal volume of 0.9% saline and used for the estimation of malondialdehyde (MDA) and super oxide dismutase (SOD).

Lipid per oxidation (MDA)

The method of Stokes Dormandy was followed [5]. Values were expressed as nanomoles per deciliter of RBC taking the molar extinction coefficient as 1.56×10^5.

Glutathione (G-SH)

Whole blood G-SH was measured by the method of Ernest Beutler [6].Values was expressed as milligrams per deciliter.

SOD activity

Was measured using the method of Beauchamp and Fridovich[7]. Values were expressed as units/gm Hb. The hemoglobin content of the erythrocytes was determined by cyanmethemoglobin method [8].

Statistical analysis

Statistical analysis was done using Kruskal-Wallis (H) and Mann Whitney "U" test for comparison of different parameters. Correlation between the values was estimated by spearman's rank correlation.

Results

The erythrocytes MDA levels are very highly increased in both OSCC patients ($P<0.001$) and tobacco chewers/smokers group when compared to normal control subjects. The whole blood G-SH levels are decreased significantly ($P<0.001$) in both OSCC patients and tobacco chewers/smokers compared to control subjects. The SOD activity in the erythrocytes was significantly low ($P< 0.001$) in OSCC patients ,where as statistically significant ($p<0.05$) increase in SOD activity was seen in tobacco chewers/smokers group (Table 1).

The correlation between MDA and the antioxidants G-SH and SOD in various groups is given in Table 2. There is a negative correlation between MDA and G-SH in OSCC group and tobacco chewers/smokers groups

Table 1. *Erythrocyte MDA (n moles/dl), G-SH (mg/dl) and SOD activity (U/gmHb) in OSCC patients, tobacco chewers/ smokers and normal subjects (Mean± SD)*

PARAMETERS	No.	Controls	Tobacco chewers/ smokers	OSCC
MDA±SD (n moles/dl),	25	437.94±96.78	622.55±100.66	722.73±151.63
p-value		-	0.001 vhs	0.001vhs
G-SH±SD (mg/dl)	5	53.06±7.79	40.67±6.85	40.69±7.04
p-value		-	0.001 vhs	0.001 vhs
SOD±SD (U/gmHb)	25	8144.82±1122.4	8701.98±1292.04	5910.77±1419.22
p-value		-	0 .041 sig	0.001 vhs

No.= Number of samples; p-value calculated by Mann Whitney test;
SD = Standard Deviation; sig - Significant; vhs = Very highly significant

Table 2. *Correlations*

Group				GSH	SOD
Normal Controls	Spearman's rho	MDA	r	-.376	-.452
			p	.064	.023 sig
			N	25	25
		GSH	r	__	.202
			p	__	.332
			N	__	25
Tobacco chewers/smokers	Spearman's rho	MDA	r	-.354	.226
			p	.082	.277
			N	25	25
		GSH	r	__	-.093
			p	__	.660
			N	__	25
OSCC	Spearman's rho	MDA	r	-.619	.419
			p	.001 vhs	.037 sig
			N	25	25
		GSH	r		-.269
			p		.020 sig
			N		25

Discussion

The present study shows significant increase in lipid peroxides in OSCC patients and in tobacco chewers/smokers group. The increase in MDA is very highly significant in OSCC patients compared to normal healthy control subjects. Oxygen derived free radicals known as reactive oxygen species (ROS) are involved in neoplastic transformation [9]. The role of ROS in the initiation, promotion and progression of carcinogenesis and the protective role of antioxidant enzymes has been the subject of much speculation with conflicting reports in literature. Nagini, Manoharan, Ramchandran [10] have reported significantly decreased lipid peroxidation levels in oral cancer patients. Their findings suggest a decreased susceptibility of oral tumour tissue to lipid peroxidation. Tumour progression is associated with low levels of malondialdehyde [11,12]. An inverse relationship has been observed between the levels of lipid peroxidation and the rate of cell proliferation [13].

In contrast, enhanced lipid peroxidation with decrease in antioxidant enzymes in oral cancer patients was reported by Beevi, Rasheed, Geetha [14], Manoharan, Kolanjiappan, Suresh, Panjamurthy [15] and Khanna, Thapa, Khanna et al[16] .

Our study reveals similar findings, and increase in MDA in OSCC patients and tobacco chewers/smokers groups is statistically very highly significant.

The magnitude of oxidative stress depends not only on ROS levels but also on the body's defense mechanisms mediated by various cellular antioxidants. Disruption of this delicate oxidant/antioxidant balance in the body seems to play a causative role in carcinogenesis[17, 18] .

Our study indicates highly significant decrease in the levels of SOD activity and G-SH , in OSCC patients. Whereas in tobacco chewers/smokers group statistically significant increase in SOD activity and significant decrease in G-SH were observed. There is a negative correlation between MDA and G-SH in OSCC and tobacco chewers/smokers group. These results indicate that there is increased formation of MDA and inadequate clearance of free radicals by the cellular antioxidants like G-SH and SOD activity. It might be also due to higher magnitude of oxidative stress since all our patients were in advanced clinical stages with a large tumor burden.

The data reported in the literature on antioxidant enzyme SOD in different human cancer types are controversial. Our results showed an increase in SOD activity in smok-

ers and decreased SOD activity in OSCC patients . These findings are consistent with those of others. Guven, Ozturk B, Sayal A, Ozeturk A, Ulutin [19] , Hulea, Olinescu R, Nita S, Kummerow [20] have reported a significant increase in SOD activity in patients with gastric cancer and smokers respectively. The increase in SOD activity observed in our study in smokers may be an adaptive mechanism to counter act the oxidant factors .

In OSCC patients SOD activity is significantly decreased . These results show that the antioxidant enzyme SOD responsible for clearance of ROS is not less in smokers/chewers but is in the OSCC patients. This indicates that MDA increase is not due to tobacco consumption but a consequence of OSCC.

Studies have shown that a diet rich in antioxidants may alleviate oxidative stress to some extent [21]. However the degree of effectiveness with which the antioxidant system can be restored with dietary modifications and nutritional supplements, so that cancer patients can actually be benefited remains to be elucidated.

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