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EVALUATION OF OXIDATIVE STRESS IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Oral Squamous Cell Carcinoma (OSCC) is one of the most common malignancies in India and thus there is a need for prognostic markers as it can make a crucial contribution to the prediction of outcome. In diseased condition, body can enhance the endogenous defense mechanism. However, it has been observed that whenever the level of the cellular antioxidant system goes down or reactive oxygen species reach abnormally high levels, oxidative damage to the cell occurs, which subsequently lead to several pathological conditions including cancer. We designed this study to evaluate the oxidative stress and antioxidant levels in OSCC patients. TBARS levels were increased, whereas the endogenous antioxidant levels were found to be declined in patients with oral cancer. As the disease progresses from precancerous to cancerous state, levels of antioxidants declined further. Hence, these antioxidant markers would be suitable for predicting the prognosis of oral cancer.

KEY WORDS: **superoxide dismutase, oral cancer, catalase, antioxidant enzymes, glutathione peroxidase**

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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. This form of cancer accounts for high rate (about 50%) of morbidity and mortality[1]. Its incidence exhibits a marked geographical variation, with preponderance in developing countries like India [2.] Cells under aerobic environment are always threatened by reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS scavenging is done by enzymatic and non-enzymatic antioxidants[3]. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The non-enzymatic antioxidants include vitamin E, vitamin A, vitamin C and reduced glutathione (GSH). When the balance between ROS production and antioxidant defense is lost, oxidative damage to the cell occurs. Lipid peroxidation, oxidative destruction of lipids, leads towards cellular pathology and ultimately to cell death if they are excessively generated[4]. Oxidative stress has been reported to be involved in oral precancerous conditions such as oral submucous fibrosis (OSMF) and leukoplakia[5]. The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The overall mortality rate of OSCC remains high and still at approximately 50%. In this scenario, investigation of prognostic markers could make a crucial contribution to effective intervention and improving the outcome. Extensive studies documented the role of oxidative stress in oral cancer by estimating various antioxidant enzymes and lipid peroxidation in saliva[3], plasma[6] and tumour tissues[6, 7]. Previous studies clearly stated the involvement of these markers in the oxidative damage in OSCC. However, there were no simultaneous studies on the status of antioxidant enzymes and oxidative stress in oral precancerous conditions in comparison with healthy subjects. Literature review revealed conflicting results about antioxidant levels in OSCC patients. The present study is thus designed to evaluate the levels of oxidative damage and antioxidant enzymes in patients with OSCC as compared to precancerous patients and healthy control subjects.

MATERIALS AND METHODS

Twenty age- and sex-matched healthy controls formed the first group (HC, n =20). Clinically diagnosed and histopathologically confirmed cases of oral pre cancerous (OPC, n = 20) which include leukoplakia, erythroplakia and oral submucous fibrosis, were included in the second group. Twenty histopathologically confirmed oral squamous cell carcinoma (OSCC, n = 20) cases formed the third group. The age ranges from 40 to 60 years. The study was approved by Institutional Human Ethical Committee of Rajah Muthiah Medical College and informed consent was obtained from all subjects before sample collection. Patients with diabetes mellitus or any other systemic diseases were excluded from the study. From each subject, 5 ml of blood was collected in an EDTA tube for obtaining plasma. Samples were separated by centrifuging the blood sample at 3000 rpm for 5 min. Lipid peroxidation (Thiobarbituric acid reactive substances) was estimated by the method of Mahfouz etal[8]. SOD was assayed by the method of Kakkar et al[9] based on the 50% inhibition of the formation of NADH-phenazine methosulphate- nitro blue tetrazolium (NBT) formazan at 520 nm. The values are expressed in U/ml where U is the amount of enzyme required to inhibit 50% NBT reduction. The activity of catalase was assayed by the method of Sinha[10], based on the utilization of H_2O_2 by the enzyme. The color developed was read at 620 nm. The values are expressed in U/ml where 1Unit is the micromoles of H_2O_2 utilized per minute. GPx was estimated by the method of Rotruck et al[11] with modification. A known amount of enzyme preparation was incubated with H_2O_2 in the presence of GSH for a specified time period. The amount of H_2O_2 utilized was determined by the method of Ellman. The values are expressed in U/dl where U is micromoles of GSH utilized per minute. GSH was estimated by the method of Ellman[12] and the yellow color developed was read at 412

nm when 5,5'-di thiobis 2-nitro benzoicacid (DTNB) was added to compounds containing sulfhydryl groups.

Statistical analysis

The values are expressed as mean ± SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by student's t test. The values are considered statistically significant if the p value was less than 0.05.

RESULTS

Table I shows the status of TBARS and antioxidants (SOD, CAT, GPx and GSH) in controls, precancerous and oral cancer subjects. The activities of SOD, catalase, GPx and GSH levels were significantly decreased in oral cancer and precancerous patients as compared to control subjects. The plasma TBARS level was significantly increased from normal to oral cancer patients.

Table I

Status of TBARS and antioxidants in HC, OPC and OSCC subjects

Parameters	Group I(HC)	Group II(OPC)	Group III(OSCC)
TBARS(nm/ml)	2.9 ±0 .49	3.3 ± 0.47 [a]	5.2 ± 0.49[ab]
SOD(U/ml)	18.28 ± 1.3	14.96 ± 1.6 [a]	10.4 ± 2.4 [ab]
CAT(U/ml)	9.76 ± 0.92	6.3 ± 1.77 [a]	1.7 ± 0.46 [ab]
GPx(U/ml)	0.215 ± 0.04	0.190 ±0.05 [a]	0.128 ±0.03 [ab]
GSH(mg/dl)	41.15 ±1.89	32.5 ± 2.3 [a]	26.3 ± 2.4 [ab]

Values are expressed as mean ± SD.
a - significantly different from healthy controls p<0.05
b - Significantly different from precancerous patients p<0.05.
HC- healthy controls, OPC – oral precancerous, OSCC – oral squamous cell carcinoma

DISCUSSION

In order to understand the alteration of the antioxidant status in the progression of oral cancer, we studied the level of antioxidants in precancerous and cancerous patients. We also analyzed oxidative stress marker, TBARS. In our study, we found that the levels of antioxidants were decreased in oral precancerous and OSCC patients when compared to healthy controls. The results suggest that antioxidants like SOD, catalase, GPx and GSH levels would be utilized during the progression of OSCC. In this study, we noticed a significant increase in the level of TBARS in OSCC patients. This is in accordance with the hypothesis that the cancer cells produce large amount of free radicals.[13] In addition to that there would be inadequate clearance of free radicals by the cellular antioxidants as observed in our study. In primary OSCCs, the antioxidant level was declined in precancerous cases when compared with controls and it was further decreased in OSCC patients. This reveals that as the disease progresses the level of antioxidants tends to decline. All the patients participated in our study had the habit of tobacco chewing with or without additives, whereas controls were devoid of any such habits. Smoking, alcohol consumption and betel nut chewing are leading risk factors for the development of oral cancer. There was an inverse relationship between the oxidative stress and the antioxidants. Such correlation has been previously reported [14] and it was shown that SOD and GPx values were decreased in not only OSCC but also in oral leukoplakia and oral submucous fibrosis. Kumar et al[15] found decreased levels of antioxidants with a corresponding increase in the level of free radicals in patients with OSCC when compared with control subjects. Khanna etal[16] and Sulthan Beevi et al [17] have stated that the levels of antioxidants are considerably decreased in oral cancer patients

than in normal individuals. Our observation is also in accordance with their findings. However, Gokul et al[18] and Neetha et al[19] have reported an increase in the antioxidant level and explained that the increase in antioxidants could be a compensatory one as a consequence of increased lipid peroxides. In this study, we also noticed, as disease advances there was decline in antioxidant status irrespective of increase in lipid peroxidation. Prabasheela et al[20] have reported a similar finding in breast cancer patients. Further studies are in progress to evaluate the enzymatic and non enzymatic antioxidants in various clinical stages of oral cancer patients.

CONCLUSION

Estimation of lipid peroxide level along with the activities of antioxidant enzymes in oral cancer as well as precancerous patients could help to assess the prognosis of the patients. Regular assessment of antioxidant status and lipid peroxidation as a screening protocol in high risk subjects could be useful in the follow up of precancerous lesions, which may help to decrease the morbidity and mortality of oral cancer patients. Further supplementation of antioxidants to precancerous and cancerous patients may improve the survival outcome and the life quality.

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Conflict of interest

The authors declared no conflict of interest

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