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Brief Report

Salivary superoxide dismutase levels in oral leukoplakia and oral squamous cell carcinoma; a clinicopathological study

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Key Words
Leukoplakia; Oral cancer; Saliva; Superoxide dismutase

Abstract
In the South-East Asian countries oral cancer is one among the commonly occurring malignancies due to tobacco use and quid chewing habits. Early stage diagnosis of the disease is reported to have a better prognosis. Sialochemistry studies involving antioxidants have recently gained importance as biomarkers for oral cancer risk assessment. The aim of this study was to estimate the levels of superoxide dismutase in saliva of patients with oral cancer, oral leukoplakia and healthy controls. Three study groups comprising of 25 oral cancer patients, 25 oral leukoplakia patients and 25 healthy controls were involved in the study. Saliva sample collected from the patients were evaluated superoxide dismutase by nitroblue tetrazolium (NBT) chloride method. The data obtained were analyzed using the one way ANOVA test. There was a significant difference between the levels of salivary superoxide dismutase in between oral cancer, oral leukoplakia groups when compared to healthy controls. The levels of salivary superoxide dismutase were significantly lower in oral cancer when compared to oral leukoplakia. These results suggest that salivary superoxide dismutase level estimation could be used to determine the risk of oral cancer as a non-invasive modality.

INTRODUCTION

Oral cancer (OC) accounts for about 40% of the malignancies occurring in the Indian subcontinent [1]. Potentially malignant disorders such as oral leukoplakia (OL) and/or erythroplakia carry an increased risk for malignant transformation to OC in the oral cavity [2]. Reactive oxygen species induced DNA damage occurs as the result of chemical attack originating from tobacco [3, 4]. The scavenging of the ROS is done by enzymatic and non-enzymatic antioxidants [5]. The enzymatic antioxidant includes superoxide dismutase (SOD), catalase, glutathione peroxidase. The non-enzymatic anti-oxidants include lipid soluble vitamins, vitamin E, vitamin A, water soluble vitamin C and glutathione [5]. Superoxide dismutase, first discovered by Mc Cord and Fridovich [6], is an intracellular enzyme, which, along with other members of the antioxidant system, scavenges oxygenated free radicals such as the superoxide anion or the hydroxyl radical [7, 8]. Studies have reported alterations in the serum levels of SOD in malignant conditions [9, 10]. The aim of our study was to evaluate the levels of salivary SOD in oral leukoplakia and oral squamous cell carcinoma patients.

SUBJECTS AND METHODS

The study involved 75 subjects with age range of 20 to 65 years reporting to the Department of Oral Medicine and Radiology. Twenty-five clinically diagnosed and histopathologically confirmed cases of oral leukoplakia formed the first group (OL). Twenty-five histopathologically confirmed oral squamous cell carcinoma (OC) cases were enrolled into the second

group. Of the 25 oral squamous cell carcinoma cases, 6 were stage I, 12 were stage II, 5 were stage III, and 2 were stage IV. Buccal mucosa was the most common site of occurrence (18) followed by alveolus (3), tongue (2), and lip (2). Twenty-five age- and sex-matched healthy controls formed the third group (HC). Each of the study groups had five female subjects. Patients with diabetes mellitus and patients with history of previous malignancy were also excluded from the study.

Un-stimulated saliva samples were collected from each of the patients by the spit method in the calibrated test tube. Care was taken to see that the volunteers did not consume food, smoke or chew gum at least one hour before the saliva collection procedure. Salivary SOD levels were determined by Beauchamp and Fridovich method [11]. Saliva was centrifuged at 10,000 rpm for 15 min. The supernatant was used for the assay. For each sample analyzed a corresponding control was maintained.

The beakers as TEST (2.5 ml of methionine, 0.3 ml riboflavin, 0.1 ml NBT and 0.1 ml of salivary filtrate); STANDARD (2.5ml methionine, 0.3 ml riboflavin, 0.1 ml NBT, 0.1 ml phosphate buffer 0.05 M - pH 7.8]; and CONTROL (2.5 ml methionine, 0.3 ml riboflavin, 0.1 ml phosphate buffer 0.05 M - pH 7.8, 0.1 ml salivary filtrate) were subjected to illumination for 10 min in an illumination chamber lined with aluminium foil and fitted with a 15W fluorescent lamp. Following illumination, immediately the optical density of all the reaction mixtures was read at 560 nm; units of enzyme present in the sample were calculated using the formula and expressed as U/mg protein.

The data obtained were subjected to statististical analysis using SPSS version 17 software. One-way ANOVA was used to compare the data among the groups and the differences were considered statistically significant if P values were less than 0.05.

RESULTS

The mean salivary SOD in groups OL and OC were 0.52 and 0.34 U/mg protein, respectively. The mean salivary SOD level of the group HC was 0.95 U/mg protein**.** The mean salivary SOD levels of the male subjects in OL, OC and HC groups were 0.54, 0.35 and 0.97 U/mg protein, whereas the mean salivary SOD levels of the female subjects 0.53, 0.34 and 0.94 U/mg protein, respectively (Table 1).

When the mean salivary SOD level of OL group was compared to HC group a statistically significant difference (P = 0.01) was observed. There was a highly statically significant difference when group HC was compared to OC (P = 0.001). The mean salivary SOD level of OL was higher than the mean salivary SOD level of OC; the difference was statistically significant (Table 2).

Table 1. Mean salivary SOD levels in the male and female subjects of the study groups

Study groups	Male subjects	Female subjects
OL	0.54 U/mg protein	0.53 U/mg protein
OC	0.35 U/mg protein	0.34 U/mg protein
HC	0.97 U/mg protein	0.94 U/mg protein

Table 2. Statistical intergroup differences groups using ANOVA test

Study groups	n	Mean salivary SOD	P value
OL	25	0.52 U/mg protein	0.01
HC	25	0.95 U/mg protein	
OC	25	0.34 U/mg protein	0.001
HC	25	0.95 U/mg protein	
OL	25	0.52 U/mg protein	0.01
OC	25	0.34 U/mg protein	

DISCUSSION

Superoxide dismutase has been considered as of one the most important antioxidant enzymes that regulate the cellular redox state in normal and tumorigenic condition [12]. It has been proposed to be a type of tumor suppressor and this is supported by studies done *in vivo* and *in vitro* showing reversal of tumorigenicity when over-expression of manganese SOD (MnSOD) [13]. Our study showed a consistent decrease in the levels of salivary SOD levels in oral leukoplakia and oral cancer groups. Similar findings were reported in a recent Indian study on erythrocyte SOD in oral cancer and precancer [14].

A study was conducted to evaluate the levels of salivary SOD in oral in smokers; the results revealed significant decrease in the levels of SOD [15]. Erythrocytic SOD was significantly reduced in individuals with tobacco habits [16]. In another immunohistochemical study increased expression of MnSOD in buccal mucosal squamous cell carcinoma was associated with better disease-specific survival [17].

There has been a recent surge in the studies where levels of SOD have been evaluated in human saliva. A study revealed cupper/zinc SOD in human saliva might be useful for estimating the level of oxidative stress caused by cigarette smoke [18]. A more recent study has indicated that decrease in concentrations of major antioxidants like SOD in the saliva of patients with cysts may increase the risk of neoplastic transformation especially in advanced age [19]. Few researchers have even stated that further research should be aimed at examining the possibility of administration of agents as antioxidants or saliva substitutes to the oral cavity of smokers [20].

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It was found that mean SOD activity in gingival crevicular fluid and saliva of smokers were lower when compared to controls and mean SOD levels were lower in heavy smokers when compared to light smokers [21]. In our study we found that the levels of salivary SOD decreased in oral leukoplakia and oral cancer patients when compared to healthy controls. The results suggest that salivary SOD levels could be a useful biomarker in oral carcinogenesis.

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