Evaluation of Salivary Super Oxide Dismutase as a Biomarker in Tobacco Users with and Without Potentially Malignant Disorders- A Comparitive Study

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Abstract
Background: *In the South-East Asian countries oral cancer is one among the commonly occurring malignancies due to tobacco use. Early stage diagnosis of the disease is reported to have a better prognosis. Saliva comprises of a wide range of analytes, oxidative stress related molecules that can be utilized as biomarkers for clinical applications. Sialo chemistry studies involving antioxidants have recently gained importance as biomarkers for oral cancer risk assessment One such oxidative stress related biomarker is superoxide dismutase. The objective of the study was to estimate and correlate the level of salivary super oxide dismutase in controls, tobacco users with and without Potentially Malignant Disorders, so that these levels can be used as a prognostic biomarker in the progression intoPMDs.*
Materials and Methods: *Study subjects were selected from department of Oral medicine and Radiology, Dayananda Sagar College of Dental Sciences, Bengaluru.*
The sample size for the study was 60 patients comprising three groups- Group 1 – control group, Group 2 – Tobacco users without PMD and Group 3 - Tobacco users with PMD with 20 patients in each group. 1 ml of unstimulated saliva was collected from each patient using a spit method. Samples were subjected to centrifugation at 10000 rpm for 15 min .samples are transferred to wells and add working solution to each well.incubate the plate for 20 min at 37^0C. The resulting chromophore is then extracted and the signal at OD 450 nm is determined by micro platereader.
Results: *There was a statistically significant decrease in the level of salivary SOD from control group to tobacco users without PMD to tobacco users withPMD.*
Conclusion: *Salivary SOD can be used as a promising biomarker in the very early diagnosis of oral cancer caused by tobacco use.*
Key words: *Potentially malignant disorders, Super oxide dismutase(SOD), Saliva, Tobacco*

I. Introduction

Oral cancer is the sixth most common malignancy worldwide.Around 300000 patients are estimated to have oral cancer worldwide with prevalence rate of 1.30%[1]. Despite advances in cancer therapies, the five-year survival rate for oral cancer has remained at approximately 50% over the past three decades. As majority of oral cancers arises from potentiallymalignant disorders it is important to diagnose them as early as possible[2]. A major challenge for early diagnosis of the at risk tissue is due to limited ability to differentiate oral potentially malignant disorders at high risk of progressing into invasive Squamous cell carcinoma from those at lowrisk[2].

Oral cavity is exposed to various substances like tobacco use,betel nut chewing and can cause the mucosa to undergo changes, as these substances contain carcinogens and tumour promoters. Tobacco use is known as one of the most important risk factors for oral cancer. Tobacco consumption has a direct correlation with DNA damage, which is an important factor in carcinogenesis.

Reactive oxygen species, free radicals and reactive nitrogen species in inhaled cigarette smoke may play a key role in theonset and development of several inflammatoryoralpathologies.

Free radical formation is naturally controlled by antioxidants[3].

Antioxidants arecapable of deactivating or stabilizing free radicals before they injure cells. Antioxidants are present in all body fluids including saliva. Saliva may constitute a first line of defense against oxidative stress and has protective effects against microorganisms,toxins and oxidants. The enzymatic antioxidant includes superoxide dismutase (SOD), catalase, glutathione peroxidase. Superoxide dismutase, first discovered by Mc Cord and Fridovich , 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 . Studies have reported alterations in the serum levels of SOD in malignantconditions

Today saliva is often used to diagnose systemic and local diseases. The main advantage of this medium is the easy, non invasive sampling method compared with that for blood[4]. Superoxide dismutase, first discovered by Mc Cord and Fridovich , 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[5] . Studies have reported alterations in the serum levels of SOD in malignant conditions.Thus the present study is an attempt to determine whether SOD can be used as a biomarker and the correlation of salivary SOD in normal and in tobacco users with and without potentially malignantdisorders.

II. Material andMethods

This case control study was carried out on patients of Department of oral medicine and radiologyat dayanandasagar college of dental sciences fromjanuary 2017 to march 2018. A total 60 subjects of three groups.

Study Design: case control study.
Study Location: Dayananda sagar college of Dental Sciences.
Study Duration: January 2017 to march 2018.
Sample size:60.

Study group will consist of 60 subjects, divided into three groups with 20 patients in each group.
Group 1(20 normal healthy patients who volunteer for the study; n=20)
Group 2 (20 patients who are tobacco users without PMD ; n=20)
Group 3(20 tobacco users with potentially malignant diseases ; n=20)

INCLUSION CRITERIA
Subjects within the age range of 20-70 yrs
Group I- 20 clinically healthy controls free from oral inflammatory conditions like periodontitis and who do not have the habit of using tobacco or alcohol.
Group II- 20 patients with tobacco habit with no clinical alteration of mucosa.
Group III- 20 clinically diagnosed and histopathologically confirmed cases of tobacco users with PMD.

EXCLUSION CRITERIA
• Patients under going treatment for potentially malignantdisorders.
• Patients with cancer and taking treatment for cancer (surgery ,chemotherapy,radiotherapy).
• Patients having any systemic diseases where there is increase in levels of Super oxide dismutase levels suchas diabetesmellitus.
• Other oral conditions known to decrease salivary super oxide dismutase levels likeperiodontitis.

III. Methodology

Patients were selected after taking a detailed case history and after considering the inclusion and exclusion criteria. The history of a patient was recorded on a preformatted case sheet. Patients with potentially malignant disorders were selected after confirmation from histological report. The informed consent was obtained from the patient before collecting the sample and also before doing any investigative procedure. Care was taken to see that the volunteer did not consume food or chew gum at least 1 hour before and smoke three hours before the saliva collection procedure.

Unstimulated whole saliva measuring 1 ml was collected using a spit method, wherein patient was asked to sit during the procedure .Then patient was asked to spit the pooled saliva into the sterile centrifugation tube. Sample was transported in a thermacol box containing ice pack. The sample was subjected to centrifugation at 10,000 rpm for 15 min and precipitate is stored at−80°C. Supernatant was collected and stored in the refrigerator at 4°c until the test is carriedout.

IV. Results

Out of the 20 subjects in the group 1 i.e healthy control group the mean of concentration salivary SODwas found to be0.80U/mg.Out of the 20 subjects in the group 2 i.e Tobacco users without PMD the mean of concentration salivary SOD was found to be 0.56 U/mg Out of the 20 subjects in the group 3 i.e Tobacco users with PMD the mean of concentration of salivary SOD was found to be0.35U/mg. (Table 1 and Graph 1) To evaluate the effects of duration of tobacco habit on the levels of salivary SOD, ANOVA test was used. Smokers with potentially malignant disorders have low p value(0.16) when compared to smokers without potentially malignant disorders(0.48).

To compare the effect of frequency of tobacco consumption on the salivary SOD concentration, ANOVA teat was applied. Smokers with potentially malignant disorders have low p value(0.59) when compared to smokers without potentially malignant disorders(0.90).(Table 2)

	N	Minimum	Maximum	Mean	Std deviation
Smokers with PMD	20	.26	.44	.35	.05
Smokers without PMD	20	.39	.88	.56	.11
Healthy control group	20	.54	1.10	.80	.15

Table 1: MEAN DISTRIBUTION OF THE SOD PROTEIN ENZYME
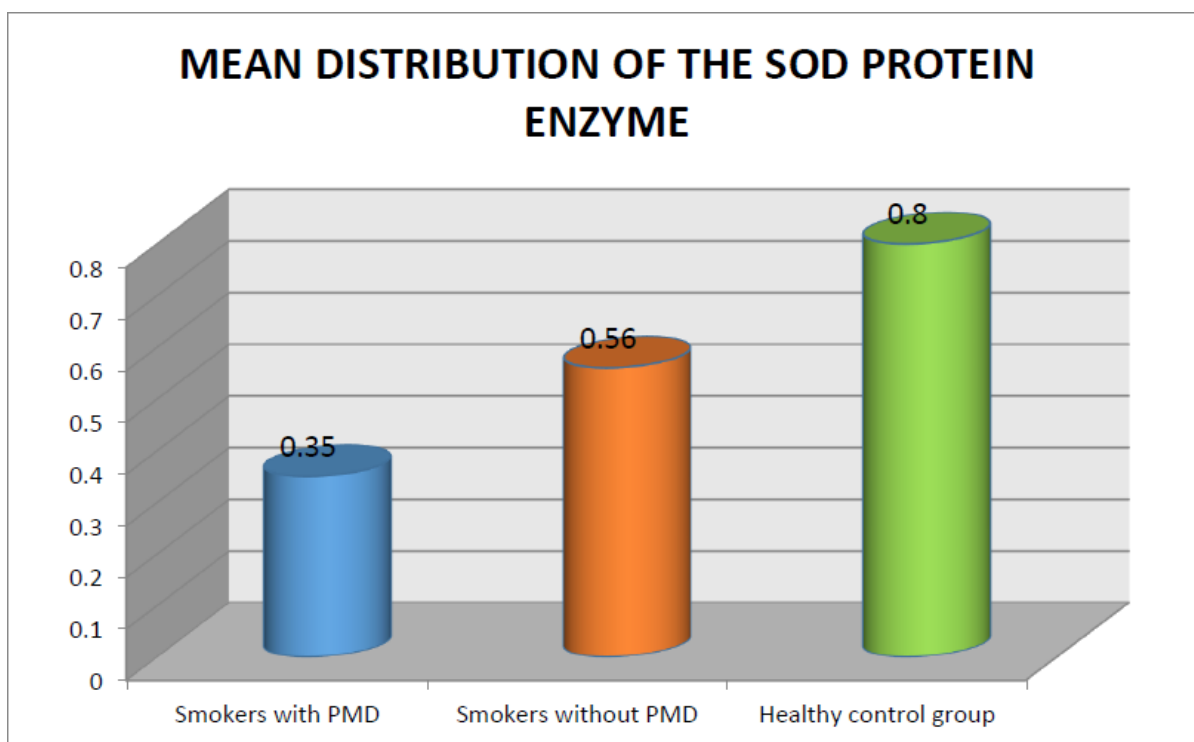
Graph 1 MEAN DISTRIBUTION OF SOD PROTEIN ENZYME

		P Value
Smokers with PMD	SOD* Frequency	0.59
	SOD * Duration	0.48
Smokers without PMD	SOD* Frequency	0.90
	SOD * Duration	0.16

Table2: PERSON'S CORRELATION OF SOD WITH FREQUENCY AND DURATION

V. Discussion

Majority of oral cancers arises from potentially malignant disorders it is important to diagnose them as early as possible as it there is high risk of progressing into invasive Squamous cell carcinoma[6]. There are many ways to diagnose potentially malignant disorders but biopsy is gold standard. Even though biopsy and pathohistological findings are gold standard in diagnosis of potentially malignant disorders it is an invasive procedure. To over come this condition several methods which are non invasive like biomarkers have been used as alternative method. Biomarkers are mostly present in serum and saliva[7].

Even though the determination of serum biomarkers is accepted as a valuable tool for diagnosis, it is an invasive procedure and also expensive when compared to the salivary biomarkers[7,8]. Superoxide dismutase is one of such biomarker which has been considered as of one the most important antioxidant enzymes that regulate the cellular redox state in normal and tumorigenic condition.

It was first discovered by Mc Cord and Fridovich, 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. Three different types of superoxide dismutase (SOD) consist of Fe-SOD, Mn-SOD, and Cu-

Zn-SOD.Cu-Zn-SOD was found in1969 by McCord and Fridrich and is a 32-kDa molecule, which is present in all body tissues and some body fluids, including saliva[9].

Smoking has a major role in developing oral cancerous and precancerous lesions. Saliva is the first body fluid to encounter cigarette smoke. The salivary antioxidant system plays a very important role in the anticarcinogenic capacity of saliva and includes various enzymes and molecules, such as super oxide dismutase(SOD)[9]. The activity of SOD elevates protect the body against the harmful effects of smoke. Reactive O2- is converted by SOD into H2O2. In the next step, H2O2 is converted into H 2 and O 2by salivary enzymes, ie catalase, peroxidase, and glutathione peroxidase).

Several studies were conducted to evaluate the relation between smoking habit and super oxide dismutase levels.A comparative study conducted by Barvandetalover 30 individuals with smoking habit and 30 individuals with non smoking habit showed a The mean value of superoxide dismutase activity was significantly higher in the smoking group (P less than 0.001), while no detectable activity level was found in nonsmokers[10].

Super oxide dismutase levels are lower in potentially malignant disorders, which leads to the disturbance in antioxidant defence mechanism leading to increase in production of free radicals, reactive oxygen species which causes cancer[11,12].

In the present study, we have observed a consistent progressive decrease in the salivary SOD acid level from normal to tobacco users without PMD to tobacco users with PMD. Based on our findings and previous reports, we can suggest that salivary SOD can be used as a biomarker in prevention of PMDs to oral cancer.

VI. Conclusion

In our study super oxide dismutase levels in tobacco users with PMD when compared with the tobacco users without PMD and normal subjects has shown significant changes. The levels were found to be statistically decreased from healthy control to tobacco users with PMD to tobacco users without PMD.

As significant decrease is observed in the levels of super oxide dismutase in saliva with good positive correlation, this study suggests the use of super oxide dismutase as a potential adjunctive biomarker for PMDs and oral cancers. Furthermore, saliva could be used as a reliable, non-invasive tool in diagnosis, screening and monitoring of oral potentially malignant conditions and oral cancers.

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