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ORIGINAL RESEARCH

Evaluation of Salivary Antioxidant Enzymes among Smokers and Nonsmokers

Tajinder Kaur Saggu, KMK Masthan, Mahesh Pundaleek Dudanakar, Shams Ul Nisa, Shankargouda Patil

ABSTRACT

Background: Cigarette smoke contains various oxygen-free radicals which are considered as the main causes of damage to biomolecules when exposed to cigarette smoke. Saliva is the first biological fluid that encounters inhaled cigarette smoke (CS) and has an antioxidant defense system able to counter toxic activities of free radical species. So, the aim of this study was to compare the levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in saliva of smokers and nonsmokers.

Materials and methods: Unstimulated saliva of 200 males (100 smokers and 100 nonsmokers) was collected. The saliva was centrifuged and the activity of salivary superoxide dismutase and glutathione peroxidase was measured according to a specific assay.

Results: The mean value of superoxide dismutase activity was significantly higher in the smoking group than in the nonsmoker group, while the levels of GSH-Px activity was significantly higher in the nonsmoking group than in the smoking group.

Conclusion: Cigarette smoke leads to an alteration in salivary antioxidant activity. Evaluating the variations in the level of antioxidant enzymes (SOD and GSH-Px) in smoker's saliva might be useful for estimating the level of oxidative stress caused by cigarette smoke. Thus, it may help in patient's education regarding the ill-effects of smoking and determining the evolution and progress of various oral diseases.

Keywords: Antioxidant enzymes, SOD, GSH-Px, Cigarette smokers, Cigarette nonsmokers, Saliva.

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INTRODUCTION

Cigarette smoking is well known to be a major cause of various health disorders. It contains many toxic components, such as carbon monoxide, hydrogen cyanide, benzopyrene and oxygen radicals.[1,2] These components can predispose to different systemic disorders, such as cardiac diseases, cancers, precancerous lesions and pulmonary disorders.[3-7]

Their levels depend on the balance between their rates of production and their rates of clearance by the endogenous antioxidant systems, including superoxide dismutase (SOD), catalase, the glutathione redox cycling enzymes, glutathione peroxidase (GSH-Px) and reductase (GRd) and glutathione itself.[8]

One of the principal reactive oxygen species produced in aerobic organisms is O_2^-, which is highly cytotoxic. With the cytotoxicity of this oxidant, exposure to cigarette smoke results in increased levels of antioxidant enzymes, such as catalase (CAT), copper/zinc superoxide-dismutase (Cu/Zn SOD), peroxidase (POx) and glutathione peroxidase

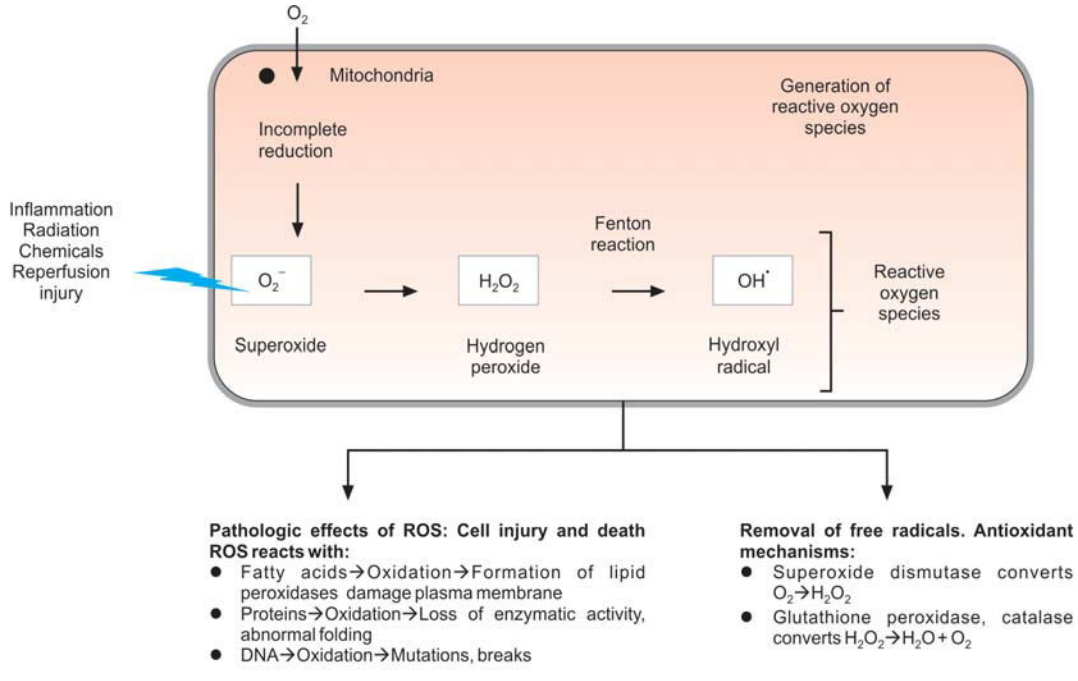


Fig. 1: Diagrammatic representation of production of free radicals, their effects and removal by antioxidant enzymes

(GSH-Px).[9] The higher reactive O_2 is converted to H_2O_2 by SOD. CAT, POx or GSH-Px can in turn convert H_2O_2 to molecular oxygen and water (Fig. 1).[10,11]

Under physiologic conditions, these systems tend to maintain a stable state called redox homeostasis.[12] The imbalance between the formation of free oxygen radicals and inactivation of these species by antioxidant is capable of causing damage to various cellular and extracellular constituents.[13]

Saliva is the first body fluid to encounter cigarette smoke.[14] The salivary antioxidant system plays a very important role in the anticarcinogenic capacity of saliva and includes various enzymes and molecules, such as uric acid and peroxidase system.[14]

Therefore, in the present study, the effect of smoking on the salivary SOD and GSH levels were measured and compared with that of the nonsmokers.

MATERIALS AND METHODS

Subjects

Unstimulated saliva samples were collected from 200 male patients (aged 20-60 years) who were visited to Sree Balaji Dental College and Hospital, Chennai, for routine dental check-up. Among these patients, 100 were smokers (smoking more than 10 cigarettes per day since 5 years) and the remaining 100 were nonsmokers (who had never smoked cigarette). All the participants were informed about the nature of study. All the subjects were healthy without any previous history of systemic disease.

Collection of Saliva

The participants were asked not to eat or drink 2 hours prior to saliva collection. The smokers were also prohibited from smoking for 1 hour prior to sample collection. Sampling was performed in a quiet room between 9 am and 12 pm to prevent any variations which may be attributable to the circadian rhythm. The participants were instructed to rinse the mouth using distilled water. The unstimulated saliva was collected for atleast 5 minutes in a container and kept at low temperature. Following saliva collection, the samples were centrifuged to remove cell debris for 5 minutes at 2000 to 2500 rpm. The supernatant was stored at 4ºC until tests were performed.

Determination of Antioxidant Enzymes

The levels of salivary Cu/Zn SOD and GSH-Px were measured using standard procedures.

Salivary SOD Analysis

Superoxide dismutase was assayed by the method of Misra and Fridovich (1972). The collected saliva was diluted with water. Around 0.25 volume of chilled ethanol and 0.15 volume of ice-cold chloroform were added. The mixture was shaken well for a minute at 4°C and then centrifuged. The supernatant was taken for the enzyme assay. Tube containing 0.5 ml of the carbonate buffer and 0.5 ml of the EDTA solution, 0.5 of enzyme was added. Their final volume was made to 2.5 ml with distilled water and the reaction was initiated by the addition of 0.2 ml of epinephrine and the increase in absorbance at 480 nm was measured in a spectrophotometer.

Salivary GSH-Px Analysis

Glutathione peroxidase was assayed by the method of Rotruck et al (1973). Around 0.2 ml each of EDTA, sodium azide, glutathione and hydrogen peroxide together with suitable volume of buffer and enzyme were mixed together to give a final concentration of 0.08 mM, 1.0 mM, 0.4 mM, 0.25 mM and 0.08 mM respectively in a total incubation volume of 2 ml. Incubation was carried at 37°C and the reaction was terminated at 1 minute intervals by the addition of 5% TCA. A 'zero time' was also carried out simultaneously by addition of TCA prior to the enzyme. To determine the residual glutathione content, the contents were centrifuged and to 2 ml of the supernatant added 8 ml of phosphate solution followed by 1 ml of DTNB and read immediately at 412 nm in a spectrophotometer.

Statistical Analysis

The mean values of SOD and GSH-Px between the saliva of smokers and nonsmokers were analyzed using the Student t-test. The data were processed using the statistical package SPSS version 15.0. A probability value of $p < 0.05$ was set as statistically significant.

RESULTS

The mean levels of salivary SOD were significantly higher (< 0.001) in the smoking group than in the nonsmoking

Table 1: Mean level of SOD enzyme in saliva from smokers and nonsmokers

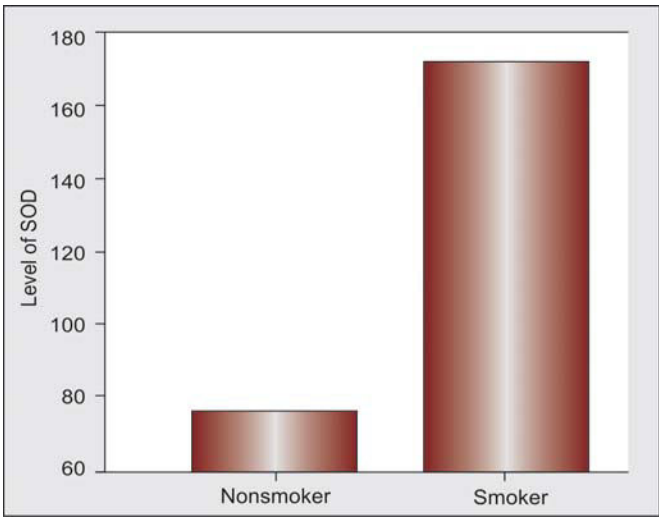
	Group	*N*	*Mean*	*Std. deviation*	*p-value*
SOD	Nonsmoker	100	77.2050	4.84625	<0.001*
	Smoker	100	171.5220	11.58909	

*p-value (< 0.001): Highly significant; SOD: superoxide dismutase

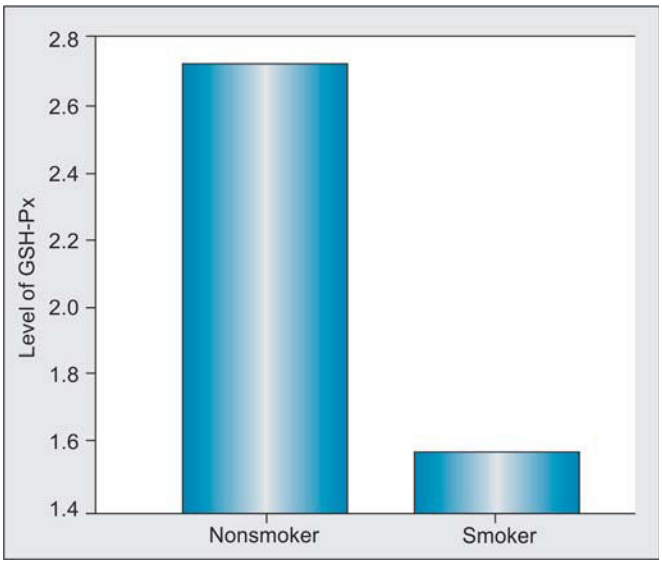
Table 2: Mean level of GSH-Px enzyme in saliva from smokers and nonsmokers

	Group	*N*	*Mean*	*Std. deviation*	*p-value*
GSH-Px	Nonsmoker	100	2.7180	0.52960	<0.001*
	Smoker	100	1.5610	0.39642	

*p-value (< 0.001): Highly significant; GSH-Px: Glutathione peroxidase



Graph 1: Mean level of SOD enzyme in saliva from smokers and nonsmokers



Graph 2: Mean level of GSH-Px enzyme in saliva from smokers and nonsmokers

group is shown in Table 1 and Graph 1, whereas the specific activity levels of GSH-Px was significantly higher (<0.001) in the nonsmoking group than in the smoking group shown in Table 2 and Graph 2.

DISCUSSION

Oxidative stress status may lead to large number of diseases, including precancerous and neoplastic lesions of the oral cavity which may be due to the altered levels of salivary antioxidant system that fails to cope with the altered level of oxidative stresses originating due to cigarette smoke. The main objective of our study was to measure the activity of antioxidant enzymes in the saliva of smokers and nonsmokers.

The results of our study showed that the mean levels of SOD were significantly higher in the saliva of smokers than nonsmokers. The present study is consistent with the results of previous studies.[3,15,16] The elevated levels show the defensive system of the body occurring to reduce the free radicals produced by cigarette.

GSH-Px acts as a powerful antioxidant defense system in our body, which converts H_2O_2 produced by SOD into nonharmful molecular oxygen and water.[3] Our results indicate that exposure to cigarette smoke caused a statistically significant decrease in the levels of GSH-Px in the saliva of smokers than nonsmokers. These findings were supported by various previous studies.[3,17,18] We found out that the cigarette smoke may alter the detoxification of hydrogen peroxide through a decrease of GSH-Px activity.[17] The overproduction of H_2O_2 may leads to increase consumption of reduced glutathione and thus sufficient amount of reduced glutathione may not be available for the detoxification of H_2O_2 leading to elevated oxidative stresses that is involved in a large number of diseases, including precancerous and neoplastic lesions of the oral cavity.[3,17]

The altered levels of antioxidant enzymes may have a consistent role in the mechanism by which the toxic effects of cigarette smoke initiate oral inflammatory diseases, promote precancerous transformation and destroy the oral cavity homeostasis.[18]

CONCLUSION

Measurement of antioxidant enzymes in human saliva might be useful for estimating the level of oxidative stress caused by cigarette smoke.

The purpose of the current study was to measure the antioxidant levels of the saliva in smokers and nonsmoker patients. This analysis may well be of great importance for further understanding the relation between saliva and free radicals. Therefore, more research aimed at examining the altered levels of salivary antioxidants profile in smokers should be considered.

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