Caspian J of Dent Res

Original Article

Salivary superoxide dismutase activity in the consumers of paan containing tobacco

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Abstract

Introduction: The habit of smokeless tobacco chewing is one of the known risk factors for oral cancer among the residents of southeast of Iran. Most likely, the antioxidant defense system in dealing with free radicals induced paan and prevention of oral cancer is important. In this study, the activity of super oxide dismutase is compared in the saliva of paan consumers and non-consumers.

Methods: In this study, Unstimulated saliva of 87 subjects (47 paan consumers and 40 non-consumers) who referred to the Oral Medicine Department of Dentistry School of Zahedan was collected. The activity of super oxide dismutase enzyme was measured by standard biochemical methods (Mc Cord and Fridovich) and the obtained data were analyzed by statistical software SPSS-15 through non-parametric Mann-Whitney test.

Results: The mean activity of super oxide dismutase was significantly higher in the paan consumers group (4.4±1.6 u/mg) compared to non-consumers (3.59±1.8 u/mg, p=0.027).

Conclusions: The results of this study demonstrate that consumption of paan leads to increased activity of salivary super oxide dismutase.

Keywords: Antioxidants, Saliva, Smokeless tobacco, Superoxide dismutase

Received: 28 Oct 2013 **Accepted:2 Feb 2014**

Citation for article: *Farhadmollashahi L, Nakhaie AR, Honarmand M, Arbabikalati F, Ghahari P. Salivary superoxide dismutase activity in the consumers of paan containing tobacco. Caspian J Dent Res 2014; 3: 8-13.*

[DOR: 20.1001.1.22519890.2014.3.1.7.7]
[DOI: 10.22088/cjdr.3.1.8]

فعالیت سوپراکسید دسموتاز بزاقی در مصرف کنندگان پان محتوی تنباکو

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چکیده

مقدمه: جویدن تنباکوی غیر تدخینی به عنوان یکی از ریسک فاکتورهای شناخته شده سرطان دهان در ساکنان جنوب شرقی ایران میباشد. احتمالا سیستم دفاع آنتی اکسیدانی در مقابله با رادیکالهای آزاد ناشی از مصرف پان و همچنین در جلوگیری از ایجاد سرطان دهان مهم است. در این مطالعه فعالیت آنزیم سوپر اکسید دسموتاز در بزاق افراد مصرف کننده پان و افراد غیر مصرف کننده، مقایسه شده است.

مواد و روش ها: در این تحقیق بزاق غیر تحریکی ۸۷ فرد مراجعه کننده (۴۷ مصرف کننده پان و ۴۰ نفر غیر مصرف کننده) به بخش بیماری های دهان دانشکده دندان پزشکی زاهدان جمع آوری شد. میزان فعالیت آنزیم سوپر اکسید دسموتاز بر پایه روش استاندارد بیوشیمیایی (Mc Cord and Fridovich) اندازه گیری شد و اطلاعات به دست آمده توسط نرم افزار آماری SPSS 15 و توسط آزمون نان پارامتریکmann_ hitney، آنالیز شد.

یافته ها: میانگین میزان فعالیت آنزیم سوپر اکسید دسموتاز در گروه مصرف کننده پان (۴/۴±۱/۶ u/mg) به طور معنی داری بالاتر از گروه غیر مصرف کننده (۳/۵۹±۱/۸ u/mg، p=۰/۰۲۷) بود.

نتیجه گیری: نتایج این مطالعه نشان میدهد که مصرف پان موجب افزایش فعالیت سوپر اکسید دسموتاز بزاقی میشود.

واژگان کلیدی: آنتی اکسیدان، بزاق،تنباکوی بدون دود، سوپر اکسید دسموتاز

Introduction

Paan is a combination of areca nut, slaked lime, catechu, tobacco, sweeteners, andspices (1, 2) and contains various carcinogenic compounds including reactive oxygen species, Arecoline (3), Tobacco specific nitrosamines (3, 4).

It isarisk factor fororal cancer, hypertension, dyslipidemia, miscarriage, low birth weight, diabetes, and asthma exacerbation (5, 6). Based on recent research, long-term use of smokeless tobacco can produce free radicals (7). The seinclude super oxideanion (O_2^-), hydroxyl radical ($HO^•$), peroxy radicals ($ROO^•$), and hydrogen peroxide (H_2O_2) (8). Free radicals are also called reactive oxygen species (ROS) which form following chewing areca nut and catechu at pH>9.5 (9). Free radicals can change the structure of intracellular and extracellular components such as proteins, lipids, and DNA and interfere with cell function (10).

Antioxidants are the body's defense system that neutralizes the destructive effects of ROS and minimize damage to cells. As the first defensive line, salivahas a protective antioxidant system that fights against oxidant-induced damage (11). One the most important antioxidant enzymes which regulate

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[DOI: 10.22088/cjdr.3.1.8]

oxidation-reduction process of cell sinnormal and tumorogenic conditionsis superoxide dismutase (SOD) (12). There are three types of superoxide dismutase including Fe-SOD, Mn-SOD and Cu-Zn SOD. SOD contains copper and zinc and is found inall body tissues as well as in some body fluids, in particular saliva (13).

SOD converts O_2 to H_2O_2 during its catalytic activity. (14) So far, several studies with contradictory results were carried out on the antioxidant enzymes such as SOD in the saliva of smokers (13-17), while no study was performed in this regard on smokeless tobacco consumers.

In the present study, we intended to compare the activity of SOD; the body's most important antioxidant enzyme, in the saliva of paan consumers and non-consumers. This study could lay the ground for research on the prevention of adverse effects of paan in oral cavity through antioxidant defense system of saliva.

Methods

Subjects: According to previous studies (16) the sample size in confidence interval 95% and power of test 80% was determind. In this cross sectional study, 47 paan consumers who used daily atleast one packet of 10 gram paan for atleast one year and 40 age and sex matched non-consumers referred to Dentistry School of Zahedan were selected through simple sampling method. Any factors that might lead to imbalance oxidant/antioxidant system in the exclusion and inclusion criteria were considered.

Inclusion criteria:

1. Healthy individual
2. Desire to participate in the study

Exclusion criteria:

1. Suffering from any systemic disease.
2. Consumption of immuno suppressive and non-steroidal anti-inflammatory drugs, antioxidants and vitamin supplement sins last three months.
3. Smoking and consumption of alcohol.
4. Oral cavity diseases such asaphthous, leukoplakia, periodontitis (pocket>3mm), etc.

All participants were informed about the study and a written consent was obtained regarding their participation in the project. The study, was approved by the Ethics Committee of Zahedan University of Medical Sciences.

Collection of saliva

The participants were asked to avoid eating, drinking, and brushing 2hours before sampling. All samples were collected between 9 am to 11 am.

During sample collection, whilst seated and slightly bent forward, the subjects evacuated their saliva 1-2 times per minute for at least 5 minutes in sterile tubes (17). The test tubes were coded and sent immediately to Biochemistry Lab of Zahedan University of Medical Sciences. Then in the laboratory, they were centrifuged (Clement 2000) for 10 minutes at a speed of 2000 rpm. The super natant was separated and maintained at -70°C.

Assay of SOD activity

The required materialsfor experimentation were purchased from Merck, Germany. The enzyme activity was measured according to Mc Cord and Fridovich method (18). 50µL of sample was mixed with 2.9 ml of the solution was prepared via mixing of 100 ml PBS 50 mM (pH 7.4) containing EDTA 0.1 mM and 2 µm olcytochrome C, with 10 ml sodium hydroxide 0.001 Ncontaining 5 µm olxanthine, and the reaction was started by adding of 50 µL the solution containing xanthine oxidase 0.2U/ml and EDTA 0.1 mm.

The absorbance of each sample was measured with aspectrophotometer (Pharmacia-Biotech) at 550 nm wavelength. In controls, 50 ml of distilled water was used instead of sample.

After the calculation of changes in absorbance in each sample for four minutes, the mean of absorbance changes were calculated for every minute. Then, the activity of each sample (in U/mg) was calculated based on molar absorption coefficient of cytochrome C and the amount of protein present ineach sample.

statisticalanalysis

The data obtained from paan consumer and non-consumer groups were analyzed by SPSS-15 statistical software through descriptive statistics for mean and standard deviation and Mann-Whitney non-parametrictest.$p \leq 0.05$ was considered statistically significant.

Results

Paan consumers included 29 males and 18 females with a meanage of 27 years and non-consumers included 22 males and 18 females with a mean age of 31 years. Table 1 shows that the mean SOD activity was significantly higher in the paan consuming group

[DOR: 20.1001.1.22519890.2014.3.1.7.7]
[DOI: 10.22088/cjdr.3.1.8]

($p \leq 0.05$). It was also found that there was not a significant difference between age and gender of two groups; this was expected regarding matching of variables of the two groups ($p>0.05$).

Table 1. Superoxide dismutase activity and demographic characteristic in subjects of the study groups

Study groups	Consumers (Mean±SD)	Non consumers (Mean±SD)	P value
SOD activity (u/mg)	4.4±1.6	3.59±1.8	0.027
Age (year)	27±11	31±9	0.176
Male	29	22	0.63
Female	18	18	

Discussion

The results of the present study showed that the activity of super oxide dismutase in saliva was significantly higher in paan consumers compared with non-consumers. It seems that the increase of this enzyme, as a component of the antioxidant defense systemin saliva, is to reduce the damaging effects of free radicals produced by the consumption of paan. Exposing to the compounds in the paan induce microsomalcy to chrome P_{450} as a source of reactive oxygen species (ROS).

Super oxide anionand hydrogen peroxideare formed especially following disruption and uncoupling of cytochrome P_{450} in the catalytic cycle (19). Oxidant toxicity caused by smoking may lead to increase antioxidant enzymes such as SOD (17). Super oxide dismutase converts super oxideanion to hydrogen peroxide which then H_2O_2 is removed by glutathione peroxidase (GPX) or catalase (9).

No study was performed on enzymatic antioxidants such as SOD in saliva of paan consumers, thus, it is difficult to compare the results of this study with other studies; this issue may be considered as a limitation of our study.

The results of the present study are consistent with the research of Bahar vandand et al. who found that smoking increases the activity of salivary super oxide dismutase (13).

Several investigators also showed that the mean levels of SOD were significantly higher in the saliva of smokers than non smokers (14,17,20). Our results were inconsistent with the studies of Abdolsamadi and Agnihotri. In the study of Abdolsamadiand et al., the activity of salivary SOD was significantly lower in smokers compared with non-smokers (16). gnihotri and et al., showed that the activity of SOD in saliva and gingival crevicular fluid of smokers was reduced compared with the control group and was lower in heavy smokers than light smoker (15). The difference between these results and ours may be due to the measurement of this enzyme in subjects with periodontal disease.

Zappacosta and et al., studied the level of glutathione, uric acid, and total antioxidant activity in saliva of smokers (before and after smoking a cigarette) and non-smokers.

In this study, no statistically significant difference was seen between the two groups in terms of uric acid concentration and total antioxidant activity of saliva, however, the glutathione level was significantly higher in smokers and decreased significantly after smoking (21). Reznick and et al., studied the activity of antioxidant enzymes invivo and invitro.

In in vivo study, the activities of antioxidant enzymes decreased during the first half an hour after consumption but then returned to 90-100% of previous status due to new saliva secretion (22).

In consistenciesin the results of the studies could be due to the differences in the type of consumed tobacco, duration and consumption pattern, method of enzyme assessment, subjects' age, research sample (salivaor blood), research method (in vitro or invivo) and type of antioxidant agent.

Shrestha and et al., compared the status of non-enzymatic antioxidants between the consumers of masala paan (containing tobacco) and control group. In this study, the levels of vitamin C, vitamin E, and albumin were significantly lower in paan consumers than incontrol group (19).

The difference between these results and ours may be due to the measurement of these antioxidants in plasma of subjects as well as studying the non-enzymatic antioxidants in the mentioned research. Karincoaglu and et al., studied antioxidant enzymes of catalase and SOD in saliva of patients with aphthous and healthy subjects. Salivary SOD and catalase levels were significantly higher in the patients group than the controls, but the serum levels of SOD and CAT were decreased in them.

They argued that the salivary defense mechanisms which act through antioxidant system cause the whole

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[DOI: 10.22088/cjdr.3.1.8]

body to send its stored antioxidants to the site of injury during aphthous occurrence, resulting in the increase of salivary antioxidant agents (18). This research corresponds to the present study since in both, the activity of salivary SOD increases, however during aphthous, the enzymatic changes precede the appearance of the lesion, while paan consumptionalters the antioxidant system in consumers.

Goku and et al., showed that the antioxidant enzymes SOD and catalase were significantly reduced in tissue samples of oral squamous cell carcinoma group than the control. While, the SOD levels in the erythrocytes were higher in patients in comparison with the control group.

They emphasized that the imbalance of oxidant/antioxidant system as a risk factor in cancer may be considered (23). Finally, it can be mentioned that the periodic assessment of salivary antioxidant system in paan consumers can play an important role in the early treatment of paan damaging effects in oral cavity.

Conclusions

The results of this study showed that salivary super oxide dismutase enzyme activity in paan consumersis higher than the non-consumers.

Acknowledgments

We would like to thank the Zahedan University of medical sciences for financial support of this research project.

Funding: This study a part thesis and research project (Grant No: 5539-T) supported and funded by Zahedan University of Medical Sciences.
Conflict of interest: There was no conflict of interest.

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