RESEARCH ARTICLE

Effects of Tobacco on Salivary Antioxidative and Immunologic Systems

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Abstract

Background: Tobacco use is a harmful habit that causes adverse effects on oral health and plays a most important role in cancer development. Saliva is the first fluid that is exposed to tobacco and its antioxidant system plays an important role in anti-cancer potential; therefore, this study was designed to compare the antioxidant activity and immunologic system of saliva in tobacco users (smokers and smokeless tobacco users) and non-users. **Materials and Methods:** In this cross-sectional study, unstimulated saliva samples of 75 healthy individuals from three equal groups were investigated. Participants in group A had no periodontal disease and never smoked. Group B had no periodontal disease, never used smokeless tobacco, but had 3 years history of smoking at least 10 cigarettes daily. The twenty-five participants in group C had no periodontal disease and had 1 year history of chewing a 10-g tobacco packet daily. Activity of salivary superoxide dismutase (SOD), salivary glutathione peroxide, and salivary IgA concentration was evaluated. Data were analyzed using SPSS (version 18) and running Kruskal-Wallis test. Statistical significance was set at $p<0.05$. **Results:** There were significant differences in the activities of the two enzymes and salivary IgA levels among the three groups. Activity of glutathione peroxide was higher in smokeless tobacco users. ($P<0.001$) while that of superoxide dismutase was higher in non-tobacco users than users ($P<0.001$). Salivary IgA levels were higher among smokeless tobacco users ($p=0.04$). **Conclusion:** Based on the results of the present study, the use of tobacco products decreases the antioxidative activity of the saliva and increases salivary IgA levels at the same time.

Keywords: Tobacco- saliva- glutathione peroxide- superoxide dismutase- IgA

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Introduction

Smoking is an irritating habit that results in deleterious effects on the oral health and has the most important role in the induction of precancerous and cancerous lesions and periodontal diseases. Cigarette smoke has a large number of toxic components such as carbon monoxide and hydrogen carbide that can induce systemic disorders such as cancer and pulmonary diseases. Reactive oxygen species might induce cytotoxic changes in the intra- and extra-cellular components, resulting in cellular function disturbances. Tobacco can alter the antioxidative capacity of the saliva; however, the reasons behind these alterations are not clear (Battino et al., 2002). Recently, an imbalance between the levels of free radicals and reaction oxygen species has been reported. On the other hand, antioxidative agents might have a key role in the initiation and progression of several inflammatory conditions of the oral cavity (Preston, 1991; Kosecik et al., 2005; Pasupathi P, 2009). Cigarette smoke is a significant source for free radicals, and tobacco smoke contains oxidating agents (Yildiz et al., 2002). Saliva is the first body fluid to come into contact with the cigarette smoke; therefore, its antioxidative system has an important role in the anticancerous capacity of the saliva. This antioxidative system consists of different molecules and enzymes such as uric acid, superoxide dismutase, catalase, and peroxidase system (Baharvand et al., 2010).

Superoxide dismutase (SOD) is found in all the tissues and some fluids such as saliva as a component of the antioxidative system of the body (Baharvand et al., 2010). The activity of this enzyme in the saliva protects the body against the deleterious effects of cigarette smoke and transforms the free oxygen radicals (O-2) to H2O2, water, and oxygen in the next stage. Only a few studies have been carried out investigating the effect of smoking on the antioxidative system of saliva in healthy participants without periodontal disease (Baharvand et al., 2010).

Reznik et al., (2003) showed that smoking decreases the activity of salivary superoxide dismutase; however, in a study by Kanehira et al., (2006) the salivary levels of superoxide dismutase were higher in smokers. A study by Baharvand et al., in 2010 demonstrated that the activity

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of superoxidase as a component of the antioxidative system of the body was higher in smokers compared to non-smokers. It appears that such an increase can decrease the production of free radicals in smokers (Baharvand et al., 2010). In another study by Abdolsamadi et al., (2011), the activities of salivary superoxide dismutase, glutathione peroxidase, and peroxidase enzymes in smokers were significantly lower in smokers compared to those in non-smokers.

Understanding which tobacco products affect secretory immune system is important since it is a key defense line against mucous pathogens.

Bonding to bacteria, IgA agglutinates them and inhibits their adhesion to mucous surfaces. It also prevents destruction of periodontal tissues by inhibiting the activity of some hydrolases produced by bacteria. Regarding contradictory results about the effect of tobacco use on salivary antioxidant system. (Waszkiewicz et al., 2012), further studies seem necessary in this respect. The present study was undertaken to evaluate the effect of tobacco use on the antioxidative system and saliva immunologic system.

Materials and Methods

The protocol of the present cross-sectional case– control study was approved by the Ethics Committee of Zahedan University of Medical Sciences. Informed consent were obtained from all the participants. Out of 75 participants, 25 healthy individuals were assigned to group A. Participants in group A had no periodontal disease and never smoked.Twenty five participants were allocated to group B. They had noparticipants periodontal disease, never used smokeless tobacco, but had 3 years history of smoking 10 cigarettes daily. The 25 participants in group C had noparticipants periodontal disease and had 1 year history of chewing a 10-g tobacco packet daily . The exclusion criteria were as follows: Participantshaving systemic conditions, having a history of aphthous stomatitis or any other oral disease, being pregnant or breastfeeder, taking vitamin supplements during the previous 3 months, participantstaking any medications for longer than 3 months, participantshaving periodontal pockets measuring >3 mm in depth.

All three groups were homogenous in terms of age and gender. Salivary samples were collected participantsin the morning. All the participants rinsed their oral cavities with physiologic serum before the sampling procedure. Then, each subject's non-stimulatory saliva was collected in special containers for 5 minutes. The salivary samples were immediately centrifuged and transferred into freezer at -20°C.

Determining the activity of salivary superoxide dismutase

The suggested technique by (Kakkar et al., 1984) was used in the current study to determine the activity of salivary superoxide dismutase (SOD). This technique relies on the inhibition of the appearance of blue color of tetrazolium formazan by SOD in the mixture containing phenazinemethosulfate-NBT (NADH) (Kuthan et al., 1986).

Determining the activity of salivary glutathione peroxidase

The activity of salivary glutathione peroxide was determined with the technique presented by Amini et al. In this technique, glutathione peroxidase reduces H2O2 and results in the oxidation of glutathione. Then, the oxidized glutathione is reduced again by glutathione reductase and NADPH, forming reduced glutathione and NADP+. Finally, oxidation of NADPH to NADP+ is measured by spectrophotometry at a wavelength of 340 nm. The activity of glutathione peroxidase is reported in milligram of protein (Amini et al., 2009).

Determining the concentration of salivary IgA

ELISA was used to determine the salivary IgA concentration using DiaMetra company kit (Italy).

Statistical analysis

Data were analyzed using SPSS (version 18). Kruskal-Wallis test was used for the comparison of groups. Statistical significance was set at $p<0.05$.

Results

In the present study, the participants consisted of 28 smokers (B group), 25 non-smokers (A group), and 24 users of non-smoked tobacco (C). All the participants were male. The mean ages of the participants were 36.35±9.5, 37.5±10.3, and 36.12±10 years in smokers, non-smokers and non-smoked tobacco groups, respectively indicating no significant difference (P=0.6).

The salivary flow rate was 3.2±0.4 mL/5 min in the smokers, 3.2±0.75 mL/5 in non-smokers, and 3.1±0.5 mL/5 min in non-smoked tobacco users. With respect to salivary flow rate, no significant difference was observed (P=0.86).

Table 1 presents the activities of superoxide dismutase and glutathione peroxidase enzymes as well as the salivary concentrations of IgA. There were significant differences among three groups in terms of two enzymes activities and salivary IgA levels.

Two-by-two comparisons showed a significant difference between smokers and non-tobacco user regarding activity of glutathione peroxide (P<0.001), with higher activity in non-tobacco user. In addition, comparison of smokers and non-smoked tobacco users showed a significantly higher level of activity in the smokeless tobacco users (P<0.001). The enzyme activities were higher in non-tobacco user compared to smokeless tobacco users (P<0.001).

Two-by-two comparison of superoxide dismutase activity revealed significantly higher activity in non-tobacco users than smokers (P<0.001). In addition, comparison of smokers and smokeless tobacco users revealed a significantly higher activity in the smokeless tobacco users (P<0.001). However, there was no significant difference between non-tobacco user and smokeless tobacco users considering enzyme activity (P=0.3).

Comparing smokers with non-tobacco user, two-by-two comparison demonstrated a significant difference in the IgA salivary levels (P<0.001) while higher levels were observed in smokers. With respect to IgA salivary levels,

Table 1. The Activities of Superoxide Dismutase and Glutathione Peroxide, and Salivary IgA Levels in the Study Groups

Group/ Variable	Smokers	Not-tobacco user	Smokeless tobacco	P value
Superoxide dismutase (U/mg Pr)	2.1±1.6	4.7±2.2	4.08±2	<0.001
Glutathione peroxidase (U/mg Pr)	1±4.9	1.5±10.8	8.2±2.6	<0.001
IgA (ng/mL)	175.3±166.1	64±105.8	167.7±100.5	0.04

no significant difference was seen between smokers and smokeless tobacco users (P=0.09). Salivary IgA levels were higher among smokeless tobacco users compared to non-tobacco user (P=0.03).

Discussion

Cigarette smoke is a mixture of thousands of chemical agents, including nicotine, acrolein, and phenyl acetyl oleid. In addition, it contains free radicals that can cause cellular damage and alter the antioxidative potential of the saliva. On the other hand, superoxide dismutase serves as one of the most important enzymes of the antioxidative system.

The results of the present study showed that the activity of superoxide dismutase and glutathione peroxidase was much lower in smokers compared to non-tobacco user that is consistent with the results of several previous studies. Abdolsamadi et al., (2011) and Ahmad Mohammad (2013) showed that the activity of superoxide dismutase in the saliva of non-smokers is less than that in non-smokers (Amini et al., 2009; Abdolsamadi et al., 2011; Waszkiewicz et al., 2012; Ahmed, 2013). Kelin et al., (2003) reported that the activity of salivary SOD decrease up to 70% in smokers (Klein et al., 2003). In addition, Reznick et al., (2003) reported that even after smoking one cigarette there is a rapid decrease in the activity of salivary peroxidase in both smokers and non-smokers.

Superoxide dismutase plays an important protective role against peroxidation of lipids, converts superoxide radicals into hydrogen peroxide, and decreases the toxic effects of free radicals.

In contrast to the present study, Kanehira et al., (2006) held that the activity of salivary SOD is higher in smokers compared to non-smokers. Although the level of glutathione peroxide was higher in nonsmokers, they did not evaluate the activity of SOD.

Consistent with the present study, Guica et al., (2010) showed a significant decrease in glutathione peroxide levels in smokers; while, SOD levels did not significantly increase in smokers and non-smokers. Moreover, a study by Greabu et al., (2008) revealed a significant decrease in salivary levels of glutathione peroxidase in smokers.

In a pilot study by Naga Sarisha., (2011), smokers, non-smoked tobacco users, and controls were explored for erythrocyte Antioxidant nzymes-Superoxide dismutase (SOD) and Glutathione Peroxidase (GPx). In line with the current study, a decrease in SOD levels was observed in smokers and an increase in salivary glutathione peroxidase levels was seen in this group. Furthermore, glutathione peroxide levels increased with an increase in the use of non-smoked tobacco (Naga Sirisha and Manohar, 2013).

Russo et al., (2011) found a decrease in the expression of SOD, GPX, and CAT enzyme genes in human neuroblastoma cells exposed to thickened cigarette smoke.

On the other hand, Shetty et al., (2013) reported a higher activity of salivary SOD in patients with leukoplakia and oral cancer compared to the controls that can be due to this fact that the majority of participants with leukoplakia and oral cancers were smokers suggesting SOD level as a biomarker for oral cancers.

In the present study, IgA levels in smokers and smokeless tobacco users were higher compared to non-tobacco users, which is in contrast to results of the majority of previous studies. In a study by Waszkiewicz et al., (2012), 37 male smokers addicted to alcohol were compared to control group. The salivary IgA levels were significantly lower in former group; however, the effect of non-smoked tobacco was not evaluated in aformentioned study.

Doni et al., (2013) also reported lower levels ofsalivary IgA levels in smokers and non-smoked tobacco users.

Golpasand et al., (2013) showed that smokers have higher dental caries rate and lower salivary IgA levels compared to non-smokers (n=15 in each group); however, non-smoked tobacco users were not evaluated in above mentioned study.

The results of the present study are consistent merely with Gregory's study conducted in (1991), in which salivary IgA levels in smokeless tobacco users were higher than those with no such habit; however, they exhibited lower levels of lysozyme and lactoferrin.

Gregory concluded that non-smoked tobacco affects the secretory epithelial cells of minor salivary glands and such an effect might be systemic, affecting all the mucous secretions, which might be confirmed by the results of the present study. However, further evaluations are necessary.

The discrepancies between the results of previous studies and the present study might be attributed to differences in sample sizes, the number of cigarettes smoked, subject inclusion and exclusion criteria, environmental conditions, and social habits.

Based on the results of the present study, the use of tobacco products decreases the antioxidative activity of the saliva and increases the salivary IgA levels at the same time.

However, further studies with larger sample sizes and different doses of smoking and chewing tobacco to find a relationship between tobacco use and saliva immune system and antioxidant is necessary.

Statement conflict of Interest

All author declares no conflict of interest.

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