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Short Communications

Comparison of thiobarbituric acid reacting substances and total antioxidant capacity in saliva of smokers and nonsmokers

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Abstract

Introduction: The aim of this study was to evaluate thiobarbituric acid reactive substances (TBARS) as lipid peroxidation marker and total antioxidant capacity (TAC) in saliva of smoker and non-smoker men.

Materials & Methods: This case-control study was performed on 104 men including smoker (52) and non-smoker (52) men, referred to the Oral Medicine Department in Babol Faculty of Dentistry. 2 ml of unstimulated saliva was collected and specimens were transferred to the Biochemistry Laboratory using dry ice and freezed. Data were analyzed using SPSS 18 and Mann Whitney test.

Results: Findings indicated that the levels of TBARS and TAC in saliva of smokers were significantly higher than control group.

Conclusion: Higher level of TBARS in smokers can show the evident and dangerous role of cigarette and its chemical compounds, and increased level of TAC in smokers can prove the hypothesis of compensatory mechanism of antioxidant system.

Keywords: Oxidative stress, Antioxidants, Smokers, Thiobarbituric acid, Saliva

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مقایسه مقادیر ترکیبات واکنش دهنده با تیوباربیتوریک اسید و ظرفیت تام آنتی اکسیدانی در بزاق مردان سیگاری و غیر سیگاری

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چکیده

مقدمه: هدف از این مطالعه،بررسی ترکیبات واکنش دهنده با تیوباربیتوریک اسید(TBARS) بعنوان شاخص پراکسیداسیون لیپیدها،و ظرفیت تام آنتی اکسیدانی (TAC) در بزاق مردان سیگاری و غیرسیگاری بوده است.

مواد و روش ها: این مطالعه مورد–شاهدی بر روی ۱۰۴ مرد (۵۲ سیگاری و ۵۲ غیرسیگاری) مراجعه کننده به کلینیک دندانپزشکی دانشگاه بابل اجرا گردید. نمونه بزاق تحریک نشده تهیه و به آزمایشگاه بیوشیمی منتقل و سپس در فریزر ۲۰- oC تا زمان انجام آزمایش نگهداری شد. مقادیر TBARS و میزان ظرفیت تام آنتی اکسیدانی اندازه گیری و برای آنالیز داده ها از SPSS 18و تست Mann- Whitney استفاده شد.

یافته ها: نتایج نشان داد TBARS و TAC، در بزاق سیگاریها بطور معنی داری بالاتر از گروه کنترل بوده است.

نتیجه گیری: بالاتر بودن TBARS در سیگاریها میتواند نقش بارز و خطرناک مصرف سیگار و ترکیبات شیمیایی آن را نشان دهد. افزایش TAC در سیگاریها، فرضیه مکانیسم جبرانی سیستم آنتیاکسیدانی را تایید مینماید.

واژگان کلیدی: استرس اکسیداتیو، آنتی اکسیدان ها، سیگاری ها، تیوباربیتوریک اسید ، بزاق

Introduction

Tobacco consumption is one of the major risk factors for increasing the overall burden of diseases.[1] Free radicals are produced not only during normal metabolic activities, but also more often in certain pathological conditions. These radicals have destructive effects on biological molecules including nucleic acids, carbohydrates, and particularly, proteins and lipids.[2] Some studies have indicated that the progression of inflammation in response to the microbial plaque accumulation is reduced in smokers compared to non-smokers, and gingivitis and gingival bleeding on probing are less common in smoker people.[3] The results of Shiva et al. in 2016 demonstrated that cigarette smoking could reduce the total antioxidant capacity and increase the lipid peroxidation parameters.[4] The aim of this study was to evaluate and compare the levels of thiobarbituric acid–reactive substances (TBARS) and total antioxidant capacity (TAC) in saliva of smoker and non-smoker men.

Materials & Methods

The study was approved by the Ethics Committee of Babol University of Medical Sciences with the code of MUBABOL.REC.1389.6 the patients referred to *the* Department of Oral Medicine in Babol Faculty of Dentistry were clinically examined. In addition, their history of chronic diseases, thalassemia, anemia, infectious, cardiovascular, inflammatory and liver diseases, the use of complementary drugs, antibiotics and multivitamin were studied. According to the standard conditions, 2 ml of whole unstimulated saliva was collected from the subjects who did not have acive periodontitis, eat food and use toothbrushes at least one

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hour prior to sampling. This sampling was taken in a sitting position on a normal chair and in a quiet environment at about 9-10 in the morning. Samples containing food particles and sputum were removed from the collection.

Measurement of TBARS: Measuring the amounts of TBARS more specifically, malondialdehyde (MDA) as a marker of lipid peroxidation is mainly performed using spectrophotometric method. To prepare the TCA-TBA-HCl reagent, dissolve 15 g of trichloroacetic Acid and 375 g of TBA in 100 mL of 0.25 N HCl.

Then, 1 ml of saliva sample was added to 2 ml of TCA-TBA-HCL reagent in the test tube and mixed vigorously. The solution was heated in a boiling water bath for 15 minutes. After cooling, centrifuge was performed for 10 minutes and the clear supernatant was used to measure. The absorbance of the samples was read at 532 nm. Then, the standard curve was drawn using absorbance of the standard samples (1,1,3,3, tetra ethoxy propane) and the TBARS concentration of the samples was obtained based on the standard curve.

Measurement of TAC: TAC was determined by the ferric reducing antioxidant power (FRAP) protocol. Ferric to ferrous ion reduction at low pH due to the presence of antioxidants causes a blue colored ferrous-tripyridyltriazine complex with a maximum absorption at 532 nm, which constitutes the basis of the FRAP assay. The changes in absorbance were investigated between the tested and standard specimens at this wavelength, and the results were reported in micromole/liter. All data were analyzed using SPSS 18 and Mann Whitney test.

Results

This study was conducted on 104 men aged 20-50 years in two groups of smokers and control without interventional criteria (Table 1). The values of TBARS as lipid peroxidation marker were significantly higher in saliva of smokers than control group (P <0.001). Based on the results of the current study, the TAC values were significantly higher in saliva of smokers than control group (P<0.0001).

Table1. Mean and SD of TBARS and TAC in smokers and control groups

Index	Group	Number	Mean± SD	Pvalue
TBARS (Micromol.l)	Smokers	52	0.584±0.210	0.001
	Control	52	0.394±0.173	
TAC (mmol.l)	Smokers	52	0.848±0.262	0.0001
	Control	52	0.713±0.263	

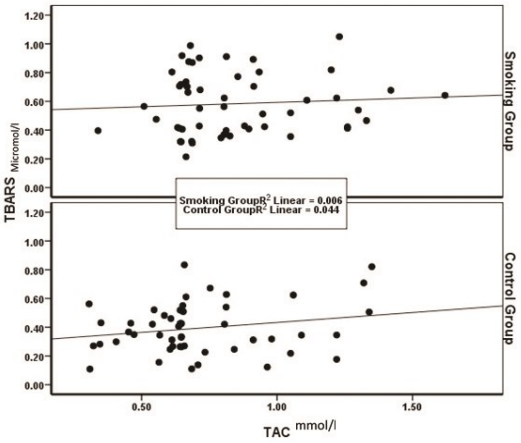


Figure1. The correlation between salivary TAC and TBARS values in two groups of smokers and controls

Discussion

Based on the findings of the present study, the values of TBARS as lipid peroxidation marker were significantly higher in saliva of smokers than control group. The results of the present study are consistent with those of some studies in other societies in terms of salivary lipid peroxidation levels.

Given that saliva is the first biological fluid exposed to cigarette smoke, easier to access and non-invasive method for sampling, this biologic fluid is very important for many studies. Pryor in his review article summarized the results of studies on free radicals in cigarette and discussed about its carcinogenic effects. [5]

Shiva et al. in 2016 studied on saliva of 50 smokers and non-smokers. Oxidative stress values in the smoker group were significantly higher than the control group. As a result, cigarette smoking can increase the lipid peroxidation parameters such as MDA. Moreover, the duration of cigarette smoking has a devastating effect on the body that causes various diseases. [4]

Motallebnejad et al. studied on 60 children aged 12-15 years old and observed that the TAC level was significantly lower in passive smokers than in non-smokers. Furthermore, the lipid peroxidation level was lower in non-smokers, which was not significant. [6] Another study was carried out on 10 smokers and 39 non-smokers and investigated the effect of CS on the activity of salivary peroxidase under mental stress conditions. It was found that salivary peroxidase activity was higher in smokers than in non-smokers and the activity of myeloperoxidase which plays an important role in the response to acute psychological stress is inhibited by CS. [7] Interpretation of this conclusion is

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that increased salivary TAC in smokers is a compensatory mechanism in response to the large amounts of free radicals found in CS and to oxidant compounds in smokers' saliva, which proves oxidative hazards of cigarette. TAC measured by FRAP method is one of the most reliable and comprehensive assays in determining the salivary antioxidant status and represents sum of the antioxidant compounds of the studied sample. This method was used in the current study although in various studies, some enzymatic or non-enzymatic antioxidants were studied.

In a study conducted on unstimulated saliva of 30 smokers and 30 non-smokers, it was shown that the activity of superoxide dismutase (SOD) enzyme was significantly higher in smokers than in non-smokers. [8] Giuca et al. studied the effect of cigarette smoke(CS)on salivary SOD (SOD) and glutathione peroxidase enzymes. Although GSH-Px activity had a significant decrease in smokers, there was no difference between control and case groups in the values of SOD activity. Of course, it should be noted that 10 of 24 persons in the control group were ex-smokers whose presence had an impaction the results. They also suggested that there was a significant difference in the values of enzyme activity between those who had not smoked for less than ten years and more than ten years. [9]

Kanehira et al. evaluated SOD and GSH-Px on the unstimulated saliva of 44 old smokers and 44 non-smokers aged over 65 years old and found that SOD level was significantly higher in smokers than in non-smokers, while the activity levels of peroxidase and GSH-Px enzymes were much higher in non-smokers. [10] The current study demonstrated that there was a positive and significant correlation between TBARS and TAC indicators, and this confirmed the hypothesis of the compensatory mechanism of the antioxidant system. This hypothesis states that when the body is exposed to higher levels of invasive free radicals and oxidizing compounds, the antioxidant defense system may be more active to resist with this condition, but excessive oxidants exceed from the antioxidant capacity of the body can lead to molecular and tissue damage, resulting in oxidative stress and a variety of disorders and diseases such as cancer and cardiovascular diseases.

Conclusion

Higher level of TBARS in smokers can indicate the evident and dangerous role of cigarette smoking and its chemical compounds in the occurrence of oxidative stress and peroxidation of compounds. Increased level of salivary TAC in smokers is a compensatory mechanism in response to the large amounts of free radicals in CS and to oxidant compounds in the saliva of smokers, which itself proves the risk of oxidative CS.

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Authors' Contributions

Mohammad Moballegholeslam: designing, concept and literature search, clinical study, data collection and manuscript preparation. Soleiman Mahjoub: designing of the study, clinical study, intellectual concept, data collection and editing. Mehrdad Taghibakhsh: designing. Ali Bijani: data and statistical analysis.

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