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

Mohammad Moballegholeslam 1, Soleiman Mahjoub2✉, Mehrdad Taghibakhsh 3, Ali Bijani 4

1. Postgraduate Student, Student Research Committee, Babol University of Medical Sciences, Babol, IR Iran. ORCID (0000-0002-1603-039X)
2. Professor, Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
3. Assistant Professor, Oral Medicine Department, Islamic Azad University of Tehran Medical Sciences, Tehran, IR Iran.
4. Assistant Professor, Social Determinants of Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.

✉Corresponding Author: Soleiman Mahjoub, Faculty of Medicine, Babol University of Medical Sciences, Babol, IR Iran.
Email: smahjoub20@gmail.com    Tel: +9809396371055 ORCID (0000-0002-9775-804X)

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 freeze. 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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http://www.CJDR.ir
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 active periodontitis, eat food and use toothbrushes at least once a day. Their clinical examination was performed by the Head of the study team for detection of gingivitis and gingival bleeding on probing.

The saliva samples were separated immediately after collection and then centrifuged at 3000 g for 10 minutes. The supernatant was collected and used for the determination of TBARS and TAC.

The levels of TBARS were measured using thiobarbituric acid (TBA) test as previously described by Uchida et al.\(^5\) The absorbance of the reaction was measured at 532 nm.

The total antioxidant capacity (TAC) was measured using ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as previously described by Sivasankaran et al.\(^6\) The absorbance of the reaction was measured at 734 nm.

Results

The results of the study showed that the levels of TBARS were significantly higher in smoker men compared to non-smoker men (p < 0.05). However, there was no significant difference between the levels of TAC in smoker and non-smoker men (p > 0.05).

Discussion

Smoking is a major risk factor for chronic diseases, and it has been shown that smoking can increase the levels of reactive oxygen species (ROS) and decrease the levels of antioxidants.\(^7\) The results of the current study are consistent with previous studies that have shown a significant increase in the levels of TBARS in smoker men.\(^4,5\)

Conclusion

The results of the current study showed that smoking can increase the levels of TBARS, which may indicate an increased risk of chronic diseases. Further studies are needed to investigate the role of smoking in the progression of chronic diseases.
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
generously. 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**

<table>
<thead>
<tr>
<th>Index</th>
<th>Group</th>
<th>Number</th>
<th>Mean± SD</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>Smokers</td>
<td>52</td>
<td>0.584±0.210</td>
<td>0.001</td>
</tr>
<tr>
<td>(Micromol/l)</td>
<td>Control</td>
<td>52</td>
<td>0.394±0.173</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>Smokers</td>
<td>52</td>
<td>0.848±0.262</td>
<td>0.0001</td>
</tr>
<tr>
<td>(nmol/l)</td>
<td>Control</td>
<td>52</td>
<td>0.713±0.263</td>
<td></td>
</tr>
</tbody>
</table>

**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. [3]

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. [5]

Another study was carried out on 10 smokers and 39
non-smokers and investigated the effect of CS on the
actvity 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. [6] Interpretation of this conclusion is
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 impact on 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.

References


