Original Article

Investigation of the Salivary Antioxidants and Oxidative Damage among Smokers and Non-Smokers with Dental Caries

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Abstract

Background: Salivary oxidative stress is thought to be involved in the progression of dental caries. However, evidence from previous studies provides equivocal results with some data supported the link between dental caries and salivary oxidative stress, while others did not find any relationship. **Objectives:** This study was designed to investigate the levels of salivary oxidative stress among smokers and non-smokers with dental caries. **Study Design:** Cross-sectional study. **Materials and Methods:** One hundred adult males, who visited the Conservative and Operative Clinics at the College of Dentistry, Babylon University, Iraq for the period from November 2021 until April 2022, were recruited in this study and were randomly subdivided into three groups; G1: smokers with dental caries, $N = 35$; G2: non-smokers with dental caries, $N = 35$; G3: non-smokers without dental caries (control), $N = 30$. Markers of salivary total antioxidants (TAC) and Malondialdehyde (MDA) were measured in all studied groups. The potential correlations between these markers and other factors such as smoking duration, number of smoked cigarettes per day, and subject's age were also measured. **Result:** The levels of TAC were significantly ($P = 0.01$) greater in patients with dental caries compared to those levels in a control group, while the levels of salivary MDA were not significantly ($P \geq 0.05$) differed among all studied groups. Smoking had no significant ($P \geq 0.05$) effects on both markers of oxidative stress (TAC and MDA). All measured correlations between makers of oxidative stress and smoking duration, number of smoked cigarettes per day, and subject's age were insignificant ($P \geq 0.05$). **Conclusions:** Salivary antioxidants may be involved in the progression of dental caries. However, further studies are needed to establish whether markers of oxidative stress (TAC and MDA) are the causes or results of dental caries.

Keywords: Caries, oxidative stress, saliva, smoking

Introduction

Caries is one of the serious oral health problems and special attention has been given to minimizing its progression over the world.[1] Caries is recognized by localized, transmissible, pathological infectious processes that lead to destroying the hard dental tissue. Dental caries has usually associated with the presence of acidogenic and acidophilic bacteria in dental plaque. Both *Streptococcus* spp. and *Lactobacillus* spp. are among the most common bacteria producing acids from sugars found in food. The produced acid could cause demineralization initiating the cariogenic process.[2,3]

Cigarette or tobacco smoke is believed to be a candidate driving the pathogenesis of several kinds of oral diseases. The harmful effects of cigarette compounds could be linked to the higher prevalence of different kinds of oral diseases such as periodontal diseases, dental caries, and neoplastic diseases of oral tissues among smokers.[4-6] The potential increment of ROS derived from cigarette compounds could be paralleled with the reduction of salivary antioxidants, which in turn, have more oxidative damage. However, there is a paucity of studies addressing the link among smoking, dental caries, and oxidative stress.[4]

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Findings from the literature have indicated that saliva has a crucial role in the progression or regression of dental caries. The process of dental caries is controlled by salivary protective mechanisms.[7] Saliva is a heterogeneous liquid equipped with a natural defense system. It is an abundant source of enzymatic and non-enzymatic antioxidants that serve as a protection system against the produced reactive oxygen species (ROS), bacteria, viruses, and fungi.[8]

However, levels of these salivary antioxidants might be downregulated or the production of ROS could exceed the levels of endogenous antioxidants, leading to oxidative stress (OS) that is recognized by the elevation of oxidative damage. Oxidative damage could target all different macro-molecules such as lipids, proteins, and DNA.[9] OS could be a candidate mechanism driving the growth and differentiation of cariogenic bacteria.[10]

Previous research indicated that salivary markers of oxidative damage were greater among patients with dental caries,[11] but it is not fully understood whether these greater levels of oxidative damage are caused by the elevation of ROS or by the downregulation of antioxidants.[8] Total antioxidant capacity (TAC) refers to the sum of both enzymatic and non-enzymatic antioxidants. It is believed that the antioxidants work collectively rather than individually and measuring a single antioxidant could not reflect the comprehensive antioxidant status as TAC does.[9] Few studies have measured the levels of salivary (TAC) in the context of dental caries and most of them indicated that patients with dental caries had significantly greater salivary TAC compared to those without dental caries.[12-18]

On the other hand, malondialdehyde (MDA) is an end product of lipid peroxidation and it is commonly used as a measure of lipid oxidative damage.[19] Until now, few studies are measuring the levels of MDA among adults with dental caries and some of them indicated that the lipid damage could be greater among patients with dental caries[20,1] or not changed.[21,22]

The lack of research on measures of salivary antioxidants alongside oxidative damage among smokers in relation to dental caries makes it necessary to assess them. Therefore, this study aimed to investigate the potential correlation between smoking and levels of salivary antioxidants (measured by TAC) and oxidative lipid damage (measured by MDA) among patients with dental caries.

Materials and Methods

Subjects

This study was conducted at the College of Dentistry/ University of Babylon for the period from November 2021 until April 2022. Formal consents were obtained from all subjects who were recruited in this study. One hundred samples of male saliva whose age ranged from 20-50 years were collected and randomly grouped into three sub-groups, namely, group 1: normal (not electronic) smokers with dental caries (N = 35); group 2: non-smokers with dental caries (N = 35); and group 3: non-smokers without dental caries (control, N = 30). Dental caries was diagnosed by a special dentist and deep carious lesions from both anterior and posterior teeth- according to DMFT were considered.[23]

Exclusion criteria

Patients with systemic diseases, taking antibiotics, and having mouth wash at the time of sample collection were excluded from the study. All procedures used in the current study were guided by the Helsinki declaration and followed by approval issued by the Ethical committee of the College of Dentistry, University of Babylon, Iraq in October 2021.

Sample collection

Saliva samples were collected properly in a contamination-free procedure for each patient according to the method.[24] Non-stimulated clean saliva was collected in a sterile laboratory cup. Each collected sample was then centrifuged at 3000 rpm for 10 minute to separate the undesired free salivary particles. A clear salivary solution was aspirated, stored in an Eppendorf tube, and kept frozen at –20°C until the measures of total antioxidants and oxidative damage were made.

Total antioxidant capacity (TAC) measurement

CUPRAC (cupric reducing antioxidant capacity) spectrophotometric method was used to measure the levels of Salivary TAC.[25] Briefly, the total antioxidant found in both samples and standard converts copper II to copper I. This reduced form of copper was selectively reacted with the chromogenic reagent marker to form a stable complex solution. The color of this complex was measured in maximum absorption at ~450 n. The readings of samples would be compared with a standard curve generated by Trolox which acts as a standard. The levels of TAC were expressed as micro Mollar copper reducing equivalents or in micro Mollar Trolox equivalents.

Malondialdehyde (MDA) measurement

Salivary lipid peroxidation was estimated by Thiobarbituric Acid Reactive Substances (TBARS). The principle of this method depends on the MDA-TBA product formed by boiling MDA and TBA (90–100°C) in acidic conditions. This product was measured at 530–540 nm.[26] The following steps were used in this assay, 100 µl of saliva and 2 ml of 1:1:1 ratio TBA–TCA–HCl reagent were added to the test tube. Then the reactive tube was placed in a water bath (15 minutes), followed by centrifugations (15 minute at 2000 rpm) and finally, the absorbance of the supernatant was measured by spectrophotometer (532 nm) against a

reagent blank. The preparation of the reagent blank was the same procedure above except change the sample with distilled water.

Statistical analysis

Data were firstly measured for normality using the Shapiro–Wilk test and the transformation was made for skewed data. One-way ANOVA was used to test for the significant changes in oxidative stress biomarkers (TAC and MDA) among the studied groups. If the changes were significant, Turkey post hoc tests were then applied to determine the changes between groups. Pearson correlation was used to test the potential correlations between markers of oxidative stress and other factors (smoking duration, number of smoked cigarettes per day, and subject's age). Data were expressed as mean ± standard deviation and $P \leq 0.05$ were considered statistically significant. All statistical procedures were made by Minitab (Version 17; Minitab Inc., State College, PA) software.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 109 on 18-10-2022.

Results

The results of our study indicated that patients with dental caries have significantly increased salivary levels of TAC compared to the control group ($P = 0.01$). However, the levels of salivary TAC were not significantly different among smokers, non-smokers, and the control group ($P \geq 0.05$) [Table 1].

On the other hand, levels of salivary lipid oxidative damage (measured by MDA) were not significantly influenced by both smoking and dental caries ($P \geq 0.05$), as shown in Table 2.

Pearson correlations indicated that the levels of salivary oxidative stress (TAC and MDA) were not significantly correlated with smoking duration, the number of smoked cigarettes per day, and the subject's age [$P \geq 0.05$; Table 3].

Discussion

Smoking is thought to be a factor involved in the increment of salivary total antioxidants. It is suggested that smoking could enhance the oxidative burst caused by neutrophils and ultimately lead to an increase the oxidative stress in the body systems.[27] However, the findings from the current study indicated that there were no significant effects of smoking on the levels of total antioxidants in which both smokers and non-smokers had similar levels of TAC compared to their levels observed in the control group who had no smoking. Consistent with our findings, many studies did not find any significant effects of smoking on TAC.[28-31] The potential reason for the lacking of TAC increment among smokers could be due to the idea that the organism tries to balance the OS even if the balancing capacity of the OS is limited. Saliva contains enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase and non-enzymatic antioxidants such as glutathione, uric acid, vitamin C, E, and β-carotene. In this regard, smoking could increase some of these antioxidants and decrease others. So measuring total antioxidants in saliva observed in this study could mask the individual effect of an antioxidant that was not measured here.[32] Contrary to our results, TAC is found to be lower in smokers compared to nonsmokers.[33] However, inconsistency found among studies could be due to the variations in the type of smoking and tobacco,

Table 1: Levels of salivary total antioxidant among studied groups

Groups	Sample size	Mean ± SD	*F*-value	*P*-value
Smokers with DC (G1)	$N = 35$	$929.00^{a} \pm 548.00$	4.64	0.01*
Non-smokers with DC (G2)	$N = 35$	$925.50^{a} \pm 411.30$		
Control (G3)	$N = 30$	$624.40^{b} \pm 216.80$		

DC: dental caries; SD: standard deviation

*Significant

Means that do not share a letter are significantly different

Table 2: Levels of salivary malondialdehyde (MDA) among studied groups

Groups	Sample size	Mean ± SD	*F*-value	*P*-value
Smokers with DC (G1)	$N = 35$	10.06 ± 4.54	2.21	0.11
Non-smokers with DC (G2)	$N = 35$	11.28 ± 5.88		
Control (G3)	$N = 30$	13.20 ± 5.89		

DC: dental caries; SD= Standard deviation

Means that do not share a letter are significantly different

Table 3: The correlations matrix between markers of oxidative stress (TAC and MDA) and other factors (duration of smoking, number of smoked cigarettes per day, and the subject's age)

	TAC	MDA	Age	Duration	Smoked cigarettes
TAC					
R	1				
P					
MDA					
R	0.13	1	-0.119	-0.165	-0.343
P	0.251		0.291	0.412	0.08
Age					
R	0.088		1		
P	0.438				
Duration					
R	0.04			1	
P	0.841				
Smoked cigarettes					
R	0.108				1
P	0.592				

and the methods being used for antioxidant detection. In addition, the changes in the type of selected population, the nutritional status of the subjects, and sample sizes could participate in this discrepancy among studies.[33]

Regardless of smoking, patients with dental caries significantly increased their total antioxidants compared to the control group without caries and smoking. These results are consistent with previous studies.[34-36] This could be explained by the fact that the salivary antioxidants are responded to the infection occurred in dental caries.[1] Another possible explanation for the observed elevation of salivary total antioxidants among groups with caries is the triggering of the salivary peroxidase system. This system is a vital part of total antioxidants that are mainly involved in the protection against oral bacteria causing dental caries. It has been found that the peroxidation of thiocyanate ion (SCN−) catalyzed by salivary peroxidase leads to producing a more stable product (OSCN−). This latter product could compromise the growth of bacteria, hence inhibiting dental caries or slowing down the caries progress.[14] The elevation of salivary antioxidants among patients with dental caries observed in the current study could also be due to the intake diet. It is well known that antioxidants derived from the diet participate in the total antioxidant.[17] Patients with dental caries could potentially utilize more carbohydrate diets that contained more exogenous antioxidants compared to those without caries.

Oxidative lipid damage (measured by MDA) was not significantly influenced by both smoking or dental caries. Contrary to our results, salivary MDA is significantly greater in smokers compared to non-smokers subjects in many studies.[33,37] However, all these studies did not include patients with dental caries. One explanation that could be applied to clarify the difference between our study and others is the potential changes in the methods of measuring MDA, sample sizes, and the population included in the study.[22] Nevertheless, it is well documented that the lack of oxidative damage to one of the biological molecules such as lipids could not reflect the damage to others molecules (protein and DNA) that were not measured in our study.[9] Therefore, further investigations of protein and DNA damage in saliva among smokers with dental caries may expand our understanding regarding the effects of smoking on salivary oxidative damage.

Our findings also indicated that the levels of salivary MDA were not influenced by dental caries. These results are consistent with previous studies,[19-22] in which these studies indicated there is no association between dental caries and salivary lipid damage measured by MDA. Our findings are also supported by recent studies of salivary lipid damage among children and adolescents. Children and adolescents with dental caries did not change their salivary lipid damage compared to those with no caries.[21,22,38] However, other studies found a positive link between salivary MDA and dental caries.[1,20] The discrepancy between studies could be the changes in oral hygiene status among studied subjects. An educated person could probably daily brush her/his teeth.[21] In our study, we did not determine the oral hygiene status of patients and control groups, while other studies reminded the patients to get their teeth brushed before taking the saliva samples. This could potentially explain the differences in the outcomes of salivary MDA among the above studies.[22]

All correlations between levels of TAC and MDA and other factors like smoking duration and number of smoked cigarettes per day were insignificant. Lack of such correlation was also previously found between the levels

of salivary TAC and duration of smoking as well as the number of smoked cigarettes per day among smokers with periodontitis.[39] However, other studies found a positive correlation between these variables.[35] In the same direction, the correlations between levels of TAC and MDA and the ages of studied groups are also insignificant.[35,40] The study here has some limitations such as the small sample sizes and other markers of oxidative damage (DNA and protein) that could expand our understanding on the role of oxidative stress and dental caries.

Conclusion

Patients with dental caries had significantly increased levels of salivary TAC compared to the control group. Smoking, however, did not seem to have significant effects on the levels of TAC. The observed increase of TAC among patients with dental caries was not paralleled by significant increase in levels of salivary lipid damage among patients with dental caries compared to the control group. The present study also indicated there were no significant effects of smoking on the levels of lipid oxidative damage (measured by MDA). In addition, the duration time of smoking per year and the number of smoked cigarettes per day did not seem to have significant effects on the measured markers of oxidative stress.

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Conflicts of interest

There are no conflicts of interest.

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