



ORIGINAL ARTICLE

The effects of cigarette smoking and exercise on total salivary antioxidant activity



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KEYWORDS

Total salivary antioxidants;
Cigarettes smoking;
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Abstract Objectives: This study was conducted to determine the effects of both cigarette smoking and exercise on total salivary antioxidants and their impact on periodontal health status.

Material & methods: the study group consisted of 120 males, 20–25 years old selected from patients at the College of Dentistry/University of Baghdad. The sample was classified into four groups: cigarettes smokers who exercised, cigarettes smokers who did not exercise, non-smokers who exercised, and non-smokers who did not exercise. The smokers smoked 5–15 Oriental tobacco cigarettes daily for 3–5 years and did not use other types of tobacco. Physical exercises were performed for a half-hour to an hour daily either at home or the gym. Stimulated saliva was collected under standardised conditions and chemically analysed to determine the total salivary antioxidants concentration using a Total Antioxidant Capacity Assay Kit. The periodontal component of the Periodontal Disease Index (PDI) was applied to diagnose and record periodontal health status. Data were analysed using SPSS version 19.

Results: The total salivary antioxidant concentrations were significantly higher among the non-smokers than the smokers and were significantly higher among those who exercised compared to those who did not exercised ($P < 0.01$). The mean of the periodontal index was significantly higher in the group of smokers than the group of non-smokers and significantly higher among those who did not exercise compared to those who exercised ($P < 0.01$). Smoking and physical exercises recorded a significant effect on total salivary antioxidants and the mean of the periodontal index ($p < 0.01$), but there was no significant interaction between these two variables for total salivary antioxidants or the mean of the periodontal index ($P > 0.05$). Person's correlation coefficient indicated significant negative correlations between the mean of the periodontal index and the total salivary antioxidant concentrations among the four studied groups.

Conclusion: Cigarette smoking and physical exercise may alter total salivary antioxidants activity and the periodontal health status. However, there is no interaction between cigarette smoking

Abbreviation: TSAs, total salivary antioxidants

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and physical exercise regarding total salivary antioxidants and the periodontal health status. Total salivary antioxidants correlated inversely with the periodontal health status and this correlation was not affected by cigarette smoking or physical exercise.

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1. Introduction

Saliva is a complex fluid that bathes the oral cavity and performs multiple functions. It is responsible for the protection of the teeth and oral soft tissues due to its chemical composition and physical properties (Edgar et al., 2013; Ligtenberg and Veerman, 2014).

Saliva performs different types of defence mechanisms, and its antioxidant system is one of the most critical defence mechanisms found in saliva. The salivary antioxidant system consists of various enzymes including peroxidase, catalase, superoxide dismutase, and glutathione peroxidase, and also small molecules such as uric acid, vitamins E and C. The salivary antioxidants are either endogenous (produced by the body) or exogenous (from external sources, especially diet) (Schipper et al., 2007; Pink et al., 2009; Abner et al., 2011; Bjelakovic et al., 2013; Armstrong and Stratton, 2016).

Antioxidants remove the free radicals generated from the oxidative reactions that occur during cellular metabolism and functional activities. Furthermore, antioxidants play essential roles in cell signalling, apoptosis, gene expression, and ion transportation. Free radicals are highly active due to their unpaired electrons and tend to react with the other molecules, causing oxidative stress. Oxidative stress can damage the DNA, RNA, proteins, and lipids, resulting in an increased risk of cardiovascular disease, cancer, autism, and other diseases. Antioxidants can decrease the oxidative damage either directly by reacting with free radicals or indirectly by inhibiting the activity or expression of free radical-generating enzymes (Bauayed and Bohn, 2010).

Antioxidants activity is significantly affected by diseases (Blauz et al., 2008). It has been reported that salivary antioxidants level rise with an increase in caries activity and periodontal diseases (Sculley and Langley-Evans, 2002; Panjamurthy et al., 2005; Tulunoglu et al., 2006; Ahmadi-Motamyle et al., 2013; Al-Azawi, 2013). However, other studies found that salivary antioxidant activity decreased in periodontal diseases (Zhang et al., 2015; Ahmadi-Motamayel et al., 2017; Acquier et al., 2017). Salivary antioxidant activity is affected by many factors such as cigarette smoking, physical exercise, and obesity, among others (Greabu et al., 2008; González et al., 2008; AlSwuilem et al., 2014; Bakhtiari et al., 2015; Sant'Anna et al., 2016). This study aimed to determine the effect of both cigarette smoking and physical exercises on total salivary antioxidant capacity, in addition to their impact on periodontal health status.

2. Materials and methods

2.1. The study group

the study group consisted of 120 males, 20 to 25 years old selected from patients at the College of Dentistry/University

of Baghdad. The sample was classified into four groups: cigarette smokers who exercised, cigarette smokers who did not exercise, non-smokers who exercised, and non-smokers who did not exercise. The smokers smoked 5–15 Oriental tobacco cigarettes daily for 3–5 years and did not use other types of tobacco (Bakhtiari et al., 2015). The physical exercises were performed for a half-hour to an hour daily either at home or the gym (Sant'Anna et al., 2016). None of the participants were taking any food supplements or antioxidant compounds and they were free from any systemic diseases. The study design is illustrated in Fig. 1.

2.2. Collection of saliva and recoding of periodontal health status

Stimulated saliva was collected between 9 AM and 11 AM. Each participant was asked not to smoke, eat, or drink (except water) 1 h before collection. They were seated in a relaxed position in a dental chair without any heavy physical stress and were asked to chew a piece of Arabic gum for one minute and then expectorate all saliva (Tenovuo and Lagerlöf, 1994). The equipments used for salivary collection and handling are shown in Fig. 2. Each saliva sample was then centrifuged (a Universal 16 A, Hettich centrifuge as shown in Fig. 3) at 3000 revolutions per minute (r.p.m) for 10 min. The salivary supernatant was stored at (–20 °C) in polyethylene tubes for subsequent chemical analysis. The periodontal component of the Periodontal Disease Index (PDI) was applied to diagnose and report periodontal health status (Ramfjord, 1956).

2.3. Analysis of saliva samples and data

After thawing of the saliva samples, the TSAs were analysed using Abcam's Total Antioxidant Capacity Assay Kit ab-65329, designed for the quantitative measurement of antioxidants in biological fluids. The procedure's principle is that; in the presence of a protein mask, there will be a combination of either small molecule antioxidants and proteins or small molecules alone that can be measured, where Cu^{2+} ion is converted to Cu^{+} by both small molecules and proteins. The protein mask prevents Cu^{2+} reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu^{+} ion is chelated, resulting in a broad absorbance peak of approximately 570 nm, proportional to the total antioxidant capacity. Each saliva sample was analysed using UV visible recording Spectrophotometry machine (Cecil CE 7200, Cecil Instruments, Cambridge, UK) (Csillag et al., 2014; Truong et al., 2016). SPSS software version 19 (Statistical Package for Social Sciences, IBM Company, Armonk, NY, USA) was used to analyse the data by applying both descriptive statistics, including mean and standard deviations and inferential statistics, including two-way univariate analysis of variance (two-way ANOVA), two-way multivariate analysis of variance

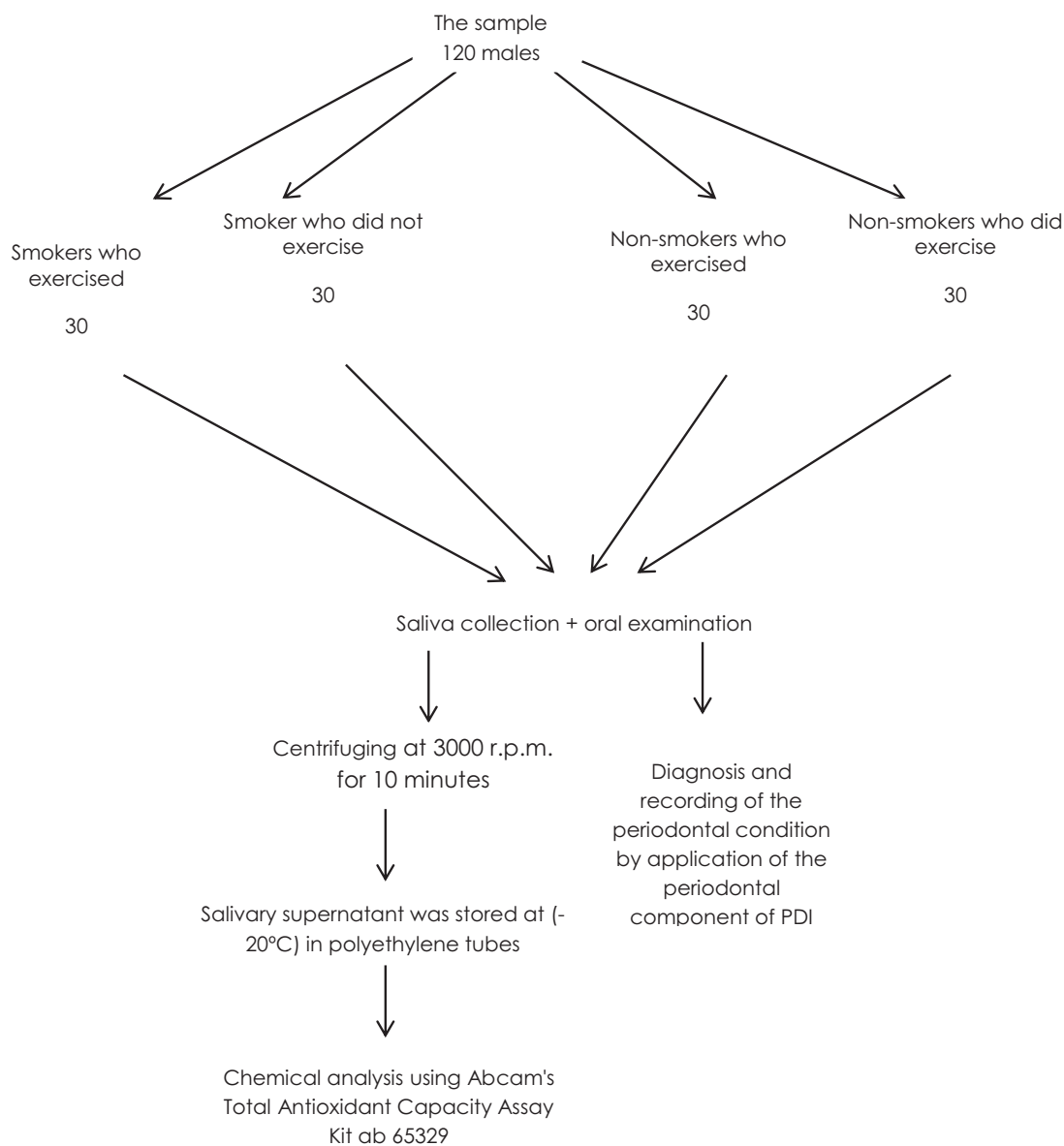


Fig. 1 Study design illustrating the group classifications and the steps to prepare the saliva samples and oral examinations.

(two-way MANOVA), and Person's correlation coefficient. The confidence limit was accepted at 95% ($P < 0.05$).

3. Results

The TSA concentrations were higher among the non-smokers than the smokers and in those who exercised compared to those who did not exercised. The non-smokers who exercised had the highest concentrations of TSAs followed by the non-smokers who did not exercise, the smokers who exercised, and finally the smokers who did not exercise (see [Table 1](#)). A profile plot estimating the marginal means of the TSAs among the study groups is illustrated in [Fig. 4](#).

Both smoking and physical exercise had a statistically significant effect on the TSAs ($p < 0.01$). The difference in the mean of the TSAs was statistically significant between the

smokers and the non-smokers and between those who exercised and those who did not exercise. There was no statistically significant interaction between smoking and physical exercises in the TSAs, as shown in [Table 2](#).

The mean of the periodontal index was higher among the smokers than the non-smokers and in those who did not exercise compared to those who exercised. The smokers who did not exercise had the highest periodontal mean followed by the smokers who exercised, the non-smokers who did not exercise, and finally the non-smokers who exercised (see [Table 3](#)). A profile plot estimating the marginal means of the periodontal index among the study groups is illustrated in [Fig. 5](#).

Both smoking and physical exercise had statistically significant effect on the mean of the periodontal index. The differences in the mean of the periodontal index were statistically significant between the smokers and the non-smokers and between those who exercised and those who did not exercise.



Fig. 2 Equipment for saliva collection and handling.



Fig. 3 Universal 16 A Hettich centrifuge.

There was no statistically significant interaction between smoking and physical exercises in the mean of the periodontal index, as shown in [Table 4](#).

There was no statistically significant interaction between smoking and physical exercises in both TSAs and the mean of the periodontal index ($p > 0.05$), as shown in [Table 5](#). Person's correlation coefficient indicated significant negative correlations between the mean of the periodontal index and the

TSAs concentrations among the four studied groups ($P < 0.01$), as shown in [Table 6](#).

4. Discussion

Studies have been conducted to determine the effect of cigarette smoking on TSAs and the impact of physical exercises on this biomarker ([Greabu et al., 2008](#); [González et al., 2008](#); [Bakhtiari et al., 2015](#); [Sant'Anna et al., 2016](#)). The present study was conducted to determine the effect of these two variables together on TSAs and the periodontal health status.

According to the present study, TSAs were statistically significantly higher among those who exercised than those who did not exercise, and physical exercise had a significant effect on TSAs. This result was in accordance with studies conducted by [González et al. \(2008\)](#), and [Sant'Anna et al. \(2016\)](#). Both [Qiao et al. \(2006\)](#) and [Carlssohn et al. \(2008\)](#) suggested that regular physical exercise leads to tissue adaptation against increased free radicals production associated with exercise and promotes adaptation to oxidative stress that results in an increase in total antioxidants activity. Furthermore, [Shih and Yen \(2007\)](#) reported that exercise enhances the production of transcription factors that stimulate the antioxidant response element (ARE) and other target genes in the cell nucleus, resulting in increased synthesis and the release of antioxidants.

TSAs were also statistically significantly higher among the non-smokers than the smokers, and smoking had a significant effect in TSAs. This result agreed with studies by [Greabu et al. \(2008\)](#); [Pendyala et al. \(2008\)](#); [Bakhtiari et al. \(2015\)](#); [AlSwuailam et al. \(2013\)](#) and [Azadbakht et al. \(2016\)](#) that found that cigarette smoking may alter TSAs activity. Cigarettes contain a large amount of free radicals that may elevate oxidative stress among smokers and minimise antioxidants activity. However, this result disagreed with [Nagler \(2007\)](#) who found that total salivary antioxidants activity was significantly higher in smokers than non-smokers. However, controversies between studies may arise from differences in smoking patterns, cigarette designs, types of tobacco, and methods of antioxidant measurement.

There was no significant interaction between smoking and physical exercise in TSAs, indicating that the harmful effect of cigarette smoking may not be affected by the beneficial effect of physical exercise or vice versa. Although cigarette smoking and physical exercise have been found to alter TSAs activity, there is controversy regarding the exact cause of these changes between the studies ([Greabu et al., 2008](#); [González et al., 2008](#); [Bakhtiari et al., 2015](#); [Sant'Anna et al., 2016](#)). Further studies are recommended to determine the importance of

Table 1 Distribution of Total salivary antioxidants concentrations (mmol/L) among the study groups.

Study populations		Physical exercise		
		Group that exercised Mean \pm SD	Group that did not exercised Mean \pm SD	Total Mean \pm SD
Smoking	Smokers	0.49 \pm 0.04	0.48 \pm 0.02	0.48 \pm 0.03
	Non-smokers	0.53 \pm 0.03	0.51 \pm 0.02	0.52 \pm 0.03
	Total	0.51 \pm 0.03	0.49 \pm 0.02	

SD: standard deviation, mmol/L: millimoles per liter.

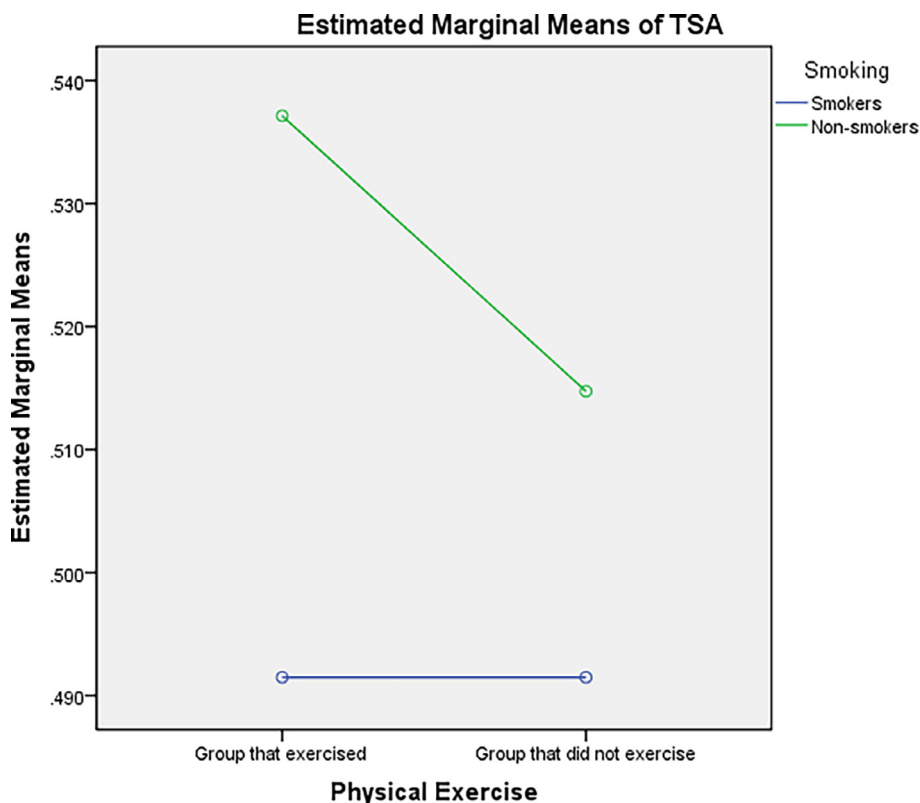


Fig. 4 Profile plot of the marginal mean of TSAs among the study groups.

Table 2 The statistical differences in total salivary antioxidants among the study groups.

Variables	Two-way ANOVA test	
	F-test	P value
Smoking	45.602	0.000**
Physical exercise	8.089	0.005**
Smoking and physical exercise	0.848	0.359

SD: standard deviation.
** Highly significant $p < 0.01$.

salivary antioxidant analysis and the relationship between saliva and free radicals.

The smokers had a statistically significant elevation in the mean of the periodontal index compared to the non-smokers, and smoking had a significant effect on the mean of the periodontal index. This result was in accordance with

the studies by Uderner et al. (2009), Jawzali (2016) and Shirzaiy et al. (2017). Cigarette smoking is one of the risk factors for periodontal diseases; cigarettes impair the oral immune response and compromise the periodontal tissue’s ability to heal due to nicotine that causes vasoconstriction of the blood vessels in the gums and the destruction of the neutrophil cells in the periodontal tissue, which are essential immune cells. Furthermore, cigarette smoking enhances calcification of dental plaque resulting in calculus formation, which is the leading cause of periodontal tissue destruction (Tomar and Asma, 2000; Hennemeyer et al., 2003).

The subjects who did not exercise had a statistically significant higher mean of the periodontal index than those who exercised. This result agreed with Merchant et al. (2003), and Al-Zahrani et al. (2005) who suggested that physical exercise improve periodontal health by enhancing blood circulation and the immune system, resulting in infiltration of the periodontal area by immune cells that fight inflammation and diseases.

Table 3 The distribution of the mean periodontal index among the study groups.

Study populations		Physical exercise		
		Group that exercised Mean ± SD	Group that did not exercise Mean ± SD	Total Mean ± SD
Smoking	Smokers	0.66 ± 0.59	0.83 ± 0.49	0.75 ± 0.55
	Non-smokers	0.26 ± 0.33	0.57 ± 0.32	0.41 ± 0.36
	Total	0.46 ± 0.46	0.70 ± 0.40	

SD: standard deviation.

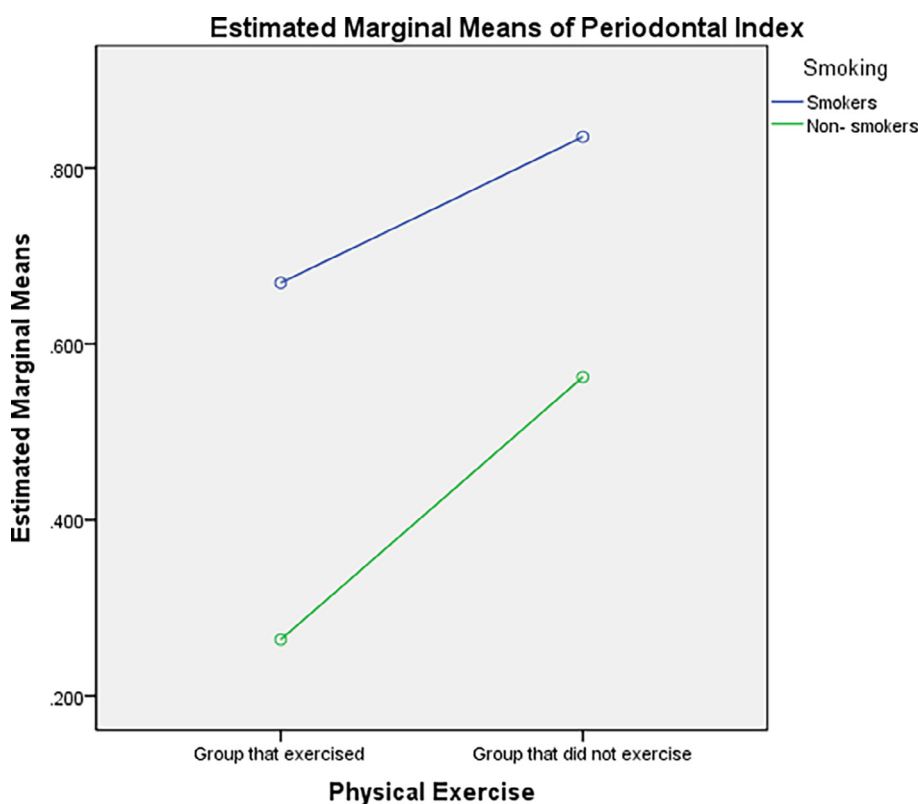


Fig. 5 Profile plot of the marginal means of the periodontal index.

Table 4 The statistical differences in the mean periodontal index among the study groups.

Variables	Two-way ANOVA test	
	F-test	P value
Smoking	16.658	0.000**
Physical exercise	7.805	0.006**
Smoking and physical exercise	0.634	0.428

** Highly significant $p < 0.01$.

Table 5 the statistical effects of both smoking and physical exercise on the TSAs and the periodontal index.

Variables	Two-way MANOVA test		
	F-test	P value	Wilks' Λ
Smoking	15.657	0.000**	0.786
Physical activities	4.016	0.021*	0.935
Smoking and physical activities	1.449	0.239	0.975

* Significant ($p < 0.05$).

** Highly significant ($p < 0.01$).

No significant interaction between smoking and physical exercise in the mean of the periodontal index was recorded. Also, no significant interaction between these two variables on both TSAs and the mean of the periodontal index together were found in this study. These results could be further supported by the significant negative correlations that were recorded between TSAs and the mean of the periodontal index

among the four groups in this study; these correlations may indicate that TSAs are affected by periodontal health status regardless of the impact of smoking or physical exercise. These results agree with those of other studies that recorded negative correlations between TSAs and periodontal health status (Pendyala et al., 2008, 2013; Zhang et al., 2015). Inflammatory conditions in the periodontal tissue enhance phagocytes especially neutrophils, resulting in the generation of oxidative stress (Asman and Bergstorm; 1992; Chapple et al., 2007). However, other studies found positive correlations between TSAs and periodontitis (Panjamurthy et al., 2005; Tulunoglu et al., 2006), in contrast to the results of the present study. There is a controversy in this field and further research may be required.

5. Conclusion

Cigarette smoking and physical exercise may alter total salivary antioxidants activity and periodontal health status. However, there is no interaction between cigarette smoking and physical exercise on total salivary antioxidants and periodontal health status. Further studies in this field are recommended. Total salivary antioxidants correlated inversely with periodontal health status and this was not affected by cigarette smoking or physical exercise. The dietary habits of the studied population may have affected the results as confounding factors; fruits and vegetables in particular contain a considerable amount of antioxidants. All of the participants in this study were selected from a single dental clinic and may have had the same socio-economic status and likely similar nutritional habits. Nutritional status should be considered in future studies.

Table 6 Correlations between salivary antioxidants of the study groups and the periodontal index.

Study groups	Group that exercised		Group that did not exercise	
	r	P value	r	P value
Smokers	- 0.71	0.000**	- 0.48	0.006**
Non-smokers	- 0.58	0.001**	- 0.60	0.000**

** Highly significant $p < 0.01$.

Ethical statement

This work had been approved by the ethical committee in college of dentistry/University of Baghdad. Furthermore, for each volunteer the objectives of the study were explained to, and they approved to participate. Involvement of human subjects in this work was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Declaration of interest

None.

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