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RESEARCH ARTICLE

Impact of smoking on oxidant/antioxidant status and oxidative stress index levels in serum of the university students

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Abstract

Background Despite frequent warnings of irreversible side effects of smoking in public media, the consumption of cigarette is increasing dramatically in both developed and developing countries. Cigarette smoke contains different kinds of chemicals, which all capable of inducing free radical production. There are studies supporting the idea that these free radicals have adverse effects in body and causing oxidative stress. Total antioxidant capacity (TAC) is considered as the total effect of all antioxidants and total oxidant status (TOS) shows the total effect of all oxidants existing in body fluids. Therefore, this research focused on the measurement and comparison of these markers in the serum of university students.

Methods This study designed to determine the total antioxidant capacity, total oxidant status and oxidative stress index levels in the serum of active smokers, passive smokers and non-smokers in university students. A total of 150 participants were included in the study. The study population consisted of 50 smokers, 50 passive smokers and 50 nonsmokers. In serum samples, the levels of TAC and TOS were measured by spectrophotometric method using Rel Assay Diagnostics kit. Oxidative stress index was calculated through the TOS/TAC formula in three groups.

Results The mean value TAC levels in serum samples of the three groups of smokers, passive smokers and nonsmokers were 1.096, 1.220 and 1.844 mmol Trolox equivalent/L, respectively, which were significantly greater in nonsmokers than smokers and passive smokers. The mean value TOS levels in serum samples of the three groups of smokers, passive smokers and nonsmokers were 13.747, 11.099 and 7.6510 μmol H2O2 equivalent/L, respectively, which were significantly lower in non-smokers than two other groups. OSI values in smokers and passive smokers were significantly higher than the control group.

Conclusions According to our findings, the antioxidant capacity in all smokers (active and inactive) was less than the control group (non-smokers). The results of this study showed that smoking reduces the activity of the antioxidant defense system and activates the oxidative stress system in the body. Based on these findings, it can be clearly concluded that the decrease in antioxidant capacity in smokers is associated with increased production of oxidants and free radicals.

Keywords Smoking · Total antioxidant capacity · Total oxidant status

Background

Despite frequent warnings of irreversible side effects of smoking in public media and by other types of advertising, the consumption of cigarette is increasing dramatically in both developed and developing countries. Based on available data, even in last years, almost one-third of population over age 30 years in the world are smokers [1]. Different prevalence surveys show that, some demographic variables such as age, sex, ethnicity, and socioeconomic status are strongly associated with cigarette smoking, especially, younger age, male sex, lower educational rank and lower socioeconomic status are positively associated with smoking cigarettes. The major cause of smoking starts at the age of 15–20 years strongly

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due to absence of neighborhood disadvantages. Started smoking from a very young ages score higher on psychological profiles of smoking dependence [2] and are less likely to stop later in life [3] implying that younger more vulnerable to nicotine dependence.

There are obvious evidences that the consequences of smoking are not only temporary, but also, there is a direct correlation between cigarette smoking and many other diseases such as cardiovascular and respiratory, as well as atherosclerosis problems [4]. Cigarettes' smoke include a number of organic compounds such as hydrocarbons, aldehydes, nitric oxide, phenols and quinone radicals. These compounds directly or indirectly lead to the production of free radicals caused by oxygen. Our body can normally manage free radicals, but if antioxidants are unavailable or if the free-radical production becomes more than usual, tissue damage can occur. Free radicals interact with biological compounds, such as lipids, carbohydrates, DNA, and proteins, which causes metabolic and structural changes in cells. This results in tissue damage in vital organs, including the heart, liver, kidneys, lungs, stomach, and brain [5].

Total antioxidant capacity (TAC) shows the amount of all antioxidants in the whole body and is a biomarker of antioxidant protection facing free radicals [6]. Cigarette smoke contains more than 4,000 chemicals, which all capable of inducing free radical production and act as highly oxidative and carcinogenic agents. There are studies supporting the idea that these free radicals have adverse effects in both smokers and secondhand smokers, causing oxidative stress [7, 8]. Our hypothesis behind the research was that decreasing amount of serum antioxidants because of cigarette smoking could have a significant role in decreasing in protective systems of antioxidants, which is considered as the cause of many pathological conditions. Total antioxidant capacity (TAC) is considered as the total effect of all antioxidants and total oxidant status (TOS) shows the total effect of all oxidants existing in body fluids [9]. Therefore, this research focused on measurement and comparison of TAC, TOS and oxidative stress index (OSI/) in the serum of active smokers, passive smoker and non-smokers in university students.

Materials and methods

Study population

The subjects (age range = 18–27 y) for this study were randomly selected from a population of students of medical sciences universities in Tehran, Iran. All subjects were healthy and confirmed no use of illegal drugs during the study period. Some eligibility criteria were as follows; normal liver/kidney function, no diagnosis of invasive cancer, not using megadoses of vitamins, not having uncontrolled diabetes or hypertension, not pregnant, good performance status, not immunosuppressed and not requiring oxygen supplementation and having normal sleep-wake cycle. All subjects were interviewed for tobacco use and questioned on the number of cigarettes smoked per day and about their exposure status with secondhand smoking. The study population consisted of 50 smokers, 50 passive smokers and 50 nonsmokers. Serum nicotine and cotinine concentrations were measured to confirm the smoking status of the subjects. All records were coded and kept secret.

Sampling

TAC and TOS levels of the subjects were measured. For the measurements, blood samples were gathered from test groups and controls between 7:00 am and 9:00 am after a night fasting period of 10–12 h. The blood samples were centrifuged at 2500 rpm for 12 min and serum was separated immediately after coagulation. The serum samples were kept at − 70 °C until TAC and TOS levels were measured.

Analysis of serum samples

In serum samples, the levels of TAC and TOS were measured by spectrophotometric method using Rel Assay Diagnostics kit. These methods are automated new generation and colorimetric. Erle's TOS method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant compounds in acidic media [10]. TAC was measured by using the automated new stable, colored 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS+). The ABTS + is decolorized by antioxidants according to their antioxidant capacities and concentrations [11]. Oxidative stress index (OSI) was calculated through the TOS/TAC formula in three groups [12].

Statistics

TAC and TOS levels among three groups (smokers, passive smokers and nonsmokers) were analyzed and because the distribution of data was not normal, the analysis was done by means of two statistical strategies: Kruskal-Wallis test and analysis of variance (one- way ANOVA) followed by Scheff's post hoc test. Results were showed as mean ± S.D. and 95 % confidence intervals. The level of significance was set to 0.05 and p values > 0.05 were assumed to be nonsignificant. Associations between TAC and TOS levels was evaluated by correlation test. All effective variables were examined by multiple linear regression analysis.

Results

A total of 150 participants [74 female (49.33 %) and 76 male (50.67 %)] with a mean age of 23.8 ± 7.0 (min = 18, max = 27, median = 24) were included in the study. The 50 participants who smoked, the 50 participants who were passive smoker and the other 50 nonsmokers were equated in terms of age and gender characteristics. Table 1 shows the results of TAC and TOS levels measurements in serum samples, in the three groups of smokers, passive smokers and nonsmokers. The mean value TAC levels in serum samples of the three groups of smokers, passive smokers and nonsmokers were 1.096, 1.220 and 1.844 mmol Trolox equivalent/L, respectively, which were significantly greater in nonsmokers than smokers and passive smokers and were significantly lower in the active smokers than in the passive smokers. The mean value TOS levels in serum samples of the three groups of smokers, passive smokers and nonsmokers were 13.747, 11.099 and 7.651 μmol H2O2 equivalent/L, respectively, which were significantly lower in nonsmokers than two other groups. OSI values in smokers and passive smokers were significantly higher than the control group. TOS and OSI levels were significantly higher in the active smokers than in the passive smokers. A significant positive correlation was found between cigarette exposure (by active or passive smoking) and serum OSI levels. The data showed that females had significantly higher levels of TAC and lower level of TOS and OSI in all groups in comparison of male subjects. A statistically significant relationship could not be found between age, BMI and TAC, TOS and OSI levels. A statistically significant negative relationship was observed between the number of cigarette per day and TAC, level.

Discussion

Tobacco use is an important causes of early and preventable deaths in the world. Smoking addiction is one of the most important public health concern due to the socioeconomic and medical problems it causes in our society, where approximately 15 million people currently smoke [13].

In this research, TAC, TOS and OSI levels were evaluated in smokers, passive smokers and nonsmokers. In our study, the 150 participants were divided in three groups of smokers, passive smokers and nonsmokers and equated in terms of gender and age characteristics. In the present study, a statistically significant difference were found among the smokers, passive smokers and nonsmoker groups in terms of TAC, TOS and OSI values.

In a study conducted by Mahmoud et al. in Mosul on 20 active smokers and 20 non-smokers, the mean TAC in smokers was significantly lower than in non-smokers [14]. Similar to our study, in Köse et al. showed that TOS and OSI were significantly higher in smokers and the mean TAC was significantly lower in smokers than in non-smokers [15]. Contrary to the present study, Aslan et al. stated that there were no significant differences between smokers and non-smokers in terms of TAC, TOS and OSI values [16]. These differences in the results of different studies indicate that TAC and TOS levels are affected by many factors such as stress, ischemia, bleeding, infection, radioactivity, medications, long-term metabolic diseases, sun exposure, smoking, and the aging process [17].

The results of this study also showed that the marker of total oxidative status in the group of smokers (both active and inactive) was significantly higher than the non-smoking group, which by calculating the oxidative stress index (OSI) this difference is quite clear and obvious. In a 2008 study, Aycicek et al. Examined changes in the antioxidant system in cord blood in smoking mothers and mothers exposed to secondhand smoke. Total antioxidant capacity of cord blood decreased significantly in the two groups of active and inactive smoking mothers compared to non-smoking mothers [18]. High and negative correlation was observed between total antioxidant capacity and total oxidant status. It seems that body mass factor has no significant effect on the values of selected markers in all study groups. Other studies also, have not reported body mass as an effective factor in the oxidative stress system [19]. Gender was identified as an influential factor in all groups. The oxidative stress system seems to be more stable in women, with a higher total antioxidant capacity. Hakim et al. examined the effect of sex factor on other biomarkers of oxidative stress system such as

Table 1 Mean serum levels (S.D.) of TAC, TOS and OSI in three groups (n = 50)

Groups	SEX	No.	BMI	TAC (mmol Trolox equivalent/L)	Pvalue*	TOS (μmol H2O2 equivalent/L)	Pvalue*	OSI	Pvalue*
Smoker	Male	27	24.599±2.573	1.040±0.194	0.025	14.212±2.296	0.021	1.439±0.459	0.015
	Female	23	21.899±1.671	1.162±0.148	0.032	13.200±2.154	0.015	1.176±0.358	0.036
Secondhand Smoker	Male	24	23.939±2.645	1.155±0.219	0.015	11.629±1.247	0.012	1.058±0.301	0.012
	Female	26	22.291±1.411	1.280±0.249	0.024	10.610±1.646	0.022	0.887±0.324	0.031
Nonsmoker	Male	25	23.621±2.455	1.704±0.482	0.032	8.065±1.965	0.025	0.549±0.295	0.028
	Female	25	22.158±1.594	1.984±0.504	0.017	7.235±1.868	0.012	0.415±0.224	0.024

*Significance level: ≤0.05

8OHdG and 8-iso-PGF2a and observed higher levels of these factors in women than men. Differences in xenobiotic metabolism between men and women may be a factor in the higher levels of oxidative stress found in female smokers. Female hormones, especially estrogen, can influence cytochrome P450 enzyme expression [20]. The number of cigarettes smoked per day has a high effect on reducing the level of total antioxidant capacity and increasing the oxidative stress index in both sexes.

Conclusions

According to our findings, the antioxidant capacity in all smokers (active and inactive) was less than the control group (non-smokers). The results of this study showed that smoking reduces the activity of the antioxidant defense system and activates the oxidative stress system in the body. Based on these findings, it can be clearly concluded that the decrease in antioxidant capacity in smokers is associated with increased production of oxidants and free radicals.

Abbreviations TAC, Total Antioxidant Capacity; TOS, Total oxidant Statu; OSI, Oxidative Stress Index.

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Author contributions RA and NR participated in the design of the study. RA did the analyses and FY interpreted the analyzed results. NR was the main investigator, supervised the work, drafted and revised the paper critically for important intellectual content and compiled the work in accordance to journal format. All authors have read and approved the final manuscript.

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Data availability The data will not be shared with a reason

Declarations Not applicable.

Ethics approval and consent to participate The research protocol was approved by Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.585).

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no competing interests.

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