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The correlation of the total antioxidant status (TAS), total oxidant status (TOS) and paraoxonase activity (PON1) with smoking

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ABSTRACT

Objective: In this study, we aimed to assess the total antioxidant status (TAS), total oxidant status (TOS) and paraoxonase activity (PON1) in smokers and nonsmokers.

Design and methods: This cross-sectional analytical study was conducted on 100 smokers and 100 non-smokers. Low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), total cholesterol (TC), triglyceride (TG), fasting blood glucose (FBG), TAS, TOS and PON1 levels of the participants were determined in the blood samples. TAS and TOS were determined by using the automated measurement method. Paraoxon was used as a substrate for measuring PON1 activity.

Results: A statistically significant difference could not be found between smokers and nonsmokers in terms of mean FBG, LDL-c, HDL-c, TC, TG, TAS, TOS, PON1, oxidative stress index (OSI) and body mass index (BMI). Mean TAS and TOS levels were higher in men than women ($p = 0.001$). As age ($p = 0.022$) and age to start smoking ($p = 0.023$) increased, TOS level decreased. As the age to start smoking ($p = 0.001$) increased, TAS level decreased whereas as BMI ($p = 0.001$) increased, TAS level also increased. A statistically significant relationship could not be established between age, age to start smoking, duration and amount of smoking, dependence score and BMI and PON1 ($p > 0.05$).

Conclusions: In our study, although no significant correlation could be established between smokers and nonsmokers in terms of mean TAS, TOS and PON1, it is a fact that TAS, TOS and PON1 in the organism are affected by many factors and therefore there is a need for more extensive studies in this regard.

Introduction

Cigarette use is one of the most important causes of early and preventable death and a significant public health concern worldwide and particularly in developing countries. There are nearly 1.1 billion smokers in the world today and more than 5 million people die because of tobacco use every year [1,2]. In Turkey, 31.2% of adults over the age of 15 (nearly 16 million individuals) are currently cigarette smokers [3].

Atoms or molecules that have one or more unpaired electrons in their outermost orbitals are called free radicals [4]. Free radicals are highly unstable and reactive molecules. Their electrons interact with other molecules within cells and damage various biological materials such as proteins, lipids, DNA and nucleotide coenzymes [5]. Substances that prevent or that can delay the oxidation of these biological materials that exist in living cells are called antioxidants, and this occurrence is called antioxidant defense [6]. In an organism, there is a balance between the production rate of free radicals and their rate of destruction. This state, which is called "oxidative balance", protects the organism by fending off the damaging effects of free radicals. An imbalance that occurs between free radical formation and antioxidant defense system in favor of free radicals is called "oxidative stress" [7]. Cigarette contains a substantial amount of free radicals which play a significant role in the impairment of oxidative balance and cause cell damage. This forms the basis of many health problems caused by smoking, such as cancer, cardiovascular and pulmonary diseases [8]. Total antioxidant status (TAS) reflects the total effect of all antioxidants and total oxidant status (TOS) reflects the total effect of all oxidants existing in plasma and body fluids [9,10].

Reactive oxygen species (ROS) and free radicals which occur in tissues can damage the biologically important materials such as DNA, proteins, carbohydrates and lipids. ROS are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms which remove them via enzymatic and nonenzymatic antioxidative mechanisms. Under certain conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidative/antioxidative balance shifts towards the oxidative status. Consequently, oxidative stress, which has been implicated in over 100 disorders, develops (10).

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Paraoxonase is a serum esterase which has arylesterase and paraoxonase activity, is the active metabolite of parathion and can hydrolyze paraoxon, which irreversibly inhibits acetylcholinesterase [11]. The paraoxonase multigene family, which is located at q21.3-22.1 region of human chromosome 7, consists of three members named as PON1, PON2 and PON3. PON1 is the most studied and best known member of the paraoxonase multigene family [12]. PON1 is a calcium-dependent enzyme composed of 354 amino acids with a molecular weight of 43 kDa and localized on HDL-c [13]. The function of PON1 is to protect LDL-c and HDL-c from oxidation and thus to prevent cardiovascular diseases and atherosclerosis [14].

In the present study, it was aimed to compare the TAS, TOS and PON1 levels in smokers and nonsmokers who applied to the Family Medicine Polyclinic of Meram Medical Faculty.

Materials and method

Study design, setting and population

This cross-sectional analytical study was conducted on 100 smokers and 100 nonsmokers who had never smoked or had not smoked at least for the last 6 months who applied to the Family Medicine Polyclinic of Meram Medical Faculty between May 2011 and December 2011. Patients with a known systemic history of hypertension, diabetes mellitus, chronic obstructive pulmonary disease, coronary artery disease, ischemia, hemorrhage, trauma, radioactivity and those with a history of antioxidant drug use within the last 2 months were not included in the study. The participants were not consuming alcohol and narcotic. A survey form inquiring about the socio-demographic characteristics and smoking status of the participants was administered through face-to-face interviews.

Nicotine dependence

The Fagerström Test for Nicotine Dependence was used to assess the nicotine dependence of the participants. According to the results of this test, participants whose total scores were 0–2 points were classified as very low dependent, 3–4 were classified as low dependent, participants with a total score of 5 were classified as medium dependent, 6–7 were classified as high dependent and those who scored 8–10 points were very high dependent [15].

Biochemistry and laboratory evaluation

Total cholesterol, LDL-c, TG, HDL-c, FBG, TAS, TOS and PON1 levels of the participants were measured. For the measurements, blood samples were taken from patients and controls between 8:00 am and 10:00 am after a night fasting period of 10–12 h. The blood was centrifuged at 2500 rpm for 10 min and serum was separated immediately after coagulation. Total cholesterol, HDL-c and TG levels of the serum were measured through routine methods using the Synchron LX System (Beckman Coulter, Fullerton CA) without delay. LDL-c was calculated using the Friedewald formula. The serum was kept at −70 °C until PON1 activity and TAS and TOS levels were measured.

TAS was measured by using the automated new generation, more stable, colored 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS+). The ABTS+ is decolorized by antioxidants according to their concentrations and antioxidant capacities. This change in color was measured as a change in absorbance at 660 nm. This process was applied to an automated analyzer and the assay was calibrated with Trolox. The results were expressed as mmol Trolox equivalent/L (9).

TOS was determined by using the automated measurement method. The oxidants in the sample oxidize the ferrous ion–*o*-dianisidine complex to ferric ion. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide (H_2O_2) and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L) [10]. Oxidative stress index (OSI) was calculated through the TOS/TAS formula.

Paraoxon was used as a substrate for measuring PON1 activity and the absorbance of the color formed through the hydrolysis of paraoxon was recorded at 412 nm and 37 °C. Basal PON1 activity was measured and the results were expressed as U/L [11].

Ethical considerations

The study protocol was approved by the Ethics Committee of Meram Medical Faculty of Konya University and written informed consent was obtained from all participants before participation in this study.

Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 16.0 was used for the statistical analysis of the data obtained in the study. Frequencies, means, standard deviations, medians, minimum and maximum values were calculated. Comparison of the means was performed using Student's *t* test and the qualitative data were compared using the Chi-square test. Correlations among the parameters were analyzed by using the Pearson's Correlation Analysis. Correlation coefficient (r) was described as a weak correlation between 0.00 and 0.24, moderate between 0.25 and 0.49, strong between 0.50 and 0.74 and very strong between 0.75 and 1.00. The results were interpreted using a 95% confidence interval and at a significance level of $p < 0.05$ [16].

Results

A total of 200 participants [78 female (39.0%) and 122 male (61.0%)] with a mean age of 31.8 ± 7.0 (min = 20, max = 50, median = 31)

Table 1
Assessment of the socio-demographic characteristics of the participants.

	Smokers (n = 100)	Non-smokers (n = 100)	Total	p
	n	n	n	
Gender				
Female	39	39	78	1.000
Male	61	61	122	
Age distribution				
21–30 years	50	49	99	0.988
31–40 years	37	38	75	
41–50 years	13	13	26	
Marital status				
Married	70	66	136	0.414
Single	27	33	60	
Widow/divorced	3	1	4	
Occupation				
Housewife	11	14	25	**0.000**
Civil servant	23	52	75	
Worker	50	21	71	
Tradesman	9	0	9	
Unemployed	4	4	8	
Student	3	9	12	
Education level				
Primary	22	18	40	**0.000**
Secondary	5	7	12	
High school	39	12	51	
University	34	63	97	