TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2009; 39 (4): 513-517
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0805-41

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The effects of cigarette smoking on serum oxidant status, and cholesterol, homocysteine, folic acid, copper, and zinc levels in university students

Aim: To examine the effect of cigarette smoking on serum oxidative damage and oxidant status in university students.

Materials and Methods: Subjects were randomly chosen from among Ankara University Faculty of Science students. The study was performed at the Ankara University Faculty of Health Sciences, Department of Nutrition and Dietetics, and the Ankara University Faculty of Medicine, Department of Biochemistry. In all, 44 volunteer (22 smokers and 22 non-smokers) students participated in the study. Malondialdehyde, sensitivity to oxidation (SO), and antioxidant potential (AOP), and total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, homocysteine, folic acid, copper, and zinc levels were measured in serum samples.

Results: Serum SO levels were significantly higher in smokers than in non-smokers (1.01 ± 0.64 and 0.49 ± 0.14, respectively).

Conclusions: Smoking history could be evidence of oxidative stress (high serum SO concentrations) and an impaired oxidant defense system.

Key words: Smoking, sensitivity to oxidation, antioxidant potential, cholesterol, homocysteine, folic acid, copper, zinc

Üniversite öğrencilerinde sigara içmenin serum oksidan durum, kolesterol, homosistein, folik asit, bakır ve çinko seviyeleri üzerine etkileri

Amaç: Bu çalışmanın amacı üniversite öğrencilerinde sigara içiminin serum oksidan durum üzerine olan etkilerini araştırmakdı.

Yöntem ve Gereç: Çalışmaya katılan kişiler Ankara Üniversitesi Fen Fakültesi öğrencileri arasından rastgele seçildiler. Çalışma Ankara Üniversitesi Sağlık Bilimleri Fakültesi Beslenme ve Dietetik Bölümü ve Tıp Fakültesi Biyokimya Bölümünde gerçekleştirildi. Çalışmaya 44 gönüllü (22 sigara içen ve 22 içmeyen) katıldı. Serum örneklerinde malondialdehid, oksidasyona duyarlılık (OD), antioksidan potansiyel (AOP), total kolesterol, HDL kolesterol, LDL kolesterol, VLDL kolesterol, homosistein, folik asid, bakır ve çinko seviyeleri ölçüldü.

Bulgular: Serum OD seviyeleri sigara içenlerde içmeyenlerden istatistiksel olarak anlamlı düzeyde yüksekti (sırasıyla; 1,01 ± 0,64 ve 0,49 ± 0,14).

Sonuç: Sigara içme artmış oksidan stres (artmış oksidasyona duyaylılık) ve bozulmuş oksidan savunma sistemi nedenidir.

Anahtar sözcükler: Sigara içme, oksidasyona duyarlılık, ontioksidan potansiyel, kolesterol, homosistein, folik asid, bakır, çinko

Received: May 12, 2008
Accepted: January 12, 2009

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Introduction

Cigarette use among young people is gradually increasing (1). In the present study the effects of cigarettes on serum oxidant status, lipid profile, cardiovascular risk factors, and on the nutritional behavior of smokers were investigated in a

sample of university students (young people who consumed a similar diet). Smoking is regarded as a risk factor for numerous diseases. Tobacco smoke contains thousands of chemicals that cause several disorders, including cardiovascular disease, cancer, and chronic obstructive pulmonary disease (2). Free radical-mediated processes have been implicated in cigarette-related diseases. Free radicals in cigarette smoke may cause oxidative damage to macromolecules, contributing to cardiovascular disease and cancer (3,4). In healthy subjects with good nutrition these free radicals are eliminated by antioxidant mechanisms, including enzymatic and non-enzymatic systems (5). Both copper (Cu) and zinc (Zn), which are trace elements in sera, are cofactors of the antioxidant enzyme, superoxide dismutase (SOD). Cu-Zn SOD converts the superoxide anion to hydrogen peroxide (H_2O_2), which is then reduced to H_2O by the antioxidant enzymes catalase and glutathione peroxidase (6).

Homocysteine is an amino acid and at elevated levels is a risk factor for cardiovascular disease (CVD). It has been reported that homocysteine thiolactone changes LDL lipoproteins by binding to apolipoprotein B free lysine groups, which results in elevated LDL aggregation and the formation of foam cells (7). Additionally, the proliferation of endothelial cells, which is necessary for angiogenesis, is suppressed by hyperhomocysteinemia (8). According to the results of a study, a 5-µmol/L increase in homocysteine concentration leads to, on average, a 70% increase in the risk of CVD (9). Folic acid (or folate) is a regulator of homocysteine.

Materials and methods

Subjects in the present study were selected from among Ankara University Faculty of Science students. The study was performed at the Ankara University Faculty of Health Sciences, Department of Nutrition and Dietetics and the Ankara University Faculty of Medicine, Department of Biochemistry. The study was performed from April 2004 to October 2006.

Smokers (n = 22; 11 female, 11 male; mean age: 21.91 ± 1.57 years) were defined as those that had smoked 10 or more cigarettes per day for at least 1 year (mean: 4.77 ± 1.80 years), while non-smokers (n = 22; 11 female, 11 male: mean, age 20.45 ± 1.37 years) were those that had never smoked. None of the participants had any chronic diseases. The difference in mean age between the 2 groups was not significantly significant. Additionally, the difference between mean body mass index (BMI) in the smoking and non-smoking groups was not significant (22.15 ± 0.72 and 21.27 ± 0.45, respectively) All the volunteer students signed an informed consent form and the study was approved by the Ankara University School of Medicine Ethics Committee.

The thiobarbituric acid reactive substances (TBARS) method was used to measure serum levels of malondialdehyde (MDA). The results are expressed as nmol/mg of protein. To measure sensitivity to oxidation, MDA levels were studied by using the TBARS method in copper-induced samples. Samples were incubated at 37 °C with 1 mm of CuSO4. The difference between induced and non-induced (basal) MDA levels were used to evaluate sensitivity of oxidation, and the results are expressed as nmol/mg of protein/hour (10). For the measurement of anti-oxidant potential (AOP), samples were incubated with xanthine—a xanthine oxidase system—in the presence of cod liver oil. After 1 h of incubation, MDA levels were measured in all samples and the results are expressed as U/mg of protein (11). Protein determination was performed using the Lowry method (12). The enzymatic colorimetric method (Integra 400 automated analyzer, Roche Diagnostics GmBH, Mannheim, Germany) was used to measure both serum TC and HDL-C (13,14). LDL-C and VLDL-C concentrations were calculated according to the Friedewald formula (15). The enzyme immunoassay (EIA) for the measurement of plasma total homocysteine was performed using the AXIS Homocysteine EIA Kit (Axis-Shield Diagnostic Ltd. Dundee, UK). Folic acid level was determined using the electrochemiluminescence method (Roche E170 automated analyzer, Roche Diagnostics GmBH, Mannheim, Germany). Measurement of Cu and Zn concentrations was accomplished with the colorimetric method (Randox Laboratories Ltd, UK).

Statistics: The results are expressed as arithmetic mean ± standard deviation (mean ± SD). For statistical evaluation Student's t-test was used and P values < 0.05 were accepted as significant.

Results

The sensitivity to oxidation and anti-oxidant potential values are shown in Table 1. Serum cholesterol, homocysteine, folic acid, copper, and zinc levels are given in Table 2. As seen in Table 1, sensitivity to oxidation was significantly higher in the smoking group than in the non-smoking group ($P < 0.05$). Although the difference was not significant, the antioxidant potential value was lower in the smoking group than in the non-smoking group. No differences were observed in MDA, serum total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, homocysteine, folic acid, copper, or zinc levels between the 2 groups.

Discussion

The present study investigated the relationship between smoking and antioxidant status, cholesterol parameters, homocysteine, folic acid, and trace elements, including Cu and Zn, in university students.

Lower antioxidant potential levels in the smokers may have been due to low-level consumption of antioxidant foods (16,17). Domagala et al. reported a significant correlation between homocysteine concentration and lipid peroxidation (18). It has been shown that elevated homocysteine levels are associated with elevated lipid peroxidation (19,20). A moderate homocysteine level is also associated with an increased risk of atherosclerosis (21).

The homocysteine level in smokers was higher than in the non-smokers (22). In another study a positive correlation was observed between the number of cigarettes smoked per day and plasma homocysteine (23); however, Genest et al. reported that there wasn't a correlation between smoking and homocysteine level (24). On the other hand, there is a negative correlation between folic acid and homocysteine concentration (25). It is shown that both dietary and supplemental folate can increase the level of homocysteine (22).

Table 1. Sensitivity to oxidation and antioxidant potential values in the smoking and non-smoking groups (mean ± SD).

	Smoking group	Non-smoking group	P
SO (nmol/mg/h)	1.01 ± 0.64*	0.49 ± 0.14	0.001
AOP (u/mg)	11.54 ± 1.74	12.69 ± 3.08	0.136
MDA (nmol/mg)	0.81 ± 0.33	0.81 ± 0.53	0.984

*$P < 0.05$ (Student's t-test between the smoking and non-smoking groups).
Abbreviations:
SO: Sensitivity to oxidation; AOP: antioxidant potential; MDA: malondialdehyde

Table 2. Biochemical parameters (mean ± SD).

Parameters	Smoking group	Non-smoking group	P
Total cholesterol (mg/dL)	158.32 ± 21.64	167.77 ± 20.01	0.141
HDL cholesterol (mg/dL)	43.79 ± 10.99	49.21 ± 12.72	0.138
LDL cholesterol (mg/dL)	93.81 ± 17.42	100.19 ± 20.15	0.267
VLDL cholesterol (mg/dL)	20.58 ± 11.97	18.27 ± 6.98	0.439
Copper (µg/dL)	96.36 ± 31.21	105.18 ± 30.57	0.349
Zinc (µg/dL)	90.14 ± 16.91	83.91 ± 16.54	0.224
Homocysteine (µmol/L)	16.03 ± 7.76	14.66 ± 5.94	0.516
Folic acid (ng/mL)	5.01 ± 1.37	5.01 ± 1.29	0.992

Faruque et al. reported lower serum Cu and higher serum Zn levels in smokers than in non-smokers (26); however, some researchers have indicated that smokers have significantly higher serum Cu concentrations and unaffected Zn concentrations, as compared to non-smokers (27,28).

Our data suggest that sensitivity to oxidation in smokers was higher than in non-smokers. Although the difference was not significant, the antioxidant potential in the smoking group was lower than that in non-smoking group. No differences were observed in serum total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, homocysteine, folic acid, copper, or zinc levels between the 2 groups. As it is known, an increased homocysteine level is a risk factor for cardiovascular disease. The smoking group had elevated homocysteine (although not statistically different than in the non-smoking group). We think that with a larger study population the difference between smoking and non-smoking groups would be significant.

In light of these findings it is concluded that smokers are more susceptible to oxidant stress as a consequence of insufficient antioxidant potential and greater oxidative burden. The consumption of antioxidant foods should be recommended to smokers in order to compensate for higher oxidant load. Additionally, they must be encouraged to stop smoking. In particular, young smokers should quit promptly before health problems arise, so as to have the optimal benefits of cessation.

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