ORIGINAL ARTICLE

Effects of acute cigarette smoking on total blood count and markers of oxidative stress in active and passive smokers

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Abstract

Background: Free radicals, as a product of cigarette smoke, are considered to have deleterious effects causing oxidative stress. Acute active smoking seems to be followed by transient leukocytosis and delayed increase in neutrophil activation. The aim of the present study was to investigate the oxidative status of smokers and passive non-smokers, as well as the impact that acute cigarette smoking has on hematological parameters.

Methods: Thirty-two healthy volunteers, 16 active smokers (Group A) aged 20-23 years and 16 age-matched, non-smokers (Group B), 18 women and 14 men in total, participated voluntarily in the study. All subjects did not have any food, drink, or cigarette smoking for eight hours before the study. Each time, two active smokers and two non-smokers were exposed simultaneously for half an hour to the smoke of two cigarettes smoked consecutively by the smokers. Blood was drawn before and after the exposure to cigarette smoke. Whole blood was analyzed immediately for total blood count parameters and serum was stored in -70°C until serum levels of malondialdehyde (MDA) and vitamin E (VitE), and total antioxidant capacity (TAC) were determined.

Results: No statistical significant difference was observed in the values of white blood cells and their subpopulations between the two groups and within the same group before and after exposure to cigarette smoke. In the group of smokers, granulocyte/lymphocyte ratio increased significantly, MDA levels showed significant elevation and protective VitE serum levels decreased significantly, whereas TAC was reduced, but not significantly, after the exposure. In the group of passive, non-smokers the results of the blood count parameters, MDA and VitE were similar to Group A, and there was a significant decrease in TAC, as well. Between the two groups, only hematocrit values and MDA levels differed significantly before the exposure to smoke, and no other significant difference was detected before or after the exposure, between active and passive smokers.

Conclusions: Acute exposure to cigarette smoking affects hematological indexes and oxidative stress biomarkers negatively, in both active and passive smokers, with similar results. The outcome seems to be even worse in passive smokers regarding oxidative stress and antioxidant protection markers. Elimination of cigarette smoking could prevent the adverse effects for smokers, as well as for healthy non-smokers in their vicinity. Hippokratia 2015; 19 (4): 293-297.

Keywords: acute smoking, passive smoking, malondialdehyde, WBC, vitamin E, total antioxidant capacity

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Introduction

Atherosclerosis is the major cause of cardiovascular diseases (CVD), initiating from childhood. As it has been proposed and proved in the past, oxidative modification of low-density lipoprotein (LDL) particles is responsible for macrophage foam cell formation, the progress of the atherogenic process and, later in life, atherothrombotic events[1]. Free radicals, atoms or molecules with one or more unpaired electrons, are highly reactive and mainly responsible for causing damage to molecules, such as proteins, carbohydrates, lipids and DNA[2].

Various exogenous factors, such as radiation, smoking, etc., cause the production of free radicals, inducing an imbalance between free radicals and antioxidant protection mechanisms, which is called oxidative stress, and enhancing LDL oxidation[3]. Smoking accounts for 17%-30% of all morbidity from cardiovascular diseases and is considered to be a preventable cause[4]. Cigarette smoke is rich in Reactive Oxygen and Nitrogen Species (ROS and RNS), such as nitrogen, alkoxyl and peroxyl radicals. These can cause the production of other free radicals, which, in turn, initiate lipid peroxidation on the

LDL particle and cause endothelial cell dysfunction[5,6].

Among lipid peroxidation end-products is malondialdehyde (MDA), a highly toxic compound, which can react with imino (=NH) and sulphydryl (-SH) groups of proteins and with deoxyribonucleic acid (DNA), causing toxic stress in cells and mutations. It is considered to be a biomarker of oxidative stress and can be assayed spectrophotometrically by the use of thiobarbituric acid[7]. Moreover, vitamin E (VitE) is a fat-soluble antioxidant, which acts as peroxyl radical scavenger, preventing the propagation of free radicals and protecting polyunsaturated fatty acids from oxidation. Thus, VitE is considered atheroprotective, although high-dosage supplementation has been shown to increase mortality from CVD[8,9]. Total Antioxidant Capacity (TAC) reflects the amount of all antioxidants in the body and is a biomarker of antioxidant protection against free radicals[10]. Finally, several studies have shown that white cell count and differential leukocyte percentages and markers of platelet activity are impaired due to cigarette smoking[11,12].

Passive smoking plays a central pathogenetic role in lipid peroxidation and atherosclerotic plaque formation[13] and has been associated with increased risk of coronary artery disease (CAD) due to arterial injury and reduced arterial elasticity[14]. Cigarette smoke contains more than 4,000 chemicals, such as polynuclear aromatic hydrocarbons, tobacco-specific N-nitrosamines and aromatic amines, all capable of inducing free radical generation and act as highly oxidative and carcinogenic[15]. While oxidative stress and hematological alterations are well documented[16,17] in active smokers, data of *in vivo* studies on passive smokers are rare. The aim of the present study was to examine the impact of passive smoking on markers of oxidative stress and on total blood count.

Material and Methods

Thirty-two healthy volunteers, 16 active smokers (Group A) aged 20-23 years and 16 age-matched, non-smokers (Group B) participated voluntarily in the study. All participants provided written consent and had no food, drink, or cigarette smoking for eight hours before the study. Blood was drawn before and after the exposure to the cigarette smoke. The procedure was as following: four people, two smokers and two non-smokers, entered a small room (2 m^2) for half an hour. During that time, each of the smokers smoked two cigarettes. The procedure was repeated for all subjects and controls. Whole blood was analyzed immediately and serum was stored in -70°C until the rest of the assays were performed.

For the purpose of the study, whole blood was assayed in BC-3200 Auto Hematology Analyzer (Mindray Medical International Ltd., Shenzhen 518057, China) for total white blood cells (WBC) and their subpopulations, red blood cells (RBC) and platelets (PLT) count, leucocyte distribution percentages, hemoglobin (Hb), hematocrit (Ht), and mean erythrocyte volume (MCV). Granulocyte/lymphocyte ratio (GLR) was then calculated. Moreover, levels of MDA, VitE and TAC were determined in serum. MDA was analyzed by a colorimetric reaction with thiobarbituric acid (TBA)[18] and VitE by reverse phase high-performance liquid chromatography (rp-HPLC) (Shimadzu LC-6A, Kyoto, Japan)[19]. Finally, TAC serum levels were assayed with the use of "Antioxidant Assay Kit" (Cayman Chemical Co., IN. 709001, Michigan, USA). The principle of the assay is based on the ability of antioxidants of the sample to inhibit the oxidation of 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] to its complex compound with metmyoglobin.

Statistical analysis was performed with IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test was used for all parameters and in both groups for evaluation of normality of distribution of values. All parameters presented normal or marginally normal distribution. Therefore, paired Student's t-test was used for parametric comparisons across groups (active and passive smoking) matched for age, expressed as means ± standard deviation (SD). Paired t-test, for parametric comparisons of related samples, was performed to test differences before and after exposure to cigarette smoke in each group of the study. Statistical significance was defined at $p < 0.05$, with p values being 2-sided.

Results

Results of total blood count and biomarkers of oxidative stress for active and passive smokers participating in the study, and the significance of their differences between groups, are shown in Table 1. In specific, values of total blood count, MDA, VitE and TAC are presented in the two groups, before and after smoking and exposure of passive smokers to cigarette smoke. In both groups, there was statistically significant decrease in values of VitE, increase of MDA and increase of GLR, after exposure to smoke.

The between-groups comparisons showed marginally statistically significant differences in Ht, Hb and MDA before acute active and passive smoking. Ht and Hb differed significantly between the two groups after the exposure to smoke.

In Figure 1 and Figure 2 the comparison of values of MDA and TAC before and after the exposure to smoke, in the Groups A and B, is presented.

Discussion

Free radicals are main constituents of cigarette smoke. There are studies supporting the notion that these free radicals have deleterious effects in both smokers and passive smokers, causing oxidative stress[13]. Moreover, it has been shown that acute active smoking is followed by transient leukocytosis and delayed increase in neutrophil activation[20]. In our study, an attempt has been made to investigate the oxidative status of active and passive smokers, as well as the impact that acute cigarette smoking has on hematological parameters, such as WBC count, MCV, etc. In specific, no statistically significant difference was observed between values of WBC count

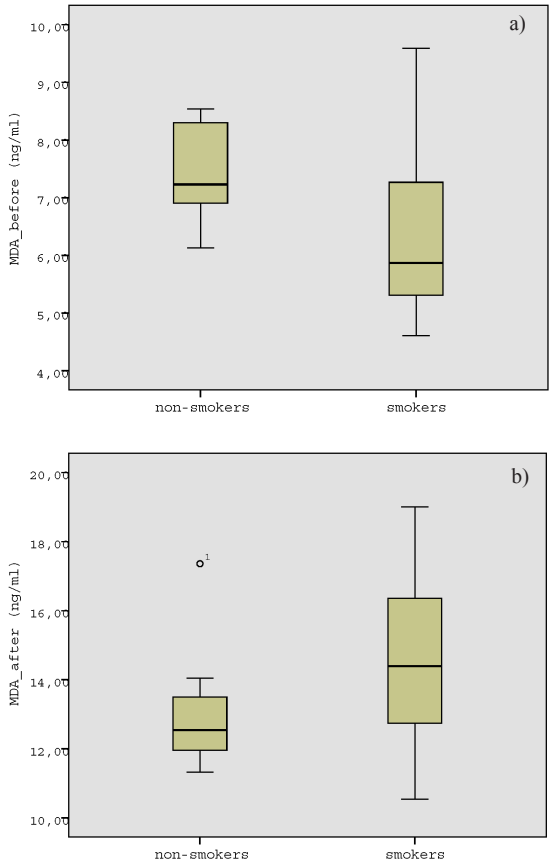

Figure 1: Distribution of values of malondialdehyde (MDA) in smokers and non-smokers a) before and b) after exposure to cigarette smoke.

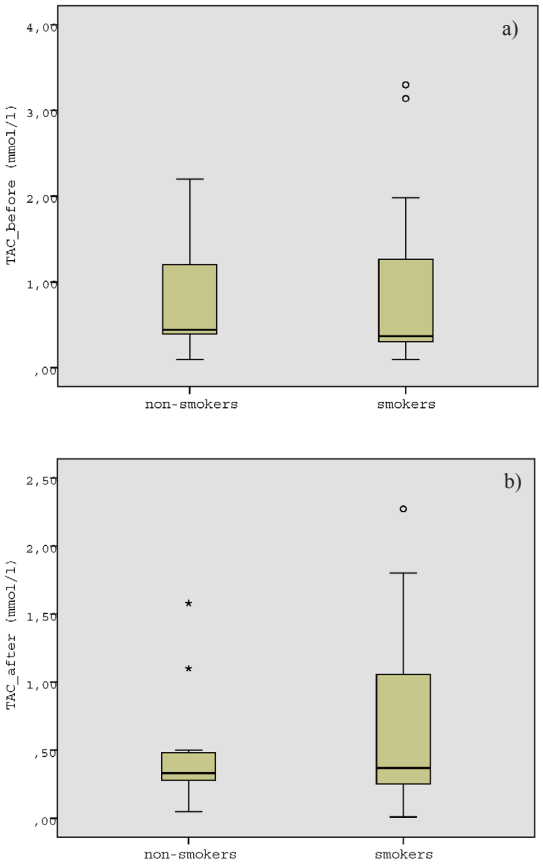

Figure 2: Distribution of values of total antioxidant capacity (TAC) in smokers and non-smokers a) before and b) after exposure to cigarette smoke.

and their subpopulations between the two groups, although higher in active smokers, and within the same group, before and after exposure to cigarette smoke.

In the group of smokers, there was no significant difference between total blood count before and after the exposure to cigarette smoke, except for GLR (granulocyte/lymphocyte ratio) which significantly increased after the exposure, showing a trend for granulocyte increase against lymphocytes. These results are in contrast to the study by Freedman et al who found a distinct increase in lymphocyte subpopulation of WBC[11]. Nevertheless, our results are in agreement with those of Blann et al in which transient leukocyte count and neutrophil activation was observed after acute smoking[20].

In contrast, in the same group, among oxidative stress biomarkers studied MDA showed a significant elevation and protective VitE a significant decrease, after smoking. Nevertheless, TAC was reduced but not significantly, which is in accordance with the study of Lim et al[21]. These findings are similar to those of van der Vaart et al in which acute cigarette smoking reduced the number of eosinophils and several inflammatory cytokines, possibly due to the anti-inflammatory effect of carbon monoxide and increased products of lipid peroxidation and degradation products of extracellular matrix proteins[22]. In the group of passive non-smokers, similar were the results regarding total blood count parameters, in contrast to the study of Sinzinger et al who found significant alterations in platelets function in passive smokers[12].

Between the two groups, only Ht values and Hb levels differed significantly after the exposure to smoke while marginally significant difference was detected in those parameters and MDA before the exposure, between active and passive smokers (Table 1). In specific, smokers presented significantly higher levels of MDA than non-smokers before smoking, fact that was expected. This result can be confirmed by the elevated levels of TAC in the group of passive smokers, although non-significant, compared to smokers. Differences between the two groups before and after exposure to smoke are also expected, as long as smokers have been shown to present higher hematocrit and hemoglobulin levels than non-smokers[23]. About MCV, there was no significant difference in the values between the two groups, in contrast to the study of Inal et al[24], in which MCV values were statistically significantly higher in smokers than non-smokers.

These results lead us to the conclusion that cigarette smoking may have many adverse results in active

Table 1: Values of parameters under study in groups A (16 smokers) and B (16 non-smokers), before and after exposure to cigarette smoke, and differences within and between groups.

Parameter	Units	Group A n=16 (Mean ± SD)			Group B n=16 (Mean ± SD)		
		Before	After	p	Before	After	p
WBC	$10^3/\mu l$	7.430 ± 1.580	7.530 ± 1.900	0.750	6.330 ± 1.300	6.55 ± 1.30	0.380
Neu	$10^3/\mu l$	4.580 ± 1.260	4.830 ± 1.490	0.204	3.750 ± 0.920	4.10 ± 1.25	0.149
Lymph	$10^3/\mu l$	2.330 ± 0.410	2.180 ± 0.350	0.234	2.10 ± 0.540	2.02 ± 0.53	0.165
MID	$10^3/\mu l$	0.480 ± 0.140	0.590 ± 0.330	0.215	0.460 ± 0.140	0.45 ± 0.17	0.549
GLR		1.9 ± 0.5	2.2 ± 0.5	0.015	1.84 ± 0.51	2.1 ± 0.60	0.040
RBC	$10^6/\mu l$	4.700 ± 0.410	4.680 ± 0.390	0.852	4.490 ± 0.590	4.480 ± 0.580	0.738
Hb	g/dl	14.4 ± 1.5*	14.5 ± 1.4**	0.375	13.0 ± 1.3*	13.0 ± 1.2**	0.691
Ht	%	40.7 ± 3.5*	40.7 ± 3.5**	0.967	37.1 ± 3.8*	37.1 ± 3.6**	0.978
MCV	fl	86.85 ± 2.8	87.0 ± 2.7	0.342	83.6 ± 9.9	83.7 ± 9.8	0.315
PLT	$10^3/\mu l$	245.080 ± 5 0.250	250.670 ± 55.530	0.420	205.500 ± 72.200	225.790 ± 43.800	0.410
MDA	ng/ml	6.3 ± 1.5†	14.6 ± 2.8	<0.0001	7.4 ± 0.9†	12.9 ± 1.6	<0.0001
VitE	µmol/l	29.8 ± 1.3	26.2 ± 1.3	<0.0001	30.7 ± 1.2	26.4 ± 1.7	<0.0001
TAC	mmol/l	0.9 ± 1.2	0.7 ± 0.8	0.120	0.8 ± 0.7	0.5 ± 0.4	0.007

WBC: white blood cells, Neu: neutrophil granulocytes, Lymph: lymphocytes, MID: monocytes, eosinophils and basophiles, GLR: granulocyte/lymphocyte ratio, RBC: red blood cells, Hb: hemoglobin, Ht: hematocrit, MCV: mean volume of red blood cells, PLT: platelets, MDA: malondialdehyde, VitE: vitamin E, TAC: total antioxidant capacity, † marginally significant difference of MDA between the two groups before exposure to smoke (p =0.077), *marginally significant difference of Ht and Hb between the two groups before exposure to smoke (p =0.073 and p =0.087, respectively), **significant difference of Ht and Hb between the two groups after exposure to smoke (p =0.043 and p =0.035, respectively).

smokers, as well as in passive smokers, with regard to oxidative stress and antioxidant protection markers. In addition, non-smokers presented a significant decrease of TAC after the exposure to smoke, in contrast to active smokers, and they may be in an even more unfavorable position. Moreover, the study of Kato et al has shown that short-term passive smoking may cause endothelial dysfunction via oxidative stress in non-smokers[25].

In conclusion, acute exposure to cigarette smoke affects hematological indexes and oxidative stress biomarkers negatively, in both active and passive smokers, with similar results. This might mean decreased antioxidant protection and increased risk for cardiovascular diseases for both groups of people. One limitation of the present study is the small number of subjects stratified, due to limited subjects that volunteered to participate, although sample size calculation had been performed. Due to small sample, larger studies are needed to confirm the results and indications of the present study, in order to convince societies for the adverse effects of cigarette smoking in everyday life and health, in general. A major effort should be made for the elimination of the bad habit of cigarette smoking, eradicating the adverse effects for smokers, as well as for healthy people in their vicinity.

Conflict of interest

All authors confirm that there is no conflict of interest.

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