Oxidative and antioxidant status in Smoking men.

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Abstract

Background: Free radicals in cigarette smoke may cause oxidative damage to macromolecules, contributing to cardiovascular diseases and cancer. Decreased plasma antioxidant concentrations in smokers may indicate cigarette smoke–related oxidative stress.

Objective: We compared the effects on serum antioxidant concentrations in confirmed active smokers with those in nonsmokers, independent of differences in dietary intakes and other covariates.

Methods: Serum samples from 60 smokers, and 40 nonsmokers aged 15-60 years were analyzed for ascorbic acid (vitamin C), α-tocopherol (vitamin E), and retinol (vitamin A), by using high performance liquid chromatography (HPLC). The measurement of serum lipid profile, and total lipid peroxidation, oxidized HDL (Ox-HDL) was done as well.

Results: Showed significantly lower serum antioxidant vitamins (A, C & E) concentrations in smokers more than in nonsmokers. Smokers had significant elevation in serum malondialdehyde (MDA) ($p<0.001$) and the percentage of oxidized non high-density lipoprotein (Ox. non HDL %) with a significant reduction in the percentage of oxidized high-density lipoprotein (Ox. HDL %) as compared to the control ($p<0.001$).

Conclusions: These results indicate that cigarette smokers have a significantly lower serum antioxidant status than do unexposed nonsmokers, independent of differences in dietary antioxidant intakes with an increased oxidative stress in smokers' sera.

Key Words: Oxidized HDL, ascorbic acid, α-tocopherol , retinol , cigarette smokers.

IRAQI J MED SCI, 2010; VOL.8 (2): 31-37

Introduction

Oxidative stress is a condition in which the cellular production of reactive oxygen species (ROS) exceeds the physiological capacity of the antioxidant defense system to render ROS inactive [(1)]. Increased production of ROS involves the oxidation of lipids and lipoproteins, DNA, proteins and other molecules in ways that impair normal cellular function, possibly resulting in impaired health and disease [(2)]. Normal cellular metabolism results in the production of ROS; however, both physical and environmental stressors can further increase ROS production. In this regard, two primary environmental stressors include cigarette smoking and high fat meals [(1)].

Cigarette smoking exacerbates ROS formation and poses a significant oxidant stress in vivo [(3)]. In one puff of a cigarette, a smoker is exposed to more than 1015 free radicals in the gas phase alone [(4)], with additional exposure in the tar phase equal to more than 1017 free radicals per gram. It has been consistently reported that cigarette smokers have elevated biomarkers of oxidative stress compared with non-smokers and this represents a potential mechanistic link between regular cigarette smoking and cardio vascular disease (CVD).

The increased oxidative stress observed in smokers may be partly due to the lower blood antioxidant capacity

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Received: 11th November 2009, Accepted: 17th February 2010.

routinely observed in smokers [5]. It is possible that the addition of other ROS generators can further promote oxidative stress in cigarette smokers. To our knowledge, no investigation to date has studied the combined effects of cigarette smoking and oxidative stress biomarkers. Therefore, in the present investigation we compared blood antioxidant status and oxidative stress biomarkers in smoking men with those of age-matched control subjects.

Subjects and Methods

A- Subjects:

This study was conducted on 60 smoking men aged 15-60 years (the mean age 46.52□6.21 years) with at least one year of smoking, they were smoking ≥10 cigarettes per day. They were selected from Al- Kadhumia Teaching Hospital, for evaluation of serum lipid profile and antioxidant vitamins. Any smoker with any medicals illness that may affect the measured parameters such as cardiac, hepatic, endocrine, metabolic diseases, and alcoholism were excluded from the study. Details of clinical state were taken from each subject.

Depending on the years of smoking, the smokers were distributed into three groups:

Group 1 (from 1-10 years of smoking): include 20 smokers, age range of 15-30 years (mean 22.52±7.43 years).

Group 2 (from 11-20 years of smoking): include 20 smokers, with an age range of 30-45 years (mean age 36.34± 5.16 years).

Group3 (from 21-30 years of smoking): were 20 smokers, of an age range of 46-60 years (mean age 56.41± 7.12 years).

Control group: Forty apparently age matched healthy non smoking men were considered as a control group (mean age 48.09±9.31 years). None of them was alcoholic, or on any drug that may interfere with the results of the study.

B-Blood specimens:

Ten milliliters of venous blood sample were taken from each smoker and control using plastic disposable syringes after 12 hours fast. The samples were transferred into clean plain test tube, left at room temperature for 15min for clotting, centrifuged, and then serum was separated into two portions:

1- For measurement of total cholesterol, triglycerides, HDL-C, total level of oxidized lipids (measured as total malondialdehyde, MDA) and specific levels of oxidized HDL (measured as HDL- MDA).

2- For measuring the concentration of antioxidant vitamins :- involve determination of serum levels of ascorbic acid (vitamin C), α-tocopherol (vitamin E),and retinol (vitamin A)

All assays were obtained by running duplicates for the test, control, & the standard. The tubes were stored at -20ºC until analysis, which was done within one month after collection.

C-Methods:

High Performance Liquid Chromatography (HPLC), with Octa Decayl Silain (ODS) C-18 Column (250x4.6mm) packed with 5µm particle size (Fisher Company, USA) was used for measurement of the antioxidant vitamin concentration (A, C, &E). They were detected by SPD-10AVP ultraviolet- visible detector, at λ-max 290nm [6].

The thiobarbituric acid (TBA) method of Buege& Aust(1978) was used to measure serum MDA. It is based on the reaction with TBA to give a pink color that is read at 535 nm. The malondialdehyde concentrations were calculated using the molar extinction coefficient of $1.5*10^5$ [7].

The levels of oxidized HDL were obtained after HDL precipitation with Mg-phosphotungestic acid. Oxidized-non HDL (oxidized LDL-VLDL) was obtained by subtracting the value of oxidized HDL from the total oxidized lipids, i.e., oxidized non-HDL =total MDA- oxidized HDL [7].

Results

- Serum lipid profile: Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), atherogenic index (expressed as LDL-C/ HDL-C) & LDL size index (expressed as TG/ HDL-C) are shown in Table 1.
- Lipid peroxides profile: The results of total lipid peroxides, (expressed as s. MDA) and oxidized lipid fractions, which included Ox. HDL (expressed as HDL-MDA) and Ox.non-HDL are described as absolute values (for s. MDA) and as percentages from the total (for oxidized lipid fractions).

These results are shown in Table 2. Serum MDA was significantly increased in smokers group 1& 2 (i.e.) years of smoking 1-10 and 11-20 when compared to the controls (P=0.03 &0.01 respectively) and it was highly significantly increased in group 3 (i.e.) years of smoking 21-30 when compared to the controls P=10^{-5}). There was, also significant variation among smoker groups when compared with each other (ANOVA-P value was 10^{-3}). There was a significant reduction of OX. HDL% fraction (P= 0.05, 10^{-3} & 10^{-4} respectively) when all groups compared with controls. Also, there was a significant variation between smokers groups when compared with each other. There was a significant elevation of Ox .non-HDL% when compared to the control (P=0.05, 10^{-3} &10^{-4} respectively), also there was a significant variation between smokers groups when compared with each other (ANOVA-P value was 10^{-3}).

-Antioxidant vitamins: The concentration of serum antioxidant vitamins (A, C, &E) are shown in table 1. They were significantly decreased in smokers when compared with controls in all groups.

Table 1: Serum lipid profile (mean ± SD) in different smokers and control groups.

Group	Years of smoking			ANOVA P-value*	Control
	1-10	11-20	21-30		
	N=20	N=20	N=20	~	N=40
Triglyceride (mmol/l) **t- test P-value***	1.75±0.65 0.05	1.82±0.52 10^{-4}	2.12±0.36 $7*10^{-4}$	0.01	1.17±0.53
total cholesterol (mmol/l) **t- test P-value***	4.82±1.11 0.05	5.3±0.69 10^{-3}	5.3±1.1 10^{-3}	0.7	4.32±0.93
HDL-C(mmol/l) **t- test P-value***	1.08±0.24 0.04	1.04±0.23 0.5	1.03±0.32 0.3	0.3	1.16±0.3
LDL-C(mmol/l) **t- test P-value***	3.01±1.05 0.07	3.06±0.55 0.05	3.1±1.23 0.05	0.9	2.65±0.92

Atherogenic index (LDL-C/HDL-C) **t- test P-value***	2.74±1.42 0.05	2.89±0.71 0.05	3.02±0.11 0.01	0.8	2.45±1.3
LDL size index (TG/HDL-C) **t- test P-value***	1.67±0.85 10^{-3}	1.74±0.76 10^{-3}	2.2±0.74 10^{-4}	$7*10^{-3}$	1.12±0.75

*Student t- test was done between each smoker and control groups.
*P-value considered significant at 0.05 or less.

Table 2: lipid peroxidation and it[s] fractions (mean±SD) in different smokers group and control.

Years of Smoking	N	S.MDA µ mol/l	t- test P-value*	OX.HDL %	t- test P-value*	OX. non-HDL %	t- test P-value*
1-10	20	0.65±0.05	0.03	65.37±7.9	0.05	33.85±1.01	0.05
11-20	20	0.76±0.2	0.01	70.7±5.95	10^{-3}	28.31±0.12	10^{-3}
21-30	20	0.97±0.25	10^{-5}	7 2.4±11.05	10^{-4}	27.19±1.05	10^{-4}
ANOVA p-value	~	~	10^{-3}	~	10^{-3}	~	10^{-3}
Control	40	0.52±0.13	~	72±14.02	~	29±14.02	~

*Student t- test was done between each smokers group and control.
*P-value was considered significant at 0.05 or less.

Table3: Antioxidant vitamins (mean ±SD) in different smokers group and control.

Years of Smoking	n	Vit. A µmol/l	t- test P-value*	Vit. C µmol/l	t- test P-value*	Vit. E µmol/l	t- test P-value*
1-10	20	1.13±0.12	0.04	24.84±1.76	0.01	10.85±1.01	0.05
11-20	20	0.81±0.09	0.01	18.31±3.19	10^{-3}	8.31±0.12	10^{-3}
21-30	20	0.63±0.11	10^{-3}	17.14±3.08	10^{-3}	7.19±1.05	10^{-3}
ANOVA p-value	~	~	10^{-3}	~	10^{-3}	~	10^{-3}
Control	40	1.56±0.23	~	39.20±1.45	~	18.39±2.08	~

*Student t- test was done between each smokers group and control.
*P-value consider significant at 0.05 or less.

Discussion

In this study oxidative stress (which is expressed as total lipid peroxide and oxidized lipid subfractions) had been measured to demonstrate the relation between smoking and oxidative stress.

The oxidation of LDL is a very complex process. The smoking state alters LDL size and composition. LDL in postprandial state appears to be more susceptible to oxidation than fasting LDL [(8)]. Oxidation of LDL leads to alteration of the apoplipoprotein B (apo B) recognition site and in the unregulated uptake of the LDL by the macrophages via the scavenger receptor, another important factor in LDL oxidation relates to ambient HDL concentrations. HDL carries important antioxidant enzymes, paroxanase and platelet activating factor acetyhydrolase, and also it serves to protect LDL from oxidation in order ways. HDL also

appears to exchange undamaged phospholipids for oxidized phospholipids in LDL; HDL, from smoker subjects is less protective than the control subjects [9].

According to the present results, there was a significant elevation of the oxidized LDL% and reduction of the oxidized HDL% in all smoker groups with increase in the years of smoking. These results are in accordance with the results obtained from Sarafian, *et al.* [10] and Morrow, *et al.* [11]. Serum lipid profiles were seen to be significantly elevated in all smoker groups and as the years of smoking increase, there are more pronounced lipid disturbances, except for the serum HDL-C which was reduced significantly when compared with control group as shown in table 1.

The changes in the serum lipids which were noticed in the smokers in the present study are in accord with previous report [12] while other report showed normal levels of serum LDL-C but of smaller and denser forms, which are more susceptible to oxidation [13].

However, the role of TG in cardio vascular disease (CVD) is a controversial subject. Many epidemiological trials do not identify hypertriglyceridaemia as an independent risk factor when the cholesterol and, in particular the HDL-C level, are taken into consideration. Nevertheless, these results must be interpreted with caution as hypertriglyceridaemia represent a very heterogeneous entity which is closely related to many factors that may affect coronary risk (tobacco consumption, hypertension, insulin resistance and sedantarity). Therefore, hypertriglyceridaemia and hypo-HDL-aemia may be the results of the same primary abnormality, as the HDL-C level is more stable, it is the parameter, which will be identified as a protective factor in epidemiological trials [14].

- Pro-oxidants and antioxidants

The results showed that, oxidative stress increased in smokers, this is clear from the highly significant elevation of serum MDA level and is agreement with the results of previous reports [15-17]. This elevation in serum MDA may be due to the loss of balance between pro-oxidation and anti-oxidation, energy depletion, and accelerated aging in target organs, such as lungs, heart, kidney and brain. Evaluation of parameters for oxidative stress is a well-accepted technique to express the extent of cell damage [16]. Previous studies have demonstrated that MDA levels increase and antioxidant capacity decreases in smokers[18], and this is in agreement with this study which indicate a highly significant increase in serum MDA levels in smokers compared to normal healthy control ($p<0.001$) as shown in table 2. This study demonstrated that current cigarette smokers have higher measures of lipid peroxidation than nonsmokersas shown in table 2. The finding of increased lipid peroxidation in smokers supports the hypothesis that smoking increases free radical–mediated oxidative damage of lipids, a putative risk factor for athero sclerosis cardio vascular disease.

Previous observational studies that assessed the extent of lipid peroxidation in smokers and nonsmokers have yielded inconsistent results. Also in cross-sectional studies that enrolled healthy volunteers, patients with angina, diabetics, and young survivors of myocardial infarction [19]. There are several studies that showed an association between smoking and oxidative damage, including one cross-sectional study that demonstrated an

association between cigarette smoking and autoantibody titer to oxidized LDL cholesterol [20- 22].

Serum levels of vitamin A, vitamin C, and vitamin E have been reported here to be lower in smokers than in nonsmokers as shown in table 3. In studies in which higher measures of lipid peroxidation were found in smokers than in nonsmokers, smokers also had lower serum vitamin E levels, which could account for the reported difference [16]. In other studies, antioxidant vitamin supplements, including vitamin C, vitamin E, and vitamin A [15,17], decreased the extent of lipid peroxidation in smokers to baseline levels of nonsmokers after only a few weeks of supplementation. In a study exclusively of smokers, a combined antioxidant supplement resulted in increased oxidative resistance to lipid peroxidation [23]. Hence, the intake of antioxidants from diet or supplements may have a major influence on the *in vitro* susceptibility of lipids to peroxidation and may account for the reported differences in lipid peroxidation between smokers and nonsmokers independent of the effects of cigarette smoke [19].

Acknowledgment

I would like to express my deepest gratitude to prof. Ghassan A. Al-Shamma for his advice and his assistance to me in reading of this study, and I am indebted to the study participants who made this study possible.

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