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Cigarette Smoking and Oxidative Stress in Patients with Coronary Artery Disease

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Abstract

AIM: To determine whether cigarette smoking, as a risk factor for CAD, affects (anti)oxidant status.

MATERIAL AND METHODS: The study included patients with CAD, divided according to their smoking status and the number of cigarettes smoked during a day. Biological markers of oxidative stress (concentration of oxidants and activity of antioxidant enzymes) were measured in all subjects.

RESULTS: The study included 300 patients with CAD, (average age of 63 ± 11 years), predominantly males. Of the total, 34.0% were active smokers, 23.0% were former smokers, and 43.0% were non-smokers. Most of the active smokers smoked 1-20 cigarettes/day. In terms of concentration of oxidants (MDA and HP) there was not a significant difference between smokers versus non-smokers. As for the activity of antioxidant enzymes (SOD, CAT and GPX), a statistically significant difference was found in the activity of GPX among the active smokers with CAD and the non-smokers with CAD (p = 0.039).

CONCLUSION: Smoking as a risk factor for CAD is closely associated with increased oxidative stress, and the number of cigarettes smoked plays an important role in increasing the level of oxidative damage and reducing antioxidant defence.

Introduction

Smoking and cigarette smoke are among the dominant risk factors for premature or accelerated development of peripheral, coronary and cerebral atherosclerotic vascular disease. Cigarette smoke contains more than 4000 identified ingredients [1] including nicotine, ammonia, acrolein, phenols, acetaldehyde, polycyclic aromatic hydrocarbons, polyphenols, then carbon monoxide, nitrogen oxides, hydrogen cyanide, trace metals [2, 3].

Two main phases have been identified in cigarette smoke, tar phase and gas phase. The two phases are rich free radicals, and non-radical oxidants. Superoxide radical (.O2-), and particularly hydroxyl radical (.OH), and peroxyl (.ROO) are able to initiate oxidative damage in the form of lipid peroxidation [4].

Because oxidative modification dominates the current concept of the pathogenesis of atherosclerosis, many studies, including that of Marangon et al. [5], have been focused on oxidative stress as potentially clinically relevant factor where cigarette smoke is associated with cancer and atherogenesis. They considered that smokers have a triple threat, first as they actively smoke cigarettes, second because of unhealthy nutrition with reduced

intake of antioxidants and finally because of consumption of large amounts of alcohol during smoking [6]. As a result, they increased oxidative stress and reduced antioxidant protection.

It is believed that smoking causes increased oxidative stress because of several mechanisms, including direct damage by radical species and the inflammatory response caused by cigarette smoking [7].

Peroxyl radicals and reactive nitrogen species cause direct damage stimulating lipid peroxidation and oxidation of proteins and DNA bases; aldehydes can deplete GSH (reduced glutathione) and modify protein -SH and -NH2 groups; cigarette smoke tar phase hydroquinone/quinine complexes diffuse across cell membranes, give rise to semiquinones and lead to the formation of superoxide radicals and hydrogen peroxide (H2O2) [8].

The damage caused by ROS (reactive oxygen species) in cigarette smoking occurs as an imbalance between production and detoxification of these species. Defence against oxidative stress is provided by a system of enzymes and antioxidants capable of preventing excess production of ROS and neutralizing free radicals [9].

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are an important line of defence against oxidative cell damage preventing lipid peroxidation, and protein and DNA oxidation.

The aim of this study was to determine whether cigarette smoking as a risk factor for coronary artery disease (CAD), affects (anti)oxidant status, and induces oxidative stress. For this purpose, we compared the biological markers of (anti)oxidant status (concentration of oxidants and antioxidant enzymes activity) in patients with CAD divided according to their smoking status, and the number of cigarettes smoked in active smokers.

Materials and Methods

The study included 300 patients with CAD, admitted to the University Clinic of Cardiology in Skopje.

Patients were examined for risk factors for CAD, with particular reference to smoking as a risk factor. Patients were divided into appropriate subgroups. According to their smoking status, the patients were separated into non-smokers, active smokers and former smokers. Non-smokers were those who during their life had never started smoking. Active smokers were those who smoked daily, over several years. According to the number of cigarettes smoked in 24 hours, active smokers were divided into: patients who smoked 1-20 cigarettes/per day, patients who smoked 21-40 cigarettes/per day and > 40 cigarettes/per day. Former smokers were those who during their life had actively smoked, but due to various influences stopped smoking.

The following parameters were determined in all patients: erythrocyte concentration of thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA), concentration of total hydroperoxides (HP) in plasma, both products of lipid peroxidation, and also the activity of erythrocyte antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX).

Fasting blood samples were collected by venepuncture of all participants, in tubes that contained EDTA as an anticoagulant. The blood was centrifuged at 4000 g for 10 minutes at 4ºC, immediately after venepuncture.

Plasma and leukocytes layer were separated, and the erythrocytes were washed three times with two volumes of saline. Then, a known volume of washed red cells were lysed with ice-cold distilled water (1:4), were left for 15 minutes in a refrigerator at a temperature of 4ºC, and cell debris was removed by centrifugation (10 minutes at 2000 g at a temperature of 4ºC). Samples of plasma and red cell lysates were kept at a temperature of -70ºC until the analysis.

Results

Basal characteristics of the study population

In our study we have included 300 patients with coronary artery disease. Within the total number of subjects, 187 (62.3%) patients were diagnosed with acute coronary artery disease and 113 (37.7%) patients with chronic (ischemic) coronary heart disease.

Patients had an average age of 63 ± 11 years, and were predominantly male (194 or 64.67% men and 106 women or 35.33%).

Risk Factors for CAD

Risk factors for CAD that were present in patients are shown in Fig. 1. All patients were divided according to their smoking status: 102 (34%) active smokers, 69 (23%) former smokers and 129 (43%) non-smokers. The group of 102 (34%) active smokers was divided into subgroups according to the number of cigarettes they smoked per day and night, which is shown in Fig. 1.

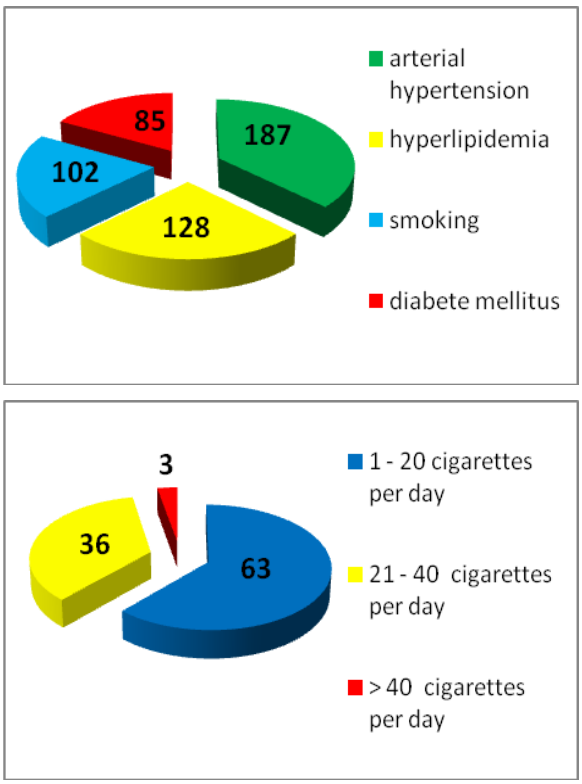

Figure 1: Risk factors for CAD (top) and active smokers by number of cigarettes smoked per day (bottom)

Biological markers of oxidative stress and smoking

We measured concentration of malondialdehyde (MDA) and the concentration of total hydroperoxides (HP) in patients with CAD, divided according to their smoking status. The results are presented on Table 1.

Table 1: Concentration of MDA and total HP (respectively) in patients with CAD, depending on smoking status

	Pts	Min	Max	Mean	Standard deviation
MDA (nmol/ml) | former smokers	69	20.153	65.915	33.791	7.605
MDA (nmol/ml) | non-smokers	129	17.260	81.857	33.474	8.178
MDA (nmol/ml) | active smokers	102	20.908	108.508	35.187	10.943
(ANOVA, p = 0.344) (Kruskal-Wallis ANOVA, p = 0.313).					
Total HP (CARR U) | former smokers	69	126.000	530.542	283.770	70.042
Total HP (CARR U) | non-smokers	129	152.753	510.103	268.875	69.398
Total HP (CARR U) | active smokers	102	111.658	484.103	289.204	78.551
(ANOVA, p = 0.180) (Kruskal-Wallis ANOVA, p = 0.077).					

Statistical analysis of these results showed that there was no statistically significant difference in mean concentration of MDA and total HP in patients with CAD according to their smoking status.

Concentration of MDA and the concentration of total HP were highest in patients with CAD who were active smokers and lowest in patients with CAD who had never smoked cigarettes.

Then we measured the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in patients with CAD, according to the smoking status. The results are presented on Table 2.

Table 2: Activity of SOD, CAT and GPX in patients with CAD, according to the smoking status

	Pts	Min	Max	Mean	Standard deviation
SOD (U/ml) | former smokers	69	10.477	571.631	128.620	114.651
SOD (U/ml) | non-smokers	127	12.118	560.085	139.121	122.408
SOD (U/ml) | active smokers	101	10.477	525.138	124.602	99.219
(ANOVA, p = 0.349) (Kruskal-Wallis ANOVA, p = 0.213)					
CATALASE (U/L) | former smokers	68	0.313	136.242	62.517	37.084
CATALASE (U/L) | non-smokers	123	0.313	147.374	67.066	40.728
CATALASE (U/L) | active smokers	94	0.430	133.137	63.889	36.780
(ANOVA, p = 0.728) (Kruskal-Wallis ANOVA, p = 0.765)					
GPx (U/ml)| former smokers	21	0.717	27.484	7.108	7.376
GPx (U/ml)| non-smokers	58	0.158	32.241	7.434	6.741
GPx (U/ml)| active smokers	38	0.158	10.510	4.362	3.030
(ANOVA, p = 0.042) (Kruskal-Wallis ANOVA, p = 0.038)					

Statistical analysis of these results showed that there was a statistically significant difference in the mean activity only of GPX in patients with CAD according to their smoking status.

In addition, the analysis showed that the activity of SOD was lowest among patients with CAD who were active smokers, and highest in patients with CAD who had never smoked cigarettes.

Active smokers according of number of smoked cigarettes per day

According to the number of smoked cigarettes per day, we examined if there is a difference in the concentration of MDA and total HP. The results are shown in Table 3.

Table 3: Concentration of MDA and total HP in patients with CAD who are active smokers according to the number of smoked cigarettes per day

	Pts	Min	Max	Mean	Standard deviation
MDA (nmol/ml) | > 40 cigarettes per day	3	21.301	55.982	35.606	8.208
MDA (nmol/ml) | 1 - 20 cigarettes per day	63	20.908	108.508	34.995	12.431
MDA (nmol/ml) | 21 - 40 cigarettes per day	36	28.108	43.964	34.212	8.535
(ANOVA, p = 0.954) (Kruskal-Wallis ANOVA, p = 0.673)					
Total HP (CARR U) | > 40 cigarettes per day	3	158.356	530.542	290.077	70.683
Total HP (CARR U) | 1 - 20 cigarettes per day	63	126.000	499.013	272.709	69.808
Total HP (CARR U) |21 - 40 cigarettes per day	36	219.138	347.661	284.062	64.272
(ANOVA, p = 0.499) (Kruskal-Wallis ANOVA, p = 0.371)					

Statistical analysis shows that there was no statistically significant difference of mean concentration of MDA and total HP in patients with CAD according to the number of smoked cigarettes.

However, the concentrations of both parameters are highest in patients with CAD who smoke more than 40 cigarettes/day.

Regarding the difference in the activity of antioxidant enzymes, SOD, CAT and GPX in patients with CAD who were active smokers, according to the number of smoked cigarettes per day, we received the following results which are shown in Table 4.

Table 4: Activity of SOD, CAT and GPX (respectively) in patients with CAD who are active smokers according to the number of smoked cigarettes per day

	Pts	Min	Max	Mean	Standard deviation
SOD (U/ml) | > 40 cigarettes per day	3	36.952	96.256	68.102	29.765
SOD (U/ml) | 1 - 20 cigarettes per day	62	12.875	525.138	125.458	101.932
SOD (U/ml) | 21 - 40 cigarettes per day	36	10.477	397.393	124.984	98.553
(ANOVA, p = 0.954)					
CATALASE (U/L) | > 40 cigarettes per day	3	18.417	50.876	29.901	18.192
CATALASE (U/L) | 1 - 20 cigarettes per day	58	0.313	147.374	70.462	42.368
CATALASE (U/L) | 2 -40 cigarettes per day	33	11.562	137.062	64.478	38.019
(ANOVA, p = 0.221) (Kruskal-Wallis ANOVA, p = 0.214)					
GPx (U/ml)| > 40 cigarettes per day	1	0.158	9.950	3.647	2.678
GPx (U/ml)| 1 - 20 cigarettes per day	22	1.370	10.510	5.412	3.393
GPx (U/ml)| 21 - 40 cigarettes per day	15	4.355	4.355	4.355	3.001
(ANOVA, p = 0.225) (Kruskal-Wallis ANOVA, p = 0.170)					

Statistical analysis shows that there was no statistically significant difference in activity of SOD, CAT and GPX in patients with CAD according to the number of smoked cigarettes.

The activity of SOD, CAT and GPX is lowest in patients with CAD who smoke more than 40 cigarettes/day and highest in patients with CAD who smoke 1-20 cigarettes/day.

Discussion

The results of our study clearly demonstrate that patients who smoke cigarettes have higher oxidative damage and reduced antioxidant status than those who never smoked. Our results showed that the concentrations of MDA and HP were higher in smokers versus non-smokers.

In their study, Metta et al. [10] proved that there is an imbalance between oxidants and antioxidant enzymes in patients who had been admitted in the intensive coronary unit with a diagnosis of ischemic coronary heart disease. According to their smoking status, patients were divided to non-smokers and smokers. There was a statistically significant difference in the activity of GPX, SOD and CAT among smokers versus non-smokers. In addition, MDA levels in plasma were significantly increased in smokers compared to non-smokers.

Also in studies of Lykkesfeldt et al. and Block et al. [11, 12] concentration of MDA in plasma and antioxidant profile were tested the in two groups of patients: smokers and non-smokers. It was proved that there is no significant difference in the antioxidant profile between the two groups, but there was a significant effect of smoking on the concentration of MDA in plasma.

Jansen et al. [13] studied the influence of smoking on the levels of several biomarkers of oxidative stress, antioxidant status and redox status, including plasma hydroperoxides. Using different assays, they confirmed that smokers have elevated concentrations of oxidative stress biomarkers and compromised antioxidant status.

Exposure to environmental tobacco smoke (ETS) is associated with increased risk of developing cardiovascular disease. A meta-analysis conducted in 1999, published a total relative risk of 1.25 (95% CI = 1.17, 1.32) and showed a dose-response relationship [14]. Even very short exposure to ETS can produce changes in platelet activation and endothelium-dependent vasodilation [15]. Cigarette smoke contains high concentrations of toxic gases and tiny particles that are easily inhaled [16]. Therefore, exposure to ETS leads to a risk of cardiovascular events associated with active smoking. The study of Megson et al. [17] proved that patients with acute myocardial infarction (AMI) who were exposed to ETS and admitted to the intensive care unit had increased oxidative stress, as demonstrated by two independent plasma biomarkers (malondialdehyde and protein carbonyl). By this way they proved the role of ETS in the development of atherosclerosis and the occurrence of AMI. In conclusion, they put forward that exposure to cigarette smoke is associated with increased oxidative stress, by significantly higher concentrations of MDA in plasma. Exposure to cigarette smoke was associated with an increased risk of death from all causes, cardiovascular deaths, and fatal/nonfatal acute myocardial infarction within 30 days of admission [18]. From here, it is plausible that oxidative stress may be involved in the mechanism by which exposure to cigarette smoke increases the risk of CAD.

In our study, active smokers who smoke > 40 cigarettes/day have higher oxidative stress than those who smoke 1-20 cigarettes/day or do not smoke, which means the number of cigarettes smoked is a significant risk factor for increased oxidative stress.

Morbidity and mortality from CAD are very high in Turkey [19]. EUROASPIRE III study showed that 20% of subjects who were hospitalized with CAD in Turkey are less than 50 years of age. Turkey has the highest prevalence of early CAD of all populations. The main reasons are the low levels of cholesterol in high density lipoprotein (HDL-cholesterol) and high prevalence of smoking, which are more common in Turkey than in other countries [20]. A study of Aksoy et al. [21], where they examined indices of oxidative stress and severity of CAD in young patients who were hospitalized with AMI, proved that oxidative stress is an important factor for the severity of CAD in young smokers. Elevated levels of biomarkers of oxidative stress reflected the seriousness of the disease and vascular damage associated with cigarette smoking in the early onset CAD.

The study of Bikkad et al. [22] confirmed the

view of increased lipid peroxidation and oxidative stress in smokers, expressed by increased concentration of MDA with a parallel reduction of antioxidant enzymes SOD and GPX. In this study, the level of SOD was significantly lower in smokers compared with non-smokers. The mechanism by which smoking causes low levels of blood SOD is unknown. Increased lipid peroxidation and antioxidant depletion in smokers may contribute for biomolecular vascular endothelial damage. The same study also reported a significant reduction in the level of GPX in smokers in comparison to the non-smokers. GPX seems to have a major role in the prevention of oxidative stress; it can also be an important anti-atherogenic enzyme. This enzyme is responsible for the removal of hydrogen peroxide and organic hydroperoxides formed during cellular oxidative metabolism.

In conclusion, smoking as a risk factor for CAD is closely linked to the increased oxidative stress. The number of cigarettes smoked per day plays an important role in increasing the level of oxidative damage and reducing the antioxidant defence, which results in increased oxidative stress.

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