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Study of lipid profile and nitric oxide in chronic smokers

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ABSTRACT

Background: Cigarette smoking is associated with an increased risk of respiratory tract infections, chronic airway disease, and cardiovascular diseases, all of which may be modulated by endogenous nitric oxide. We have investigated whether cigarette smoking reduces the production of endogenous NO. Oxidative stress also alters the lipid profile in chronic smokers. With this view, we planned to assess vasodilators by determining the level of nitric oxide and lipid profile in chronic smokers.
Aim: This study aims to: 1) determine the level of nitric oxide in chronic smokers. 2) estimate the level of lipid profile such as total cholesterol, HDL, LDL, VLDL, and triglycerides.
Methodology: Chronic smoker patients and healthy controls from Government Medical College and Hospital, Miraj, and P. V. P. G. Hospital, Sangli, were included in this study. A total of 70 clinically diagnosed chronic smokers and 70 healthy controls, all in the age group of 40 to 70 years, participated.
Results: Serum nitric oxide level was found to be significantly decreased (p <0.001) in chronic smokers as compared to the control group. Serum total cholesterol, LDL-cholesterol, and triglycerides levels were significantly increased (p <0.001) in chronic smokers as compared to the control group, and serum HDL-cholesterol level was found to be significantly decreased (0.001) in chronic smokers as compared to the control.
Conclusion: Cigarette smoking decreased exhaled nitric oxide, suggesting that it may inhibit the enzyme nitric oxide synthase. Since endogenous nitric oxide is important in defending the respiratory tract against infection, counteracting bronchoconstriction and vasoconstriction, and inhibiting platelet aggregation, this effect may contribute to the increased risks of chronic respiratory and cardiovascular diseases in cigarette smokers.

1. Introduction

Smoking is the inhalation of the smoke of burning tobacco, typically encased in cigarettes, pipes, and cigars. "Chronic" means long-lasting or long-suffering. A chronic smoker is someone who smokes 15 or more cigarettes per day or smokes more than one cigarette daily for more than one year.

Tobacco smoking is the leading source of preventable illness and premature death worldwide.[1] More than 1.2 billion smokers are globally addicted, and half will die from diseases linked to it.[2] On average, cigarette smokers die 10 years earlier than non-smokers.[3]Research has shown that the main factor influencing teenagers to start smoking is cigarette advertising. Additionally, having parents, siblings, or friends who smoke also plays a significant role in encouraging teenagers to take up smoking.

Smoking is the leading risk factor for developing COPD, responsible for 81.5% of all COPD-related deaths.

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Individuals who smoke or have chronic bronchitis are at an increased risk of developing emphysema. Cigarette smoke contains various harmful oxidants, including oxygen-free radicals and volatile aldehydes, which are likely the primary cause of damage to biomolecules.[1] These highly reactive free radicals attack essential cellular components, such as DNA, proteins, and cell membranes, leading to oxidative stress, which is implicated in the pathogenesis of COPD.[3,4]

Smoking affects numerous bodily systems due to oxidative damage caused by free radicals. Tobacco smoke is a complex mixture of over 5,000 identified chemicals, 98 of which have known toxicological properties. While most people associate cigarette smoking with breathing problems, it is also a major cause of heart disease in both men and women. Smoking causes an immediate increase in blood pressure, pulse rate, and levels of stress hormones like ACTH, cortisol, aldosterone, and catecholamines. Moreover, cigarette smoke not only harms smokers but also those around them, particularly children, through passive smoking. People who are frequently exposed to secondhand smoke are also at risk of developing health problems. It is estimated that approximately 70,000 people die from heart disease each year due to passive smoking.

Nitric oxide (NO) is a highly reactive free radical that plays a crucial role in regulating various physiological and pathological processes in the body. Despite its short half-life of approximately 4 seconds, NO penetrates surrounding tissues and activates cellular signaling pathways. NO is synthesized from L-arginine by NO synthase (NOS) enzymes in almost all cell types. Exhaled NO may serve as a marker of disease activity in various lung diseases.

Cigarette smoke contains high levels of oxides of nitrogen, including NO, which can be oxidized to nitrogen dioxide. Additionally, cigarette smoke contains peroxyl radicals, carbon-centered radicals, and other stable radicals in the tar phase.

The dark pigmentation in smoker's lung tissue is associated with iron deposits, similar to those in ferritin and hemosiderin, possibly due to microhemorrhage.[4] Released iron can contribute to oxidative stress in the lung, as it can participate in redox reactions and form hydroxyl radicals from hydrogen peroxide. Chronic smokers exhibit a cluster of lipid and lipoprotein abnormalities, including elevated total cholesterol, LDL cholesterol, and triglycerides, and lower HDL cholesterol levels, increasing the risk of cardiovascular disease.

We aimed to investigate biochemical parameters related to endothelial dysfunction, decreased NO concentration, enhanced oxidant injury, and coronary thrombosis.

2. Materials and Methods

The present study was conducted in the Department of Biochemistry, Government Medical College, Miraj, and P.V.P.G. Hospital, Sangli.

The Institutional Ethical Committee approved the study, and informed consent was obtained from each participant in the study.

2.1. Study group

It included 70 patients in the age group 40-70 years with health issues related to smoking as diagnosed by clinician. The diagnosis of the patient was done on the basis of the patient's condition, clinical history, personal history, physical examination, etc.

2.2. Control group

It consists of 70 normal healthy individuals with age and sex-matched with patients who were included in this study.

2.3. Inclusion criteria

Patients with health issues related to smoking as diagnosed by the clinician and who are willing to participate in informed consent were included in this study.

2.4. Exclusion criteria

Patients having tuberculosis, pregnant women, diabetes mellitus, liver disease, kidney disease, nephrotic syndrome, obstructive jaundice, hypothyroidism, any chronic illness, and below 18 years of age were excluded from this study.

In the fasting state of patients, 5 ml blood samples were withdrawn using disposable needles and syringes, taking aseptic precautions. Blood was collected in a plain bulb, and serum was separated and used for the estimation of lipid profile and NO.

Estimation of serum nitric oxide (as nitrite) by colorimetric method, total cholesterol by CHOD-PAP method, triglyceride by GPO–PAP method, HDL and LDL by direct method.[5–8]

2.5. Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was done by using the 'Z' test.

3. Results and Discussion

The mean level of serum nitric oxide was found to be significantly decreased in chronic smokers when compared with control (p <0.001, Table 1)

Smoking is associated with reduced levels of tetrahydrobiopterin, which might reduce enzymatic NO production by uncoupling NOS, with the resultant production of superoxide instead of NO. Superoxide can, in turn, react with NO to form peroxynitrite. The fact that NO consumption might be increased in smokers' airways. As a result, the basal endogenous NO synthesis in the airway and blood vessels of smokers is reduced. Subsequently, because

Table 1: Results of biochemical parameters in chronic smokers and healthy controls

S. No	Biochemical parameters	Chronic smokers patients (n = 70) Mean ± SD	Healthy control (n = 70) Mean ± SD
1	Nitric oxide (μmol/L)	16.24 ± 2.53	23.87 ± 1.99
2	Total cholesterol (mg/dl)	217.41* ± 13.22	160.97* ± 2.97
3	Triglycerides (mg/dl)	142.41 ± 17.74	120.91 ± 5.49
4	HDL cholesterol (mg/dl)	23.18* ± 1.99	41.33* ± 1.72
5	LDL cholesterol (mg/dl)	160.66* ± 8.01	95.91* ± 2.99
6	VLDL cholesterol (mg/dl)	33.59 ± 6.79	23.84 ± 2.99

*p <0.001, highly significant.

NO is involved in maintaining airway dilatation, smokers may have constricted airways.

S. A. Kharitonov (1995) found a decrease in NO concentration in smokers compared with nonsmokers.[9] Our study reveals the same result. Exhaled Nitric Oxide may be a marker of disease activity in a variety of lung diseases.[10] NO concentration was significantly reduced in smokers, with a significant relation between the exhaled NO and cigarette consumption. It might be related to decreased endothelial NO synthase. Smoke contains a high concentration of NO, and NO itself exerts a negative feedback inhibition on its synthase. Our study is also supported by Renata TMB (2010), Szmitko PE et al. (2003), Node K et al. (1997).[11–13]

Mean level of Serum total cholesterol, LDL-cholesterol and triglycerides levels were significantly increased (p <0.001) in chronic smokers as compared to control.

Dysfunctional NO biosynthesis, as indicated by reduced endothelial–dependent vasodilatation, precedes the morphological changes in the vessel wall associated with various atherosclerotic risk factors, including smoking.[14] Excessive generation of Reactive Oxygen Species (ROS) such as superoxide ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2) and their products has been implicated as a final common pathway for the development of endothelial dysfunction by various cardiovascular risk factors, and ROS may be responsible for the observed changes in NO biosynthesis, including the upregulation of $_e$NOS.[15]

The most abundant class of atherogenic lipoproteins in human plasma is low-density lipoproteins (LDL), which transport cholesterol from the liver to tissues. Nicotine stimulates the release of adrenaline, leading to increased free fatty acids (FFAs) through enhanced lipolysis and FFA mobilization from adipose tissue. Free fatty acids stimulate the hepatic secretion of VLDL, triglycerides, and also cholesterol.[16]

LDL only poses a threat after oxidation by free radicals, as it is reported to migrate across the endothelial membrane into the arterial wall. These oxidized components attract macrophages, which absorb and deposit cholesterol within the cell to form “foam cells”. These foam cells may initiate the formation of an atherosclerotic lesion, which can result in blockage of blood vessels. Interruption of the blood supply causes severe pain, known as angina pectoris, and may eventually cause death of the cardiac tissue.

Cigarette smoking also increases oxidative modification of LDL. Circulating products of lipid peroxidation and autoantibody titers to oxidized LDL are significantly increased in smokers. Nicotine increases the amount of bad fats (total cholesterol, low-density lipoprotein cholesterol, and triglycerides) circulating in the blood vessels and decreases the amount of good fat (high-density lipoprotein cholesterol) availability. Our findings were also supported by Frat AC et.al., (1996), and Muscat JE, et al. (1991).[17,18]

The mean level of Serum HDL -cholesterol was found to be significantly decreased (p< 0.001) in chronic smokers as compared to control.

Cigarette smoke increases oxidative stress, which is characteristic of chronic smokers. Cigarette smoking, in particular, affects HDL; lowering its concentration and modification of HDL might make it lose its protective properties or even become atherogenic.

Cigarette smoking adversely affects the lipid profile.[11] First by altering the concentration of serum lipids and secondly by modifying the lipids chemically.[19] The serum anti-atherogenic HDL level is significantly low in chronic smokers, irrespective of the number of cigarettes smoked. Cigarette smoking can decrease the level of apoA-I. ApoA-I deficiency results in impaired HDL synthesis. The maturation of HDL may be affected by reduced LCAT activity, thereby inducing rapid clearance of nascent HDL from the circulation. Our study is supported by Neki NS (2002).[20] Due to the reduced plasma HDL level the reverse transport of cholesterol is severely affected leading to the accumulation of cholesteryl esters in tissues. The result is that atherogenesis and related complications like heart attack are increased.

4. Conclusion

From this study, we conclude that free radical mediated oxidative stress appears to play a central role in cigarette smoke-mediated atherothrombotic disease. These free radicals could potentially arise directly from cigarette smoke. The negative impact of cigarette smoking on risk of cardiovascular disease seem to be mediated through its effects on lipid and lipoprotein metabolism.

5. Source of Funding

None.

6. Conflict of Interest

None.

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