Original Article

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Study of Oxidative Stress in Smokers by Estimation of Serum Malondialdehyde Uric Acid and Bilirubin

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Abstract

Background: Study of Malondialdehyde Uric Acid and Bilirubin smokers provides opportunity to explain the correlation between cigarette smoking activity and oxidative stress. Cigarette smoking is the second cause of death in the world. cause oxidative stress, which overwhelm natural radical blocking or scavenging mechanisms. Cigarette smoke may promote atherogenesis by producing oxygen-derived free radicals that damage lipids. Cigarette smoking is associated with impaired endothelium-dependent vasodilatation and cardiovascular disease (CVD). As the current report concerns solely to the study oxidative stress in smokers, the results of this study can be correlated with other biochemical, physiological and clinical aspects. *Aim:* The Aim of our study was to determine oxidative stress in smokers by estimation of serum Malondialdehyde , Uric Acid and Bilirubin in smokers as compared to non-smokers. *Method:* A Total No. of 100 subjects were selected, out of which 50 were healthy individual and 50 were smokers less than 50 years of age. Uric acid and Bilirubin were analyzed using kits on automated biochemistry analyzer while MDA was estimated spectrophotometrically using Thiobarbituric acid. *Result:* The levels of Malondialdehyde were significantly higher while the levels of Uric Acid and Bilirubin was significantly lower in smokers as compared to their levels in non smokers.

Keywords: Malondialdehyde Uric Acid and Bilirubin; Smokers; Non-Smokers.

Introduction

Cigarette smoke contains oxygen radicals and causes formation of new radicals in the body.

The smoke is formed by dispersing of the products as a consequence of melting and distillation in hot medium at gas or droplet state [1]. If the smoke is passed through Cambridge glass fiber, 99.9 percent of the particles larger than 0.1 μm remain in the filter. The part which passesthrough the filter makes gas phase, and the remaining part makes the tar phase [2,3]. Cigarette-smoking is a well-known risk factor for atherosclerosis development and its complications including cerebral and cardiovascular diseases (CVD) [4,5] through vascular endothelial damage [6] that possibly occurs through oxygen free radicals production as superoxide radicals, hydrogen peroxide and hydroxyl radicals [7,8]. Several enzymes capable of producing oxygen free radicals including xanthine oxidase, NADPH oxidase, myeloperoxidase, and endotoxin [7,8]. As cigarette smoke contains superoxide and reactive nitrogen species that readily react with various biomolecules [9]. It has been hypothesized that some of the adverse effects of smoking may result from oxidative damage to endothelial cells, which results in nitric oxide (NO) shortage [10,11]. Nitric oxide (NO) shortage regulates vascular tone that accelerates insufficiency of coronary artery and vasoconstriction in many different tissues [12].Therefore imbalance

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between oxidants and antioxidants may play an important role in the susceptible smoker [13,14]. In addition cigarette smokers have increased inflammatory responses that further enhance their oxidative stress [8,9]. Since in humans, uric acid and bilirubin are considerably the most abundant aqueous antioxidant, accounting for up to 60% of serum free radical scavenging capacity [15] and is an important intracellular free radical scavenger during metabolic stress including smoking [16,17], therefore,measurement of its serum level reflects the antioxidant capacity [15]. It was estimated that a single cigarette puff contains approximately 10 [14] free radicals in tar phase and 10 [15] radicals in the gas phase, which are capable of causing an increase in the generation of various reactive oxygen species (ROS) like superoxide ($O^{-}2$), hydrogen peroxide (H_2O_2), hydroxyl (OH•) and peroxide (ROO•) radicals. These reactive oxygen species in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation [18,19]. Cigarette smokers have an increased risk of cardiovascular diseases (CVD), possibly mediated by elevated levels of oxidized macromolecules owing to heightened ROS production. Smokers are exposed to significant quantities of ROS in both gas and tar phase. Further ROS production mediated through inflammatory processes may exacerbate those produced through direct exposure [20]. Blood of cigarette smokers routinely displays decreased antioxidant capacity and increased oxidized lipids compared to non-smokers [20]. This chapter describes the malondialdehyde (MDA) as index of lipid peroxidation. The determination of malondialdehyde (MDA) has attracted widespread interest, because it appears to offer a facile means of assessing lipid peroxidation in biological materials [21]. The primary form of bilirubin circulating in healthy individuals, is also a powerful antioxidant [22] at levels within the normal reference range. Thus, while seemingly counterintuitive, bilirubin has been inversely associated with risk of a number of disorders,associated with oxidative stress [23]. The concordance between the negative health consequences of smoking, including those recently highlighted by the Surgeon General and those associated with lower bilirubin concentrations, is striking [24].

Aim & Objectives

1. Present study has been taken up for estimation of serum Malondialdehyde ; Uric Acid and Bilirubin in smokers.
2. To have an estimate of oxidative stress in smokers.
3. To create awareness among smoker population in order to prevent occurrence of different diseases as a result of oxidative stress in smokers.

Material & Methods

Study Design

The current Prospective study was undertaken from Aurangabad ; Maharashtra.

Study Period

Dcember 2016 to December 2017.

Ethical Approval

The study was approved by the Institutional Ethical Committee of IIMSR Medical College Jalna.

Inclusion

We labeled smoker as the one who smokes atleast 10 cigarettes or Bidi per day.

The study composed of 70 male smokers and 30 nonsmokers between the age group of 20 to 50 years. All the subjects were consuming both vegetarian diet and non vegetarian diet, and belonging to different walks of community.

The subjects were volunteer participants in the study and gave informed consent.

Exclusion

Individuals who have any systemic illness or who were taking any medication or antioxidant for prophylaxis were not included to this study.

Site of Sample Collection

Samples were collected Medicine and TB Chest OPD of IIMSR Jalna.

Site of Sample Study

Central Clinical laboratory(Biochemistry section), IIMSR Jalna.

Study Subjects

Group I Non Smokers (Control) n = 30

Group II Cigarette Smokers n=70

Method

5ml blood sample was collected by standard venipuncture technique in plain bulb.Blood sample was centrifuged to obtain a clear serum sample. Serum was divided in two plain tubes i.e 2ml for uric acid and bilirubin estimation and remaining serum sample for MDA estimation.Uric acid and bilirubin estimation was done on fully automated transasia analyzer in central clinical laboratory while MDA estimation was done in biochemistry laboratory by Thiobarbituric method. MDA level of the plasma was measured by the following procedure according to Tomotsu et al. 0.5 plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. 1ml of 0.6 % TBA was added to the mixture, shaken, and warmed for 30 min in a boiling water bath followed by rapid cooling. Then it was shaken into a 4 ml of nbutyl-alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 535 and 520 nm by spectrophotometer against butanol. The standards of 5, 10, 20 nmol/ml TEP were used. The results were expressed as nmol/ml plasma [25]. Statistical analysis was done by using Chi square test by calculating p value with the help of SPSS software. Difference between the parameters of two groups was considered significant if $p < 0.001$

Results

The levels of Malondialdehyde were significantly higher while the levels of Uric Acid and Bilirubin was significantly lower in smokers as compared to their levels in non-smokers.

Table 1:

Sr. No	Parameters(mg/dl)	Reference Range	Non-Smokers (Mean+/- SD)	Smokers. (Mean+/- SD)	p value
1.	Uric Acid	3.5-7.5 mg/dl	6.15+/- 0.65	3.74+/-0.93	<0.001
2.	Bilirubin	0.1-1.0 mg/dl	37.86 +/-4.93	29.06 +/-2.98	<0.001
3.	Malondialdehyde(MDA)	2.59+/-0.24µm/l	2.74 +/-0.36	3.18 +/-0.36	<0.001

Discussion

In this study we found that levels of Uric Acid in smokers were significantly lower than that in non-smokers. This finding are in agreement with other studies that showed low serum uric acid in regular smokers [26] and reduction of antioxidants including uric acid in smokers [27,28] indicating that oxidative stress increases everytime a cigarette is smoked [26]. It even proved that administration of uric acid raises circulating antioxidant defenses and allows restoration of endothelium-dependent vasodilation [29,30].

Therefore, high serum uric acid concentrations might be protective in situations characterized by increased cardiovascular risk and oxidative stress as smoking [29], and by reducing its level it increases susceptibility to oxidative damage and accounts for the excessive free radical production [31].

We also evaluated bilirubin in smokers that bilirubin levels are significantly decreased in them as compared to their non-smoking counterparts. Similar findings were also shared by some other studies like those done by Madhavan et al., 1997; Merz, Seiberling, & Thomann, 1998; Van Hoydonck, Temme, & Schouten, 2001; Zucker et al., 2004) [32-35]. The possibility that smoking leads to reductions in bilirubin, which in turn may contribute to smoking-related disease though diminished availability of this endogenous antioxidant, is intriguing. One possible mechanism for bilirubin reduction among smokers that has been suggested (van der Bol et al., 2007) [36], but not proven (Zevin & Benowitz, 1999) [37], is that of induction of UGT 1A1 by nicotine and/or other constituents of tobacco smoke. UGT 1A1 is the uridine diphosphate glucuronosyltransferase isoform, which catalyzes conjugation of bilirubin, the major metabolic pathway responsible for its disposition.

Lastly we focused our study on oxidative stress due to lipid peroxidation by estimating last product and indicator of lipid peroxidation process i.e Malondialdehyde(MDA) and we found that MDA were significantly increased in smokers as to their non-smoking counterparts. These results are in accordance with the earlier studies, showing elevated lipid peroxidation ~ 96 ~ The Pharma Innovation Journal among smoker subjects [38-40]. Chole et al [41] reported association of lipid peroxidation with the habit of either chewing betel nut or betel leaf or tobacco or smoking in the control subjects. In another study, significantly elevated MDA levels were reported in smokers than nonsmokers in patients with lung cancer [42].

Conclusion

After exclusion of other factors affecting uric acid level, the significant low serum uric acid level in smokers was attributed to reduce endogenous production as a result of chronic exposure to cigarette smoke that is a significant source of oxidative stress. As this reduction is proportionate with smoking status and predisposes to cardiovascular disease, it is recommended for smokers to stop or reduce smoking and introduce serum uric acid estimation as routine test since its cheap and simple to reflect their antioxidant level.

From the results of MDA obtained, we conclude that oxidative stress as indicated by serum lipid peroxidation is more intense in smoker subjects as compared to non-smoker subjects. There is a strong association between increased lipid peroxidation and cigarette consumption in smoker subjects. Evaluating the serum MDA levels might serve as a valuable biomarker to identify the high risk population, which may deserve further investigation for early diagnosis and treatment.

Lastly significant decrease in bilirubin also warrants danger signals as lower bilirubin levels has been found in concordance with incidence of different carcinomas.

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