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Effects of Cigarette Smoking On Lipid Peroxidation and Serum Antioxidant Vitamins

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Abstract: *Cigarette smoke contains many oxidants capable of generating reactive oxygen species and reactive nitrogen species. These species play a key role in oxidative stress, leading to the development and progression of many disorders, including hypertension, cancer, diabetes mellitus and cardiovascular diseases. This study estimated the levels of serum antioxidant vitamins and Malondialdehyde (MDA) as evidence of lipid peroxidation. Healthy male smokers aged 15-35years from Usmanu Danfodiyo University Sokoto community who consume at least five sticks of cigarette/day were recruited for the study. Serum Antioxidant vitamins (A, C, and E) and MDA were estimated using standard methods and compared to aged matched control (non-smokers) group. The levels of antioxidant vitamins were found to be significantly lower in smokers than non-smoker P<0.05. On the other hand, MDA concentration was found to be significantly higher in smokers than non-smokers P<0.05. Interestingly, the number of cigarettes consumed/day determines antioxidants depletion, as well as MDA build up. Based on the results obtained, it was concluded that cigarette smoke depletes the concentration of serum antioxidants required to scavenge excess free radicals, consequently increasing the rate of lipid peroxidation.*

Keywords: *Antioxidants, Cigarettes, Free-Radicals, Smokers, Vitamins*

I. Introduction

Cigarette smoking has been reported to be a huge public health problem since its consumption has reached the level of global epidemic today (Can *et al.,* 2009: Salawu *et al.,* 2009). It is a well-known risk factor for the development of variety of diseases including: cardiovascular diseases, Al-zheimer's disease, age related muscular degeneration, chronic pulmonary disease, stroke, deafness, blindness, Parkinson's disease and premature death (Henderson *et al.,* 1999; Iain *et al.,* 2007; Vardavas *et al.,* 2012; Gboyega *et al.,* 2013). It has been estimated that one billion men and 250 million women currently smoke cigarettes. Approximately, 5 million people die annually from tobacco-related illnesses, projecting to be doubled by 2025 (WHO 2002).

Cigarette smoke contains mixture of over 4000 chemicals, in which many are bioactive substances such as nicotine, hydrogen cyanide, methanol, butane etc. These chemicals induce the rate of reactive oxygen species (ROS), reactive nitrogen species (RNS), and acetaldehyde generations which are collectively called free radicals. Characteristically, free radicals are highly unstable and capable of undergoing complex interactions in biological system, triggering oxidative stress, which occurs when there are not enough neutralizing molecules to counteract their side effects otherwise known as Antioxidants (Valko *et al.,* 2007; Kim *et al.,* 2007; Gandhi *et al.,* 2009).

Antioxidants are a group of molecules inherent in our biological system responsible for scavenging free radicals or protecting free radical effects. They can be synthesized endogenously in the body through diverse mechanisms or determined mainly by dietary intake, both accounting for the total antioxidant status of an individual (Akpotuzor *et al.,* 2012).Vitamins A, C, and E possessing the protective properties against oxidants hence called antioxidant vitamins (Eduardu *et al.,* 1999). Depletion of these antioxidants in the biological system may lead to oxidative stress.

Vitamin A has been thought to function as an electron scavenging antioxidant due to its effect to quench free radicals by electron donation, particularly involving singlet oxygen (Wallstrom *et al.,* 2001: Graziano *et al.,* 2007).Vitamin E, a lipophilic chain breaking antioxidant, terminates free-radical mediated lipid peroxidation especially in the cell membrane (Dan *et al.,* 2010; Veysel *et al.,* 2013). Vitamin C, a preventive antioxidant, is a radical scavenger inhibiting the production of free radicals by either hydrogen or electron donation and essential in recycling of vitamin E. It is also important in maintaining cellular functions and differentiation (Greg, 2003).

Reportedly, smokers usually have poor eating habits, ingesting lower levels of essential antioxidants and inhaling large amount of oxidants from cigarette. For several reasons, the prevalence of cigarette smoking among youth in Sokoto State, Nigeria has become rampant and outrageous. Yet, studies documenting the effects of smoking on this community are lacking. This study set out to evaluate the effects of smoking on lipid peroxidation and serum antioxidant vitamins of healthy volunteers living at Usmanu Danfodiyo University, Sokoto (UDUS) community.

II. Methods

Sample Collection, Separation and Storage

Using questionnaires, 32 healthy volunteers from UDUS community were recruited for this study. Blood samples were drawn after an overnight fasting and allowed to coagulate. The coagulated blood was centrifuged to separate the serum from the whole blood at 4000 revolution per minute (rpm) for 5 min. The supernatant was pipetted into plain Eppendorf and stored at 0^oC until required for analysis.

Estimation of Vitamin A

Vitamin A was estimated using the protocol developed by Latimer 2007. 200 µL of serum was added to 2.5 mL of acetone, mixed and centrifuged at 4000 rpm for 5 min. The absorbance of the supernatant was taken at 450 nm spectrophotometrically against the reagent blank (acetone). The concentration of Vitamin A was extrapolated using its standard curve.

Estimation of Vitamin C

The protocol developed by Baker and Frank 1968 was applied for the estimation of this vitamin. 1 mL of 10% trichloroacetic acid (TCA) was pipetted into a test tube; 400 µL of serum was added, gently mixed and centrifuged at 4000 rpm for 5 min. To a different set of test tubes, 600 µL of supernatant and 200 µL of dinitrophenyl hydrazine (DTC) were added, gently mixed and incubated at 40^oC for 30 min. 400 µL of cold 12M sulphuric acid (H_2SO_4) was added to the tubes and incubated at room temperature for 20 min. Standards and blanks were prepared by pipetting 600 µL Vitamin C standard solution and DTC respectively and absorbance was taken at 520 nm.

Estimation of Vitamin E

Estimation of serum concentration of Vitamin E was carried out using the protocol developed by Baker and Frank 1968. 200 µL each of serum, standard and distilled water were pipetted into test tubes labeled tests, standard, and blank respectively. 1.5 mL each of ethanol and Xylene were added to the test tubes and centrifuged at 4000 rpm for 5 minutes. From the centrifuged tubes, 1 mL of Xylene layer was carefully taken and mixed with 1 mL of Diphyridyl reagent. The absorbance was taken at 460nm against Xylene blank. After few minutes, 330 µL Ferric chloride ($FeCl_3$) was added to the test tubes, mixed gently and absorbance was recorded again at 520nm.

Estimation of Serum Malondialdehyde

The concentration of MDA was determined by Hartman 1983 protocol. 50 µL of serum was diluted against 450 µL of deionized water. 250 µL of (1.34%) Thiobarbituric acid (TBA) was added; an equal amount of 40% TCA was also added. The mixture was mixed and incubated for 30 minutes in a water bath at 90oC. The tubes were allowed to cool at room temperature and absorbance was taken at 532nm against reagent blank.

III. Data Analysis

Data were expressed as the mean ± standard error of the mean (SEM). The parameters were analyzed statistically using One-way Analysis of Variance (ANOVA) with Microsoft Excel; 2010 version. The limit of statistical significance was set as $P<0.05$.

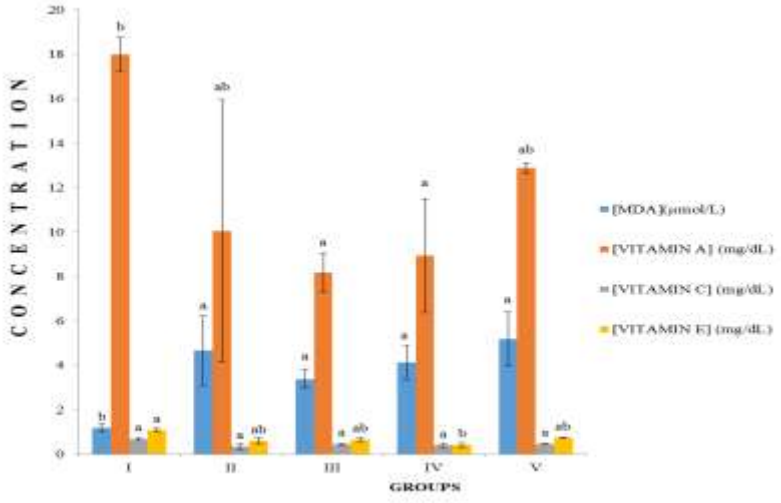

Figure 1: Lipid peroxidation and Serum antioxidant vitamins levels in smokers of different age groups.
LEGEND: Values are expressed as mean ± SEM. Group I=control, Group II=15-20years, Group III=21-25years, Group IV=26-30years and Group V=31-35years.

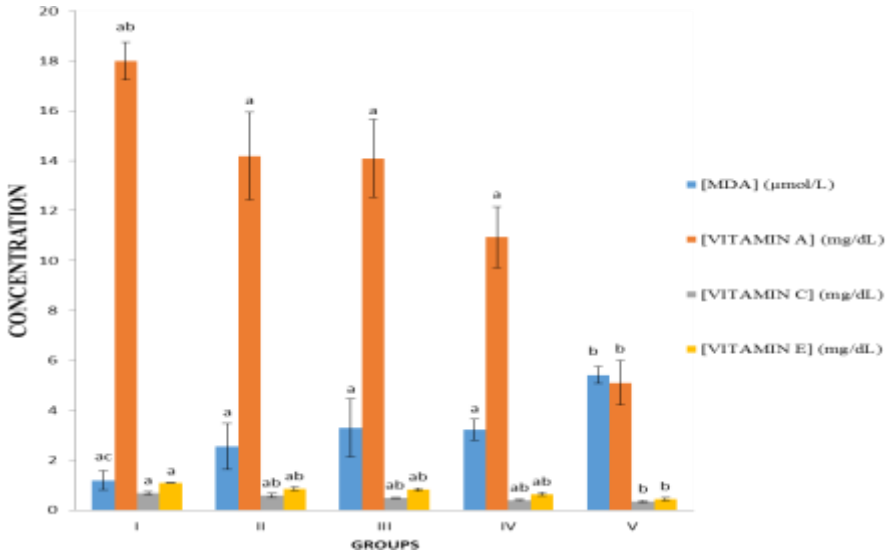

Figure 2: Lipid peroxidation and Serum antioxidant vitamin levels in different level of smoking.
LEGEND: Values are expressed as mean ± SEM. Group I=control, Group II=5-10 cigarette sticks, Group III=11-15 cigarette sticks, Group IV=16-20 cigarette sticks and Group V=21 and above cigarette sticks**.**

IV. Discussion

Cigarette smoking has been reported to contain many free radicals and other highly reactive molecules. The increased concentration of these reactive molecules in tissues would induce lipid peroxidation with concomitant release of products such as MDA (Flemming *et al.,* 1997). Similarly, this study is aligned to the above findings and it is suggested that the observed increase in the levels of MDA in the serum of smokers is linked to the increase in the levels of exogenous free radicals generated from cigarette smoke that ultimately leads to lipid peroxidation.

Vitamin A is a strong antioxidant and is the best quencher of singlet oxygen. Its lipophilic nature allows it to pass through the membrane and scavenge free radicals (Wallstrom *et al.,* 2001). Diverse observations were reported on cigarette smoking effects on serum levels of vitamin A. Some revealed a significant effect (Ascherio *et al,*. 1997; Chui *et al.,* 2009) while Biesalski (1986) has a different observation. This research is analogous to the former findings; it is likely that a lower level of this antioxidant among smokers is an upshot of its annihilation during quenching of singlet oxygen produced from cigarette smoke (Abdulrahman *et al,*. 1997).

Vitamin C, as opposed to Vitamin A is a hydrophilic molecule; its unique structure makes it an excellent electron donor which allows it to scavenges free radicals and inhibits lipid peroxidation by promoting the regeneration Vitamin E (Satyanarayana and Chakrapani, 2006; Dan *et al.,* 2010). There are incongruous reports about the effects of cigarette smoking and serum Vitamin C levels. Some revealed there is an effect (Gey *et al.,* 1992) while Mezzetti *et al.* (1995) reported a reverse effect. Conversely, this research indicated that smokers have lower levels of serum Vitamin A than non-smokers with an extremely significant difference similar to the findings of Gey (1992). This is evidence that accumulation of excess free radicals and other reactive molecules generated from cigarette smoke reduces the levels of this vitamin in the serum as observed in smokers (Christine *et al.,* 2006).

Lipid structures, like membranes, are particularly vulnerable to oxidative stress. Smokers have been reported to display evidence of more endogenous lipid peroxidation than nonsmokers (Kelly, 2002). The biological activity of vitamin E is almost entirely due to its antioxidant properties which allows it to stabilize membrane structures by effective preventing lipid peroxidation (Galan *et al.,* 2005; Christine *et al.,* 2006). In the same way, this study shows that the serum level of Vitamin E was observed to be significantly lower in smokers than in non-smokers which is in line with the findings of other studies (Mezzetti *et al.,* 1995; Palanisamy *et al.,* 2009; Veysel *et al.,* 2013). It is now evident that the levels free radicals consumed in cigarette smoke induces lipid peroxidation and ultimately leads to decrease in Vitamin E levels (Abdulrahman *et al.,* 1997)

The observed increase and decrease levels of MDA and antioxidant vitamins respectively in smokers in relation to increase in levels of cigarette consumption in this study is evident that the increase in free radicals generation, lipid peroxidation and subsequent release of MDA which decreases the levels of antioxidant vitamins in the serum as a results of their constant destruction during the neutralization of these free radicals. It is also exposed that there was no significant difference between serum levels of antioxidant vitamins and

Malondialdehyde in smokers in relation to different age groups which is in contrast to the findings of Chui *et al.* (2009) and also Christine and David (2006). This might be due to narrowing of age range (15 to 35 years) in this research, differences in the rate of cigarette consumption, duration of smoking, dietary intake of fruits and vegetables or a combination of these but significant differences were observed in relation to a number of cigarettes consumed per day, the more number of cigarette stick smoke, the lower the serum antioxidant vitamins and the more lipid peroxidation.

V. Conclusion

The present study evaluated the effect of cigarette smoking on serum antioxidants (Vitamins A, C, and E) and lipid peroxidation maker (MDA). A questionnaire was used to recruit 32 healthy male volunteers of age of 15 and 35 years. The study group was classified based on age and number of cigarettes consumed per day to evaluate the harmful effects of smoking on the serum levels of antioxidant vitamins and resultant oxidative stress. The result showed a significant decrease in serum antioxidants with a concomitant increase in MDA concentrations when compared to the control group. Interestingly, a linear relation was observed between the numbers of cigarettes consumed/day vs decreasing antioxidant levels. A similar relation was observed for cigarette consumption vs MDA accumulation suggesting that smokers consuming ≥15 cigarettes/day are more likely susceptible to various diseases. This study also highlights the effects of smoking at a young age, suggesting a progressive depletion and a sequential accumulation of antioxidants and MDA respectively, as they mature into adulthood possibly causing deleterious effects. Conclusively, cigarette smoking depletes many serum antioxidants required to scavenge excess free radicals, thus increasing the rate of lipid peroxidation.

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