Lipid Peroxide Levels, Antioxidant Status, and Protein Changes in Nigerian Smokers

Adunmo G. O[a]*, Adesokan A. A[b], Akanji M. A[c], Biliaminu S. A[d], AbdulAzeez I. M[e], Adunmo E. O[f]

[a,d,e] *Department of Chemical Pathology, College of Health Sciences, University of Ilorin, Nigeria.*

[b] *Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria.*

[c] *Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.*

[f] *Department of Family Medicine, University of Ilorin Teaching Hospital, Ilorin, Nigeria.*

[a]*Email: godwinadunmo@yahoo.com*

Abstract

Oxidative stress has been implicated in the pathogenesis of many diseases. We evaluated index of lipid peroxidation (Malondialdehyde), antioxidants (Uric Acid and Total antioxidant Status), Total Protein and Albumin levels in current smokers (n=140), Ex-smokers(n=12) and Non- smokers (N=84). Malondialdehyde (MDA), Uric acid, Total antioxidant activity, total Protein and albumin were determined by spectrophotometry. Anthropometric indices were measured and information on clinical history, daily cigarette consumption and duration were obtained through a semi-structured questionnaire. The MDA and Uric acid levels were significantly elevated in current smokers compared with non smokers ($p<0.05$) and correlated with quantity and duration of smoking. Total antioxidant status, total Protein and albumin were significantly reduced in current smokers compared with control. There was also a significant increase in serum MDA in ex-smokers compared with control ($p<0.05$) but significantly lower when compared with current smoker. There was no significant difference in uric acid in ex-smokers when compared with control. There was also a significant decrease in total antioxidant status between ex-smokers and control. The results from this study revealed an increased oxidative stress in association with cigarette smoking with reduced antioxidant capacity.

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* Corresponding author.

E-mail address: godwinadunmo@yahoo.com.

Keywords: Antioxidant status; Cigarette smoking; Oxidative stress; Malondialdehyde; Serum Proteins.

1. Introduction

Cigarette smoke contains numerous compounds, many of which are oxidants and prooxidants, capable of producing free radical [1]. Cigarette smoking leads to the uptake of many hazardous compounds. Such compounds or their metabolites may be electrophilic and thereby able to react with biological macromolecules, or they may give rise to oxidative stress by formation of reactive species or the initiation of radical chain reaction[2]. When there is an excessive addition of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues.

It has been postulated that many of the adverse effects of smoking may result from oxidative damage to critical biological substances [2]. Smokers are vulnerable to free-radical-induced damage of the cardiovascular system. They are exposed to free radicals from cigarette smoke and in general they have lower plasma antioxidant plasma levels. The obligatory use of the body's reserve of antioxidants to detoxify the tremendous level of these free radicals in smokers therefore results in severe antioxidant deficiency, thereby predisposing them to oxidative stress [2,3].

This study was aimed at evaluating the effect of cigarette smoking on lipids peroxidation, protein levels and antioxidants status in smokers, ex-smokers and non smokers in North-Eastern Nigeria.

2. Materials and Methods

A group of one hundred and forty (140) male current non –alcoholic cigarette smokers and twelve (12) ex-smokers were recruited from Maiduguri, Damaturu and Potiskum, all in North-Eastern Nigeria. Eighty-four (84) age-matched apparently healthy non-smokers were also recruited from the same metropolitan areas to serve as control. All participants in the study were included after obtaining written consent. They were between the ages of 25-60 years and current smokers must have been smoking for at least five (5) years. Those with medical conditions such as diabetes, hypertension, tuberculosis and autoimmune disease were excluded from the study. Clinical examination was done by a medical practitioner. Information on demographic, anthropometric and life style factors of each participant was obtained through a well-structured questionnaire.

2.1 Grouping of subjects

The subjects were grouped as follows:

Group I: Control

Group II: Smokers

Group III: Ex- smokers

The smokers were further grouped according to intensity of smoking (number of Cigarette smoked per day)

Group IIa: Light smokers (1-5 sticks of cigarette daily)

Group IIb: Moderate smokers (6-10 sticks of cigarette daily)

Group IIc : Heavy smokers (More than ten(10) sticks of cigarette daily)

And also according to the duration of smoking

Group IId : Short Term duration (5-10 years of smoking) smokers

Group IIe: Medium Term Duration (11-20 years of smoking) smokers

Group IIf: Long Term Duration (> 20 years of Smoking) smokers

2.2 Collection of blood sample

The blood sample from the subjects was collected after an overnight fast. The blood sample were collected between 08.30-10.00 a.m each day. The venepuncture was done in the cubital fossa, tourniquet was used but was released just before collecting the blood sample to avoid artificial increase in concencentration of serum lipids and protein. Fasting blood sample (15 ml) was aseptically collected from each participant into lithium heparin bottle and left undisturbed for an hour. The blood sample was then centrifuged at 4000Xg for 10 minutes and plasma collected was stored at $-20^{0}C$ and analysis of biochemical parameters was done within two weeks.

2.3 Biochemical Analysis

Determination of the total antioxidant activity was carried out by using the ferric reducing antioxidant power (FRAP) assay of Benzie and Strain [4].

Malondialdehyde (MDA) determination. MDA levels were determined by a method of Draper and Hadley [5] based on the reaction of MDA with thiobarbituric acid (TBA). MDA and TBA react to form a pink pigment with maximum absorption at 532nm.

Uric acid, albumin and total protein were determined spectrophotometrically using a reagent kit obtained from Randox Laboratories Limited, United kingdom.

2.4 Stastistical Analysis

Data obtained for each biochemical parameter was expressed as mean ± standard error of mean (SEM). The difference between means were compared using one way analysis of variance (ANOVA) followed by Duncan's post hoc test using SPSS version 20 (SPSS Inc., Chicago, Illioinous) and $p<0.05$ was considered significant.

3. Results

There was a significant elevation (p<0.05) in the levels of malondialdehyde with a significantly reduced level (p<0.05) of total antioxidants in smokers and ex-smokers when compared with control (Table 1).

Table 1: Plasma total antioxidant capacity, malondialdehyde and uric acid concentrations of smokers and ex-smokers

Subjects	Malondialdehyde (µmol/l)	Uric Acid (mg/dl)	Total Antioxidant Capacity (µmol/l)
Control (n=84)	2.41 ± 0.05^a	4.14 ± 0.05^a	1089.10 ± 16.48^a
Smokers (n=140)	3.78 ± 0.05^b	5.22 ± 0.06^b	867.43 ± 13.45^b
Ex-smokers (n=12)	3.01 ± 0.03^c	5.14 ± 0.12^b	988.00 ± 22.35^c

Values with different letters as superscript are significantly different at p<0.05

Values are mean ± standard error of mean

There was a significant increase (p<0.05) in plasma uric acid and malondialdehyde concentration with a significantly reduced total antioxidant capacity in smokers according to the number of cigarette smoked per day when compared to controls (Table 2).

Table 2: Effect of cigarette smoking level on malondialdehyde, uric acid and total antioxidant capacity levels of smokers

Subjects	Plasma malondialdehyde concentration (µmol/l)	Plasma uric acid concentration (mg/dl)	Plasma total antioxidant capacity (µmol/l)
Control (n=84)	2.41 ± 0.05^a	4.14 ± 0.05^a	1089.10 ± 16.48^a
Light smokers (n=26)	3.38 ± 0.84^b	4.79 ± 0.97^b	997.85 ± 32.17^b
Moderate smokers (n=66)	3.56 ± 0.58^b	5.13 ± 0.07^c	863.12 ± 15.57^c
Heavy smokers (n=48)	4.28 ± 0.10^c	5.58 ± 0.07^d	802.71 ± 22.72^d

Values with different superscripts are significantly different at p<0.05

Values are mean ± standard error of mean

Plasma malondialdehyde and uric acid concentrations were significantly elevated (p<0.05) in smokers with increase in duration of smoking when compared with controls. Plasma total antioxidants capacity, though

increased with the duration of smoking, was not significantly different (p>0.05) between medium and long term smokers (Table 3).

Table 3: Alterations in plasma total antioxidants capacity, malondialdehyde and uric acid concentrations in response to duration of cigarette smoking

Duration of smoking	Plasma malondialdehyde concentration (µmol/l)	Plasma uric acid concentration (mg/dl)	Plasma total antioxidant capacity (µmol/l)
Control (n=84)	2.41 ± 0.05^a	4.14 ± 0.05^a	1089.10 ± 16.48^a
5 – 10 years (n=53)	3.46 ± 0.05^b	4.99 ± 0.08^b	939.70 ± 18.37^b
11 – 20 years (n=48)	3.77 ± 0.08^c	5.27 ± 0.07^c	828.38 ± 20.75^c
> 20 years (n=39)	4.83 ± 0.10^d	5.66 ± 0.10^d	787.51 ± 28.57^c

Values with different superscripts are significantly different at p<0.05
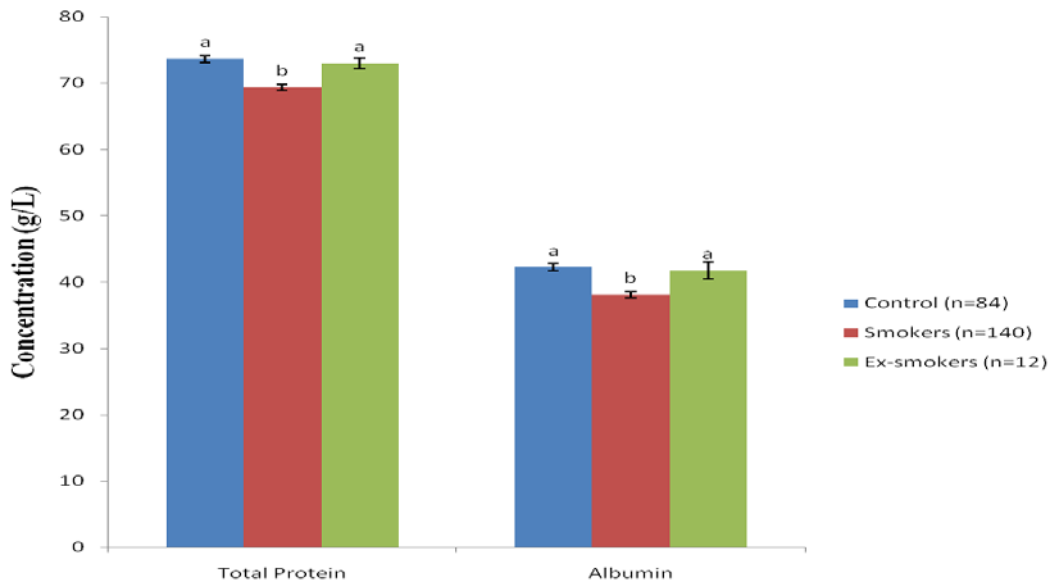
Values are mean ± standard error of mean

Figure 1 : Albumin and total protein in the plasma of smokers and ex-smokers

Values with different superscripts are significantly different at p<0.05

Values are mean ± standard error of mean

Plasma total protein and albumin concentrations were significantly reduced (p<0.05) in smokers when compared with the controls. The plasma total protein and albumin of ex-smokers and control were not significantly different (p>0.05) (Figure 1).

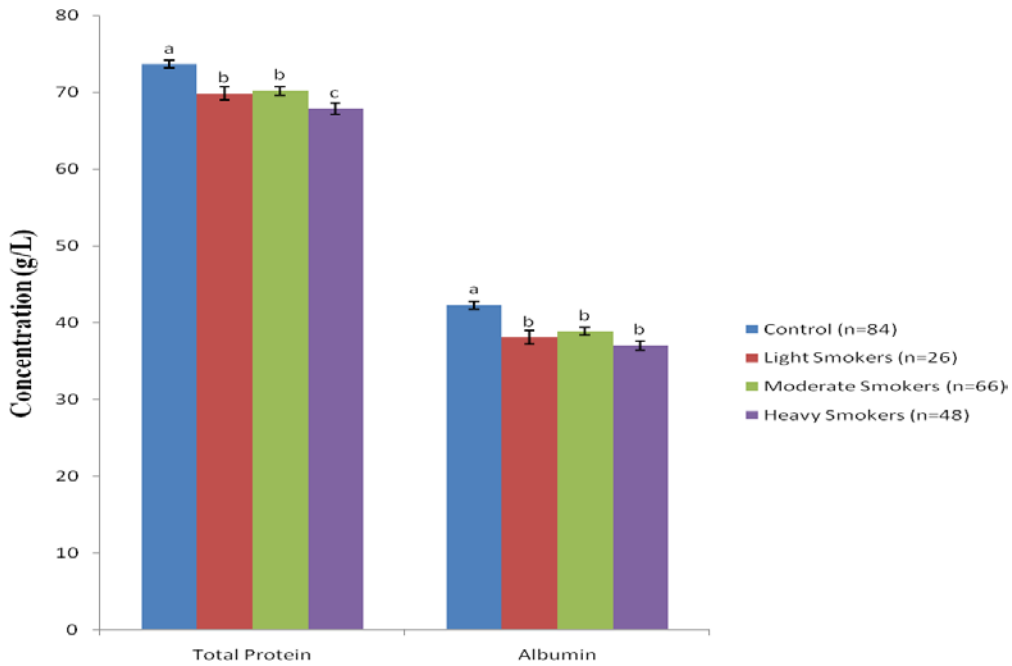**Figure 2: Effect of cigarette smoking on plasma total protein and albumin levels of smokers**

Values with different superscripts are significantly different at $p < 0.05$

Values are mean $\pm$ standard error of mean

Plasma total protein and albumin levels were significantly reduced ($p < 0.05$) in smokers when compared with controls, the decrease was not dependent on number of cigarette consumed per day (Figure 2).
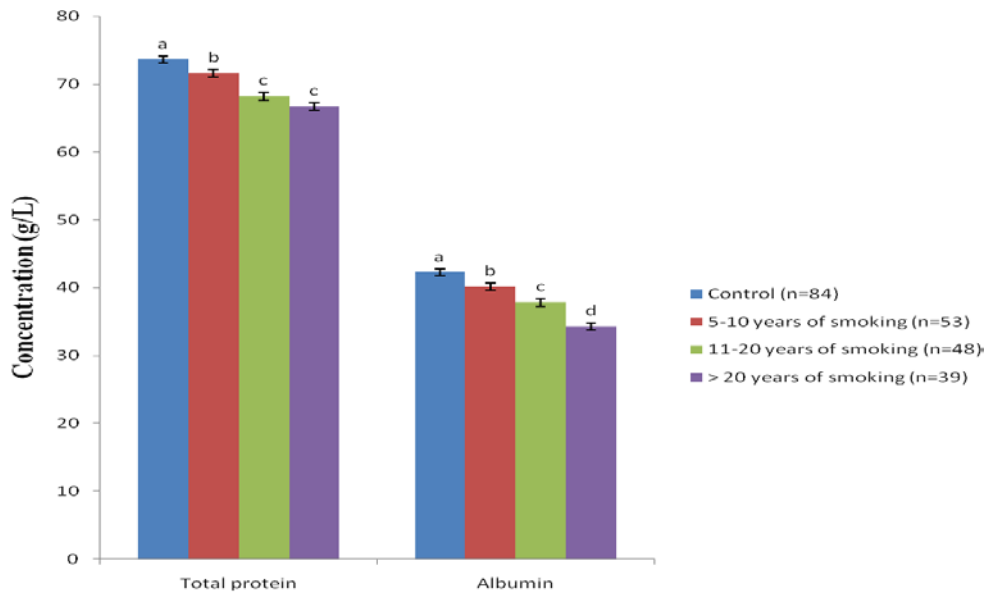

Figure 3: Effects of duration of cigarette smoking on plasma total protein and albumin levels of smokers

Values with different superscripts are significantly different at $p < 0.05$

Values are mean $\pm$ standard error of mean

There was a duration dependent significant (p<0.05) decrease in plasma albumin and total protein concentration in smokers (Figure 3).

4. Discussions

Cigarette smoke contains more than 4,000 compounds most of which are rich sources of free radicals [6]. It has been estimated that one puff of cigarette, the gas phase of the smoke, exposes the smokers to greater than 10^{15} free radicals [7]. Sustained release of free radicals imposes an oxidative stress, promotes lipid peroxidation and consequently perturbs the antioxidant defence system. Elevated level of MDA in cigarette smokers that were both dependent on duration of smoking and the number of cigarette smoked per day (Tables 1, 2 and 3) is an indication of increased lipid peroxidation. This is consistent with the finding of [8] and [9] respectively. Free radicals produced by cigarette smoke can attack polyunsaturated fatty acids (PUFAs) in the cell membranes which can result in the loss of membrane integrity. The oxidative destruction of polyunsaturated fatty acids known as lipid peroxidation is damaging because it proceeds as a self- perpetuating chain reaction [9]. The possible endothelial cell injury could lead to a host of changes in endothelial lining culminating in formation of lesion of atherosclerosis [10] and thus can lead to coronary artery and peripheral arterial diseases in smokers. Free radicals can oxidize lipids, protein, carbohydrate and DNA molecules, resulting in cell membrane damage, thereby altering cell structure and function.

Antioxidants inhibit free radical damage by preventing the formation of free radicals, scavenging them or by promoting their decomposition. Thus, the reduced plasma total antioxidant capacity (Table 1) suggest oxidative stress in smokers. Oxidative stress has been implicated in many disease conditions such as cardiovascular diseases, neurological disorder, pulmonary disease, rheumatoid arthritis, nephropathy and reproductive disorders [2]. Uric acid was significantly increased (p<0.05) in cigarette smokers when compared with control (Table 1) in this study. The rise in uric acid level indicated that smoking increase catabolism of purine. Catabolism of purines is accompanied by additional production of superoxide anion since xanthine oxidase is a potent producer of superoxide anion. Thus, cigarette smoking aggravates nucleic acids catabolism, of which a possible consequence will be damage to nucleic acids especially DNA which is more prone to oxidation [1]. In addition, the elevated level of uric acid seen in cigarette smokers might be an attempt to combat increased oxidative stress generated by cigarette smoke since uric acid is an endogeneous antioxidant.

The decreased level of total protein and albumin in smokers might be as a result of increased urinary excretion of albumin or catabolism as a result of increased free radical generation [12,13,14]. Albumin contains one reduced cysteine residue (Cys34) which constitutes the largest pool of thiols in circulation. Through the reduced Cys34, albumin is able to scavenge hydroxyl radicals [14].Thus, significantly low levels of plasma albumin in cigarette smokers could be as a result of increased free radicals generated in this group compared with the control group. An indirect antioxidant activity of albumin comes from its ability to transport bilirubin, which binds with high affinity to the molecule at Lys240 [15]. Such albumin-bound bilirubin was shown to act as an inhibitor of lipid peroxidation [15].

Bilirubin bound to albumin in the primary site has been shown to protect alpha-tocopherol from damage mediated by peroxyl radicals and to prolong the survival of human ventricular myocytes against in generated oxidative stress [14,15].

A positive association between current cigarette smoking and clinical albuminuria has been observed in a number of studies [12,18]. The mechanism of tobacco- induced albuminuria is uncertain. Increased heart rate and blood pressure among cigarette smokers have been reported which may increase renal plasma flow [17]. In addition, the reduced plasma total protein and albumin concentration may suggest that cigarette smoking may impair hepatic albumin synthesis in the liver [18]. The results of our study thus not have general spread since our subjects are from a particular zone of the country and the sample size is small.

5. Conclusions

In conclusion, these findings indicated that cigarette smoking causes a significant increase in lipids peroxidation level and decreased antioxidant levels which may result in oxidative stress with its attendant pathologies. Therefore, we advised that cigarette smokers should quit smoking. Further work should be carried out with a national geographical spread and sample size to verify the claims in this study.

Acknowledgement

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