ASSESSMENT OF TOTAL ANTIOXIDANT STATUS, MALONALDEHYDE AND SERUM LIPID PROFILE IN CIGARETTE SMOKERS IN NNEWI METROPOLIS

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ABSTRACT

Cigarette smoking is the leading cause of diseases in both developed and underdeveloped countries. This study Assessed the Total Antioxidant Status, Malonaldehyde and Serum Lipid Profile In Cigarette Smokers In Nnewi Metropolis, Anambra State, Nigeria. A total of 100 male subjects (50 smokers and 50 non smokers) were be recruited for this study. Samples collected from the subjects were used for the estimation of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), malondialdehyde (MDA) and total antioxidant capacity (TAC). All the parameters were analyzed using standard laboratory methods. The results of the smokers showed a significantly higher value in the levels of VLDL-C, (0.56 ± 0.29mmol/l; p=0.00), LDL-C (2.75 ± 0.88mmol/l; p=0.00), TG (1.23 ± 0.64mmol/l; p=0.00) and TC (4.53 ± 0.90mmol/l; p=0.08) when compared with non smokers. However, significantly lower values was observed in the level of high density lipoprotein cholesterol (HDL-C) in cigarette smokers (p=0.08) and correlates directly with intensity and duration of smoking. Again, the mean serum MDA was significantly lower in non smokers (p <0.05) although TAC was not significantly lower (p.0.05). The findings in this study showed that cigarette smoking is significantly higher in the level of lipid profile, malondialdehyde, and significantly lowers the total antioxidant status and HDL-C. This may increase the risk of heart attacks, strokes and chronic obstructive pulmonary disease (COPD).

Key Words:

Total Antioxidant Status,
Malonaldehyde Serum Lipid Profile,
Cigarette Smokers

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I. INTRODUCTION

Smoking is an escalating public health problem especially in developing countries (Reddy *et al*., 2004). Unless current smoking patterns are reversed, the World Health Organism (WHO) estimates that by the decades 2020 -2030 tobacco will be responsible for 10 million deaths per year, with 70% of them occurring in developing countries (WHO, 2001). Despite the growing problem of global cigarette use, according to World Health Organization, the prevalence pattern in Nigeria remains sparse at about 10.49% in 2009. Smoking causes an estimated 1.69 million deaths per year worldwide (Ezzati *et al.,* 2003). It has been shown that exposure to cigarette smoking (CS) increases myocardial oxygen demand and concurrently reduces coronary blood flow by causing vasoconstriction in the coronary arteries and microvasculature (Kiowski *et al.,* 1994). According to the World Health Organization (2009), second-hand smoke (SHS) also known as passive smoke is as a result of inhalation of smoke. It occurs when tobacco smoke permeates any environment, causing it's inhalation by people within that environment. Also, extensive evidence has demonstrated that cigarette smoking (Howard *et al.,* 1998) or exposure to secondhand tobacco smoke, increases the risk for cardiovascular disease (CVD). It has also been found that smoking is strongly and positively associated with an increase in the risk for myocardial infarction, sudden cardiac death, and an increase in thrombosis and atherosclerosis (Smith *et al.,* 2001). The global tobacco epidemic threatens the lives of at least one billion people. Tobacco use is a risk factor for six of the eight leading causes of death globally (WHO, 2008). Cigarette smoking is a significant risk factor in the development and acceleration of the atherosclerosis, especially those who started smoking before age of 20 years. More so, some of the risk factors associated with cigarette smoking includes cardiovascular diseases, respiratory disorders , peptic ulcers and gastro esophageal reflux diseases, blindness, bone matrix loss and hepatotoxicity, stroke, peripheral vascular disease and numerous cancers including cancer of the mouth, kidney, pancreas , larynx ,stomach, bladder, cervix, esophagus (Witschi *et al.,*2001).

The Mechanism by which cigarette smoking causes myocardial Infarction, remains obscure, but cigarette smoking have been found to alter the levels of lipoproteins. The risk of CVD among smokers may vary because of differences in smoking characteristics, such as number of years smoked, number of cigarettes per day, use of filters, inhalation pattern, and type of tobacco, or because of differences in antioxidant vitamin intake or status (Powell *et al.,* 1997). Smoking is associated with lower antioxidants concentrations, increased oxidative stress and damage and increase the risk of several chronic diseases. Many studies have shown that antioxidant nutrient supplements, especially β-carotene, vitamin C, and vitamin E, are effective in protecting the oxidation of DNA, LDL, and protein against oxidative damage by smoking Invitro (Baker *et al.,* 1999). Some previous studies also showed that vitamin E has a role in increasing blood flow and arterial vasodilatation in coronary heart diseases (Dutta and Dutta, 2003). It was found that vitamin C is important for the re-synthesis of vitamin E from the tocopheroxyl radical; hence it increases the efficacy of vitamin E as an antioxidant (Carr and Frei *et al.,* 2000). Currently, there is paucity of data on the status of lipid profile in smoker in Nnewi metropolis. Hence, the need for the Assessment of Total Antioxidant Status, Malonaldehyde and Serum Lipid Profile In Cigarette Smokers In Nnewi Metropolis, Anambra State, Nigeria.

II. MATERIALS AND METHODS

Study Area

This research was carried out at motor parks and Nkwo Nnewi market in Nnewi metropolis, Nnewi North L. G.A , Anambra State, Nigeria.

Study design

A total of 100 subjects were recruited for this study in Nkwo Nnewi Market and motor parks in Nnewi North local government area of Anambra State. Each participants were given a questionnaire to fill their bio-data, body mass index (BM I) and blood pressure (BP) were also measured using standard procedures as described by (Chobanian *et al.,* 2003; Kliegman and Berhman, 1996; Paynter and Parkin, 1991). After overnight fasting, 5mls of whole blood was collected from the subjects and the blood dispensed into a plain container, allow to clot at room temperature for one hour. Thereafter, the sera were separated from cells by centrifugation at 2500 rpm for 10 minutes and used for the estimation of the biochemical parameters (TC, TG, LDL, HDL, VLDL, MDA and TAC) using standard methods as described by (Tonks, 1967; Trinder, 1988; Trinder,1988; Assmann,1984; Wieand and Seidel, 1983; Gutteridge and Wilkins, 1982; Benzie and Strain, 1996**)** respectively.

Inclusion Criteria and Exclusion Criteria

Male smokers were included while female smokers were excluded. The subjects having risk factors that may mal the lipid profile as diabetes mellitus (DM), neoplasia, and other pathological disorders were excluded.

Ethical Approval and Informed Consent

Ethical approval for this research was obtained from the Ethical Committee of the Nnamdi Azikiwe University, Faculty of Health Sciences and Technology , College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. Also, written informed consent of the subjects were obtained.

Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) Version 20.0. The mean differences were assessed using Analysis of variance and Student-t test. ($P<0.05$). Result of parameters analyzed were expressed as mean±SD. Level of significance was set at $p<0.05$.

III. RESULTS

There were no significant differences in the mean age, BMI and SBP of the smokers and controls ($p > 0.05$).The mean TG was significantly lower non smokers ($p < 0.05$),While the LDL-C was significantly higher in the non smokers compare to the smokers ($p < 0.05$). The results also indicate that the mean VLDL-C was significantly lower in non smokers ($p < 0.05$). The mean MDA was significantly lower in non smokers ($p < 0.05$) although TAC was significantly lower ($p < 0.05$) in non smokers compared to the smokers (Table 1).

Table 1. Mean values of the parameters in smokers and non-smokers

Parameters	Smokers (n=50)	Non-Smokers (n=50)	t-value	p-value
Age(Years)	35.46 ± 8.08	32.22 ± 8.66	1.93	0.24
BMI(Kg/m^2)	22.95 ± 3.50	23.69 ± 4.3	-0.94	0.88
SBP(mmHg)	86.0 ± 7. 28	86.20 ± 6.35	0.35	0.02*
DBP(mmHg)	22.95 ± 3.50	23.69 ± 4.3	-0.94	0.88
TC (mmol/L)	4.53 ± 0.90	4.19 ± 0.98	1.79	0.08
TG (mmol/L)	1.23 ± 0.64	0.89 ± 0.42	3.11	0.00*
HDL-(mmol/L)	1.21 ± 0.59	0.78 ± 0.25	- 1.28	0.20
LDL-c (mmol/L)	2.75 ± 0.88	3.00 ± 1.05	4.81	0.00*
VLDL (mmol/L)	0.56 ± 0.29	0.39 ± 0.18	3.299	0.00*
MDA(nmol/mL)	5.17 ± 1.23	2.93 ± 1.21	9.16	0.00 *
TAC(µmol/mL)	0.56 ± 0.29	0.39 ± 0.18	3.29	0.00*

Results are expressed as Mean ± SD of Values with superscript* in a row are significant (P<0.05).

There was a significant increase in the mean age of the smokers compared with the number of years smoked (p > 0.05) (Table 2).

Table 2. Influence of daily cigarette smoking on blood pressure, BMI, TAC and Lipidprofile (p<0.05).

Parameters	Number of sticks of cigarette per day	Mean	t-value	p-value
Age (Years)	< or =5years (n=34) 6yrs and above (n=16)	34.53 ±8.46 37.44±7.04	-7.6	0.00*
BMI (Kg/m^2)	< or =5years (n=34) 6yrs and above (n=16)	22.90 ±3.59 23.06±3.41	0.47	0.64
SBP (mmHg)	< or =5years (n=34) 6yrs and above (n=16)	126.18 ±10.45 127.50±7.75	-1.23	0.22
DBP (mmHg)	< or =5years (n=34) 6yrs and above (n=16)	84.41±5.73 89.38±084	-1.01	0.32
TC (mmol/L)	< or =5years (n=34) 6yrs and above (n=16)	4.55± 0.83 4.48±1.05	-0.29	0.77
TG (mmol/L)	< or =5years (n=34) 6yrs and above (n=16)	1.23 ± 0.63 1.21±0.69	-0.38	0.70
HDL-(mmol/L)	< or =5years (n=34) 6yrs and above (n=16)	1.21 ± 0.62 1.21±0.62	0.25	0.80
LDL-c (mmol/L)	< or =5years (n=34) 6yrs and above (n=16)	2.77 ±0.83 2.72±1.01	-0.34	0.74
VLDL (mmol/L)	< or =5years (n=34) 6yrs and above (n=16)	0.56 ± 0.28 0.55 ± 0.32	-0.37	0.71
MDA (nm/ml)	< or =5years (n=34) 6yrs and above (n=16)	4.92±1.23 5.69±1.14	-0.05	0.96
TAC (µmol/ml)	< or =5years (n=34) 6yrs and above (n=16)	617.8 ± 126.87 644.28±117.22	-1.04	0.31

Results are expressed as Mean ± SD of Values with superscript* in a row are significant.

Table 3. Shows a Pearson correlation of duration (years) of cigarette smoking with the parameters. There was no correlation in the mean age, BMI, SBP, MDA, & TAC compared to number of sticks of smoked per day (P<0.05).There was a negative correlation in the HDL-C compared to the duration and number of sticks per day (p<0.05). While LDL-C/TC, TG/VLDL-C, SBP/DBP and TG/HDL-C shows a positive correlation compared to duration and number of sticks smoked per day at significant level of (P<0.05). TC/TG has no correlation at (P>0.05)

Table 3. Correlation of duration (years) of cigarette smoking with parameters.

Parameters	r-value	p - value
SBP/DBP (mmHg)	0.56	0.00**
TC/TG (mmol/l)	0.74	1.00
TG / HDL-C(mmol /l)	0.75	0.00**
HDL-C /LDL-C (mmol/l)	-0.59	0.00**
LDL-C / TC(mmol/l)	0.71	0.00**
TG /VLDL-C (mmol/l)	0.75	0.00**

Correlation is significant at value with superscript** in a row are significant (P<0.05).

III. DISCUSSION

It has long being established that one of the major constituents of tobacco i.e nicotine has a considerable influence in increasing the lipid levels in blood. Lipid have important roles in virtually all aspect of life, serving as hormone precursors aiding in digestion, providing energy storage metabolic fuel, acting as functional and structural component in cell membranes and forming insulation to allow nerve conduction or to prevent heat loss (Nader *et al.,* 1999), but their excessive concentrations are associated with various metabolic disorders. Cigarette smoking contains large number of oxidants that can be inhaled into the body. These oxidants leads to damage of all the constituents of cell including proteins, lipids, carbohydrates and DNA. The increase in oxidative stress caused by cigarette smoking is evident in the present study there were significant increase in lipid peroxidation products in smokers compared to the non-smokers (p < 0.05). The basic mechanism of lipid peroxidation is found in the radical chain reaction observed in typical autoxidation process, an oxidation by molecular oxygen (O_2) : initiation-propagation-termination (Porter, 1986). Therefore, lipid peroxidation is self-propagating and will proceed until substrate is consumed or termination occurs. In this way, lipid peroxidation is fundamentally different from other forms of free radical injury in that it is a self-sustaining process capable of extensive tissue damage (Porter *et al.,* 1995). Peroxidation of membrane lipids disrupts the lipid bilayer and structural organization. Lipid peroxidation has been implicated in a wide range of tissue injuries gland diseases example, atherosclerosis. The decrease in total antioxidant capacity and increase in the levels of malondialdehyde (MDA) with greater daily smoking quantity signifies that the extent of oxidative stress caused in an individual is directly proportional to the amount of smoking. Chronic smokers who are addicted to cigarette smoking for a longer duration of time tends to have more deleterious effects of oxidants than those who are smoking for a relatively shorter duration of time. The oxidants continuously act and leads to more and more damage of body tissues leading to severe disorders like bronchogenic carcinoma, coronary artery disease, hypertension, aging , chronic bronchitis , etc . Additional studies have also suggested that smoking duration has a stronger effect in the prediction of lung cancer risk than number of cigarettes smoked per day (Flanders., 2003). This study show that the mean levels of TC, LDL-C, TG, VLDL-C, MDA, DBP, BMI were significantly increased in the duration and intensity of cigarette smoking (P<0.05). The values of triglycerides of smokers in this study were significantly increased compared to that of non-smokers. The increase in the value of triglyceride is due to induction of lipogenic enzyme by nicotine as reported by Khurana *et al.,*(2000), where they established that there is induction of both glycerolkinase and glycerol-3-phosphate acyl transferase by nicotine. The result of this study is in line with the work of Nader *et al.,*(1999), where both recorded an increase in triglycerides in smokers compared with non- smokers. It has also been documented that nicotine stimulates the release of adrenaline from the adrenal cortex leading to increased serum

concentration of free fatty acid (FFA) which further stimulates hepatic synthesis and secretion of cholesterol (Goh *et al.,* 1973) as well as hepatic secretion of very low density density lipoprotein (VLDL) and hence increased triglyceride (Muscat *et al.,* 1991). Carl and Edward reported that clinically increased low density lipoprotein cholesterol is associated with increased risk of coronary heart disease (Carl *et al.,*1999). The findings of this present work revealed higher level of cholesterol in smokers when compared with non - smokers. This findings is in consonance with the work of Khurana *et al.,*(2000) where it was reported that increased low density lipoprotein level in cigarette smokers was due to the down regulation of low density lipoprotein receptors and failure of receptor (Brischetto *et al.,*1983). High density lipoprotein cholesterol was significantly lower when compare with non- smokers in this work. A decreased high density lipoprotein cholesterol concentration is associated with coronary heart disease. This shows that smokers are predisposed to developing coronary heart disease earlier than their non- smoking counterpart. The result of total cholesterol level was higher in smokers compared to non-smokers ($P< 0.05$). The result of this work is in line with work of Adedeji and Etukudo.,(2006),where high concentration of cholesterol was recorded in smokers when compared with the non - smoker. In contrast, Alharbi.,(2008) in their work , which was on the influence of cigarette smoking on lipid profile in male university students recorded non- significant result in total cholesterol level in smokers. The risk in the blood cholesterol levels in smokers may be through catecholamine and adenyl cylase axis including tissue lipolysis (Devaranavadgi *et al.,* 2012). Cigarette smoking may enhance oxidative stress by the following three reasons, First, tobacco is rich in pro-oxidants, which are further supplemented during smoking and chewing, Secondly, these pro-oxidants consume more antioxidants and Thirdly, smokers have a tendency for low intake of dietary antioxidants. There is evidence that smoke induced oxidative stress and lower serum antioxidant concentration plays an important role in cancer development. There is also a growing evidence that oxidative stress (OS) has a causal relationship with cancer and a weak antioxidant defense can aggravate it further. Therefore, this study tends to examined the total antioxidant activity with special attention to the influence of smoking, serum levels of total antioxidant. There were significantly lower antioxidant capacity in smokers on the compared to non cigarette smokers. Oxidative stress can be induced by a range of environmental factors, including smoking, drought etc. One of the main cellular components susceptible to damage by reactive oxygen species are lipids (by lipid peroxidation of unsaturated fatty acids in biological membranes).

The assay of thiobarbituric acid reactive substances (TBARS) is a well- established method for monitoring lipid peroxidation. Serum malondialdehyde (MDA), a biomarker of lipid peroxidation , has been extensively used to investigate oxidative stress in coronary artery disease(CAD) (Mutlu *et al.,* 2005). The production of this aldehyde is used as a marker to measure the level of oxidative stress in an organism. The important reactive aldehydes originated from lipid peroxidation are ketoaldehydes, including malondialdehyde (MDA) and glyoxal. The increased lipid peroxidation is also as a consequences of oxidative stress when the balance between pro-oxidant and oxidant, antioxidant status is impaired.

Malondialdehyde is a product of autoxidation of polyunsaturated fatty acids and is used as an index of oxidative damage (Cavalca *et al.,*2001). Dubois *et al.,*(1994), reported a significant rise in serum malondialdehyde levelsand lipid per oxidation with a decrease in antioxidant in smokers . The significant increase in malondialdehyde in the present study is similar to the results of kaur *et al*.,(2008),where they observed a significant increase in of malondialdehyde levels among smokers Verma *et al.,*(2005), also reported a significant drop in antioxidant levels where as lipid peroxides were significantly higher in acute myocardial infarction in smokers than non smokers.

IV. CONCLUSION

In conclusion, there is a greater depletion of anti-oxidants levels found in the smokers which predisposes them an increased tobacco-induce oxidative stress. Again, the lipid profile parameters alongside the lipid peroxidation marker indicate a greater risk in developing cardiovascular diseases in cigarette smokers in comparison with the non-tobacco smokers.

V. RECOMMENDATION

There is an increasing prevalence of cigarette smoking among adults and adolescents. This will in future translate to increase morbidity and mortality if nothing is done to curb the trend. It is recommended that early intervention should be made to reduce the incidence of cigarette smoking in adolescents. Parents and adults should refrain from smoking in the presence of these adolescents. Indeed, cessation of smoking prior to middle age is associated with a more than 90% reduction in cancer risk attributed to tobacco, and the risk of death diminishes soon after cessation of smoking.
However, smokers should always be advised to quit smoking as quitting smoking not only stops the oxidative damage to the body but also reverses the body antioxidant defense system and the benefits become more evident with passage of time.

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