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Evaluation of total antioxidant status, superoxide dismutase and malondialdehyde in apparently healthy active tobacco smokers in Nnewi Metropolis, South-East, Nigeria

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Abstract

Oxidative stress has been implicated in the pathogenesis of many diseases. Daily cigarette consumption and duration of smoking were obtained through a semi-structured questionnaire. Information on other risk factors like alcohol consumption were also obtained using the same tool. A total of 120 subjects were used; 60 tests subjects and 60 control subjects. The test group were subdivided into 3 groups based on duration of smoking (1- 5 years, 6 -10 years, 11 years and above). Daily cigarette consumption/number of sticks smoked was also used to subdivide the test group into 3 groups ($1 - 5$ sticks/day, $6 - 10$ sticks/day, 11 and above sticks/day). Total antioxidant status (1148.0660 ± 171.97974 µmol/L) and Superoxide Dismutase activity (8.5360 ± 3.98112IU/L) were significantly reduced in current cigarette smokers compared with control (1310.4080 ± 230.85928µmol/L, 12.6640 ± 2.26849IU/L respectively, $p<0.05$). This outcome correlated with duration of smoking (TAC = <0.001 SOD =<0.001) but not with the rate of cigarettes smoking. Furthermore, MDA levels ($1.6620 \pm .72868$µg/L) were significantly elevated in current cigarette smokers compared with control group (nonsmokers) ($1.2660 \pm .28400$µg/L, $p<0.05$) and correlated with duration of smoking (<0.001) which maybe suggestive of lipid peroxidation. These findings may indicate that smoking cigarette no matter the quantity depletes the antioxidant pool of the body exposing the body to effects of reactive oxygen species and other free radicals. Furthermore, the increase in the index of lipid peroxidation (MDA) could be suggestive of cell/DNA damage.

Keywords: Tobacco Smoking, Oxidative Stress, Lipid Peroxidation, Total Antioxidant Capacity (TAC), Superoxide Dismutase, Malondialdehyde.

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INTRODUCTION

Background

Smoking is regarded as a social activity but not without serious adverse effects. Smoking is a practice in which a substance is burnt and the resulting smoke absorbed into the bloodstream. Most commonly the substance is the dried leaves of the tobacco plant which have been rolled into a small square of rice paper to create a small, round cylinder called a "cigarette"[1]. Smoking is primarily practiced as a route of administration for recreational drug use because the combustion of the dried plant leaves vaporizes and delivers active substances into the lungs where they are rapidly absorbed into the bloodstream and reach bodily tissue. In the case of cigarette smoking these substances are contained in a mixture of aerosol particles and gasses and include the pharmacologically active alkaloid, nicotine; the vaporization creates heated aerosol and gas to form that allows inhalation and deep penetration into the lungs where absorption into the bloodstream of the active substances occurs. In some cultures, smoking is also carried out as a part of various rituals, where participants use it to help induce trance-like states that, they believe, can lead them to "spiritual enlightenment" [1].

Cigarettes are primarily industrially manufactured but also can be hand-rolled from loose tobacco and rolling paper. Other smoking implements include pipes, cigars, bidis, hookahs, vaporizers, and bongs[1].

Constituents of Tobacco

Tobacco is an agricultural product processed from the fresh leaves of plants in the genus *Nicotiana*. The genus contains a number of species however, *Nicotiana tabacum* is the most commonly grown. Tobacco smoke contains many chemicals that are harmful to both smokers and nonsmokers. Breathing even a little tobacco smoke can be harmful (passive smoking)[2]. Of the more than 7,000 chemicals in tobacco smoke, at least 250 are known to be harmful, including hydrogen cyanide, carbon monoxide, and ammonia [2].

Among the 250 known harmful chemicals in tobacco smoke, at least 69 cancausecancer. Thesecancer-causingchemicalsincludethefollowing[2]:

Acetaldehyde, Aromatic amines, Arsenic, Benzene, Benzo[α]pyrene, Beryllium (a toxic metal), 1,3–Butadiene (a hazardous gas), Cadmium (a toxic metal), Chromium (a metallic element), Cumene, Ethylene oxide, Formaldehyde, Nickel (a metallic element), Polonium-210 (a radioactive chemical element), Polycyclic aromatic hydrocarbons (PAHs), Tobacco-specific nitrosamines, Vinyl chloride.

Smoking generally has adverse health effects, because smoke inhalation inherently poses challenge to various physiologic processes such as respiration and metabolism. Diseases related to tobacco smoking have been shown to kill approximately half of long term smokers when compared to average mortality rates faced by non-smokers. A 2007 report states that, each year, about 4.9 million people worldwide die as a result of smoking[1].

Smoking is one of the most common forms of recreational drug use. Tobacco smoking is the most popular form, being practiced by over one billion people globally, of which the majorities are in the developing world[3]. Less common drugs for smoking include cannabis and opium. Some of the substances are classified as hard narcotics, like heroin, but the use of these is very limited as they are usually not commercially available.

Cigarette smoking harms nearly every organ of the body, causes many diseases, and jeopardizes the health of smokers in general[2]. On the other hand, quitting smoking lowers risk for smoking-related diseases and can increase the life expectancy of the smoker[4]. Cigarette smoking is the leading preventable cause of death in the United States[2]. Cigarette smoking only, causes more than 480,000 deaths each year in the United States. This is nearly one in five deaths[5]. Smoking caused more deaths than the following causes combined in the United States as at year 2003[6]: Human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, firearm-related incidents

More than 10 times as many U.S. citizens have died prematurely from cigarette smoking than have died in all the wars fought by the United States during its history. Smoking causes about 90% (or 9 out of 10) of all lung cancer deaths in men and women[2]. More women die from lung cancer each year than from breast cancer. About 80% (or 8 out of 10) of all deaths from chronic obstructive pulmonary disease (COPD) are caused by smoking[2]. Cigarette smoking increases risk for death from all causes in men and women. The risk of dying from cigarette smoking has increased over the last 50 years in men and women in the United States[2].

Prevalence Rate of Tobacco Smoking in Nigeria

According to the Global Adult Tobacco Survey (GATS) fact sheet for Nigeria; an initiative of World Health Organization (WHO) (2015); 10.0% of men, 1.1% of women, and 5.6% overall (4.5 million adults) currently use tobacco products in Nigeria. 7.3% of men, 0.4% of women, and 3.7% overall (3 million adults) currently smoke tobacco. 2.9% of men, 0.9% of women, and 1.9% overall (1.6 million adults) currently use smokeless tobacco[3].

7 in 10 current smokers planned to or were thinking about quitting. 6 in 10 male smokers who visited a health care provider in the past 12 months were advised to quit[3].

17.3% of adults who worked indoors (2.7 million adults) were exposed to tobacco smoke at the workplace. 6.6% of adults (5.2 million adults) were exposed to tobacco smoke at home.29.3% of adults (6.4 million adults) were exposed to tobacco smoke when visiting restaurants[3].

The median monthly expenditure on manufactured cigarettes was 1202.5 Naira. 4 in 10 adults noticed anti cigarette smoking information on the television or radio.3 in 10 current smokers who thought about quitting because of a warning label. 82.4% of adults believed smoking causes serious illness.48.6% of adults did not believe smoking causes stroke[3].

Oxidative Stress and its Markers

Oxidative stress is a condition characterize by imbalance between antioxidants and oxidants formed in the body. Examples of oxidants include Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).

The pathophysiology of oxidative stress

There is a mitochondrial dysfunction and subsequent imbalance between releasing of reactive oxygen, nitrogen or chlorine species and synthesis of defensive antioxidant capacity systems from nuclear DNA, resulting in oxidative stress. Serious consequences of the oxidative stress include, DNA damage, mutations, cell death by necrosis or apoptosis[7, 8]. For many decades, researchers have studied many markers of oxidative stress-associated tissue damage and antioxidant defense, including measurement of antioxidant enzymes– superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ceruloplasmin, and proteins such as metalothionins[8]. In the beginning of 1990's, Miller *et al.*[9] had created a new test to measure the total antioxidant status, which has been designated as total antioxidant capacity (TAC)[9]. The major advantage of this test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound. Another important test is the test to measure the product of lipid peroxidation – Malondialdehyde (MDA) and 4-Hydroxynonenal (4-HNE). These arrays of test have the common aim of establishing oxidative stress and estimate the extent too.

MATERIALS AND METHOD

Study Site

Subjects were recruited from public eateries, restaurants, motor parks and recreational centres in Nnewi Metropolis. The analysis of their

Samples were carried out in the Laboratory complex of the Department of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital (NAUTH) and Springboard Research Laboratory, Awka after receiving ethical clearance from NAUTH Ethics Committee. Control group was also recruited from apparently healthy individuals in the metropolis too. Consent of the subjects was obtained before recruitment.

Sampling Technique

The sampling technique that was employed in the recruitment of subjects is simple stratified sampling technique and involved the use of questionnaire which was administered by the researcher. The content of the questionnaire was explained to the subjects and only those who willingly obliged were recruited. No monetary remuneration was given to the subjects so as to remove bias.

Study Design

This is a cross-sectional analytical study. A total of 120 subjects (60 control subjects and 60 test subjects) were used for this study. 5mls of venous blood samples was collected from the subjects for the analysis of the parameters. The subjects were stratified based on duration of smoking and rate of smoking thus: light smokers (1 - 5 sticks of cigarettes per day), moderate smokers (between 5 - 10 sticks of cigarettes per day) and heavy smokers (greater than 10 sticks of cigarettes per day). For duration of smoking: (1year – 5years) (6years – 10 years) 11 and above.

Sample size

Tobacco

According to GAT-WHO Fact sheet on smoking (2015), the prevalence of tobacco smoking in Nigeria is 3.7%.

Sample size will be obtained using the formular by Naing *et al.,* (2006).

$$N = Z^2 \times P (1 - P) / d^2$$

Where:
N = Minimum sample size
D = Desired level of significance (0.05)
Z = Confidence Interval (1.96)
P = Prevalence rate of tobacco smoking (3.7%)

Applying the formular above the sample size calculated thus:

$$N = 1.96^2 \times 0.037 \times (1 – 0.037) / 0.05^2$$

$$N = 55.$$

Attrition size of 10% was included = 5.5

However, Sixty (60) apparently healthy tobacco smokers were recruited for this study. And sixty (60) apparently healthy non-smokers were used as control subjects.

Inclusion criteria

1. Smokers aged 18 and above who do not belong to the groups listed in the exclusion criteria.
2. Non-smokers aged 18 and above who do not belong to the groups listed in the exclusion criteria.

Exclusion Criteria

1. Battery workers.
2. Painters.
3. Known hypertensives.
4. Known diabetics
5. Pregnant women.
6. Subjects below the age of 18 years old.
7. Subjects that use tobacco snuff and opoid.

Sample Collection

5mls of whole blood was collected into a plain contain, allowed to clot, retracted and separated by centrifugation at 3000rpm for 5min, a clear serum was obtained. The serum was stored at -20°C. The analysis of the parameters was conducted at the Chemical Pathology Laboratory unit of NAUTH and Springboard Research Laboratory Awka.

METHODS

Determination of SOD Activity

Misra and Fredovich (1972) [10].

Principle

The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O_2^*) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD [10].

Reagents/ Reagents Reconstitution:

- 0.3M Epinephrine: 0.01g of epinephrine was dissolved in 17ml of distilled water
- 0.05M Cabonate buffer (pH 10.2): 0.53g of Na_2CO_3 and 0.42g of $NaHCO_3$ was dissolved in 100ml of distilled water

Procedure

80µl of sample/blank were added into a clean test tube containing 1000 µl of carbonate buffer (pH 10.2). The resulting solution was mixed thoroughly, and allowed to equilibrate by incubating at 37 °C for 5 minutes. Thereafter, 600 µl of freshly prepared epinephrine was added and the reaction mixture was read at 30 seconds interval for 150 seconds at 480 nm. The blank was treated the same way except that 80µl of distilled water was used instead of plasma. The changes in absorbances of both test and blank were determined. The % inhibition of auto oxidation of epinephrine by SOD was calculated and the plasma SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of SOD that can cause 50% inhibition of epinephrine.

Calculation:

$$\% \text{ inhibition} = (\Delta OD_{blank} - \Delta OD_{test} / \Delta OD_{blank}) \times 100$$

$$\text{Enzyme Unit (U/ml)} = (\% \text{ inhibition}/50) \times \text{dilution factor.}$$

Determination of MDA level

MDA level was determined by the colorimetric method of Gutteridge and Wilkins, (1982).

Principle

Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample [11].

Reagents/ Reagents Reconstitutions

- Glacial acetic acid
- 0.05M NaOH (0.2g of NaOH in 100ml of distilled water)
- 1% Thiobarbituric acid (TBA) in 0.05M NaOH (w/v) (dissolve 1g of TBA in 100ml of 0.05M NaOH).
- Heat the solution in a hot water bath for some minutes to dissolve TBA.

Procedure

To 0.1 ml of sample in test tube was added 1 ml of 1% Thiobarbituric acid dissolved in alkaline medium (sodium hydroxide). The mixture was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 °C) for 15 minutes. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10 minutes. Thereafter, the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was added to the blank instead of plasma. The level of MDA in the serum is expressed as nmol/ml using the molar extinction coefficient for MDA (1.56×10^5 M-1cm-1).

Calculation:

$$MDA \text{ (nmol/ml)} = (OD \times 1000000)/ E_{532}$$

Where E_{532} = Molar extinction coefficient for MDA (1.56×10^5 $M^{-1}cm^{-1}$).

Estimation of Total Antioxidant Capacity

Total antioxidant activity was estimated by Ferric Reducing Ability of Plasma (FRAP) method by Benzie and Strain, 1996.

At low pH, Antioxidant power causes the reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) that can be monitored by measuring the change in absorption at 593nm. FRAP values are obtained by comparing the absorbance change at 593 nm in mixture (test), with those containing ferrous ion in known concentration (Standard)[12].

Procedure

Initially, a working reagent comprising acetate buffer (pH 3.6), ferric chloride and tripyridyltriazine in the ratio of 10:1:1 respectively was prepared. To 60 µl of sample or standard or blank in a clean test tube, 1.8 ml of working reagent was added. The reaction mixture was mixed thoroughly, and incubated at 37 °C for 10 minutes. The resulting blue coloured solution developed was then read at 593 nm. The blank was treated the same way except that 60 ul of distilled water was added instead of plasma. The standard solution contains 1000 µmol/l of ferrous sulphate.

Calculation:

Total Antioxidant Capacity (µmol/l)   =

$$\frac{OD_{TEST}}{OD_{STD}} \times \text{Std conc. (1000).}$$

Chemicals

Reduced glutathione, epinephrine, DTNB, TBA, hydrogen peroxides were purchased from Sigma Aldrich, Germany. TCA, sodium dihydrogen phosphate, disodium hydrogen phosphate, SSA, sodium hydrogen bicarbonate, sodium carbonate, sodium hydroxide, and ammonium molybdate were purchased from BDH, England.

Equipments

Spectrophotometer (APAL PD303S, Japan), Incubator (MEMMERT, Germany), Water bath with shaker (ThermoScientific 2871, USA).

RESULTS

Table 1. The mean – standard deviation and level of significance of TAC, SOD and MDA, in the study participants and control. The mean serum concentration of Total Antioxidant Concentration (test subjects = 1148.07 ± 171.98 µmol/L, controls =1310.41 ± 230.86 µmol/L) p-value (.036) and SOD (test subjects = 8.54 ± 3.98 IU/L, controls =12.66 ± 2.27 IU/L) p-value (<.001) were significantly lowered in test subjects when compared with the control groups. The mean serum concentration ofMDA (test subjects = 1.66 ± .73µg/L, controls =1.27 ± .28µg/L) p-value (.004).

Table 1: Levels of TAC, SOD and MDA incontrols and active Tobacco Smokers (Mean and Standard Deviation)

Variables	(Test group) N = 58	(Control) N =58	t-value	p-value
TAC(µmol/L)	1148.07 ± 171.98	1310.41± 230.86	-2.12	.036*
SOD (IU/L)	8.54 ± 3.98	12.66 ± 2.27	5.85	<.001*
MDA(µg/L)	1.66 ± 0.73	1.27 ± 0.28	-2.99	.004*

Result is significant at p < 0.05
KEY: BMI – Body mass index, **TAC** – Total antioxidant capacity,
      SOD – Superoxide dismutase, **MDA** – Malondialdehyde.

Table 2. Test subjects were sub-divided into 3 groups: Group A (those that have been smoking for 1 – 5 years), group B (those that have been smoking for 6 – 10 years) and group C (those that have been smoking for more than 10 years). Multiple comparisons (ANOVA) of effect of duration of smoking on the tests parameters show significant levels. Mean serum concentration of TAC for Group A was: (1213.87 ± 122.69µmol/L), Group B TAC (1081.51 ± 81.18 µmol/L), Group C TAC (941.54 ± 126.01µmol/L) (p-value was <.001) while for SOD, Group A was: (10.076 ± 2.431 IU/L), Group B: SOD (7.85 ± 2.61 IU/L), Group C: SOD (4.64 ± 2.60IU/L) (p-value was <.001). MDA Group A: (1.31 ± .26 µg/L), Group B: MDA (1.68 ± .30µg/L), Group C (2.36 ± .75µg/L) (p-value was <.001).

Furthermore, the effects of duration on TAC, SOD, Cd and Cr were compared within the sub-groups (post-hoc). Group A: (smokers of 1 – 5 years duration) was compared against Group B (smokers of 6 – 10 years duration) TAC (003) and SOD (.037) showed significant values respectively while MDA (.067) did not show significant value. In the same vein, Group A (smokers of 1 – 5 years duration) was compared against Group C (smokers of > 10 years duration): TAC (<.001), SOD

(<.001) and MDA (<.001)all showed significant values. Group B (1 − 5 years) was compared against Group C smokers of > 10 years duration):

TAC (<.005), SOD (<.004) and MDA (<.001), all showed significant values.

Table 2: Durations of tobacco smoking on levels of TAC, SOD and MDA (Mean and standard deviation)

Variables	1-5 years (A) n = 20	6 – 10 years n = 22	>10 year n = 16	sf –Value	p –value	A vs B	A vs C	B vs C
TAC (µmol/L)	1213.87± 122.68	1081.51 ± 81.18	941.54 ± 126.01	24.64	<.001*	.003*	<.001*	.005*
SOD (IU/L)	10.08±2.43	7.85± 2.61	4.64 ± 2.60	19.35	<.001*	.037*	<.001*	.004*
MDA (µg/L)	1.31 ±.26	1.68 ± .30	2.36 ±.75	21.99	<.001*	.065	<.001*	<.001*

* The mean difference is significant at the 0.05 level

KEY:BMI – Body mass index, **TAC** – Total Antioxidant Capacity, **SOD** – Superoxide Dismutase, **MDA** – Malondialdehyde.

Table 3. Test subjects were sub-divided into 3 sub-groups: Group A Group A (those that have been smoking 1 – 5 sticks of cigarette per day), group B (those that have been smoking 6 – 10 sticks of cigarette per day) and group C (those that have been smoking more than 10 sticks of cigarette per day). Multiple comparisons (ANOVA) of the effect of rate of tobacco smoking on the tests parameters show various levels of significance.

Mean serum concentration of TAC for group A was: (1089.13±166.75µmol/L), For Group B; TAC (1106.12 ± 174.01µmol/L), Group C (1124.81±91.89µmol/L) p-value (.855). There was no statistical difference in the mean concentration of TAC within the compared groups. For SOD, Group A value was (6.74 ± 3.21 IU/L), Group B (8.67 ± 2.95 IU/L) and Group C (9.75 ± 3.55 IU/L). p-value was (.062) which was not significant. Inter –group comparison of effects of rate of smoking on MDA did no show a significant value:

Group A mean ± SD value for MDA was (1.91 ± .83µg/L), for Group B (1.56± .33µg/L) while Group C (1.51 ± .43µg/L), p-value was (.138).

Furthermore, the effects of rate of smoking on, TAC, SOD and MDA were compared within the sub-groups (post-hoc). Group A: (those that have been smoking 1 – 5 sticks of cigarette per day), was compared against group B (those that have been smoking 6 – 10 sticks of cigarette per day) TAC (1.00), SOD (.174) and MDA (.245), showed no significant values respectively. In the same vein, Group A (those that have been smoking 1 – 5 sticks of cigarette per day) was compared against Group C (smokers of > 10 sticks per day): TAC (1.00), SOD (.077) and MDA (.385), showed no significant value. Furthermore, group B (those that have been smoking 6 – 10 sticks of cigarette per day) was compared against Group C (smokers of > 10 sticks per day) and none of the parameters showed significant value: TAC (1.00), SOD (1.00) and MDA (1.00).

Table 3: Rate of Tobacco Smoking on TAC, SOD, MDA, Cd and Cr (Mean and Standard Deviation).

Variables	1-5 years (A) n = 21	6 – 10 years n = 21	>10 year n = 16	sf –Value	p –value	A vs B	A vs C	B vs C
TAC (µmol/L)	1089.13±166.75	1106.12 ± 174.01	1124.81±9	1.89	.157	.855	.003*	1.001.00
SOD (IU/L)	6.74 ± 3.21	8.67 ± 2.95	9.75 ± 3.55	2.390	.062	.174	.077	1.00
MDA (µg/L)	1.91 ± .83	1.56± .33	1.51 ± .43	2.070	.138	.245	.385	1.00

*. The mean difference is significant at the 0.05 level.

KEY:BMI – Body mass index, **TAC** – Total antioxidant capacity, **SOD** – Superoxide dismutase, **MDA** – Malondialdehyde.

Table 4. Pearson Correlation was carried out to determine the relationship between the variables (TAC, SOD and MDA) and the duration of smoking. Duration of smoking against TAC and SOD negatively correlated (p-value was significant at <.001 respectively), duration of smoking against, MDA positively correlated (p-values was <.001).

Table 4: Correlation table showing the relationship between duration of active tobacco smoking and the variables

Variables	N	r	p-value
Duration of Smoking vs TAC	58	-0.716[**]	<.001*
Duration of Smoking vs SOD	58	-0.685[**]	<.001*
Duration of Smoking vs MDA	58	0.723[**]	<.001*

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
KEY:BMI – Body mass index, **TAC** – Total antioxidant capacity, **SOD** – Superoxide dismutase, **MDA** – Malondialdehyde, **Cd** – Cadmium, **Cr**– Chromium.

Table 5. Table shows the relationship between rate of smoking and the variables. p – value for rate of smoking versus TAC, SOD, MDA, Cd and Cr were not significant. R-value for duration of smoking versus the variables were: TAC (0.096), SOD (0 81) and MDA (-0.262).

Table 5: Correlation table showing the relationship between rate of active tobacco smoking and the variables

Variables	N	r	p-value
Rate of smoking vs TAC	58	0.096	0.510
Rate of smoking vs SOD	58	0 81	0.067
Rate of smoking vs MDA	58	-0.262	0.069

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
KEY:BMI – Body mass index, **TAC** – Total antioxidant capacity, **SOD** – Superoxide dismutase, **MDA** – Malondialdehyde.

DISCUSSION

When antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction and/or disease. Then, the maintenance of adequate antioxidant levels, but not over dosage, is essential to prevent or even manage a great number of disease conditions[13]. Oxidative stress has been implicated in the pathogenesis of many diseases. In this study; Total antioxidant status and Superoxide Dismutase were significantly lower in current cigarette smokers compared with control (p<0.05). It positively correlated with duration and contrasted with quantity of cigarette of smoking. Furthermore, the MDA level was significantly higher in the test group (current cigarette smokers) compared with control group (nonsmokers) (p<0.05) and positively correlated with duration of smoking.

Lowered total antioxidant capacity found in this study is evidence that smoking overwhelms the antioxidant pool of the smokers leading to oxidative stress. This may be as a result of either or both of these factors; physiological and nutrition. The former is observed when there is outright imbalance between the generation of antioxidant and the free radical generation due to insult to the redox balance of the body antioxidant pool. This insult which in this case is cigarette smoke could have induced an overwhelming free radical generation that the body antioxidant pool depleted significantly.

Furthermore, Oxidised glutathione associated with excessive protein carbonylation has been shown to accumulate in the lungs of older smokers, raising the possibility that antioxidant defences could be overwhelmed. This is another mechanism that could be the cause of overwhelmed total antioxidant status seen in cigarette smokers in this study. Other possible mechanisms include: the depletion of glutathione and other antioxidants, the initiation of redox cycling mechanisms, enhancement of the respiratory burst in neutrophils and macrophages, inactivation of protease inhibitors such as α_1-antitrypsin inhibitor, and direct damage to lipids, nucleic acids and proteins[14].

The other factor is nutrition. It is known that exogenous antioxidant intake from whole food and nutritional supplements may influence both the antioxidant capacity of blood as well as oxidative stress biomarkers. It has been independently reported that smokers consume less antioxidant rich foods compared to nonsmokers[15, 16] and have suppressed blood levels of certain antioxidants such as ascorbic acid, tocopherol and superoxide dismutase which may influence the degree of oxidative stress[17]. Furthermore, it has been noted that nicotine causes a feeling of fullness. Therefore, cigarette smokers are less likely to eat enough quantity of food. This could result to altered production of antioxidants by the body as there could be malnoulrishment due to low food intake. Also, to a lesser extent, economic status of individual smokers may also be a contributing factor, as some smokers may not have the capacity to feed well even when there is sensation of hunger. In this study, it is worthy to note that most of smoking participants used are individuals that could be regarded as low income earners like labourers, brick layers, commercial cyclists and motor park workers.

Furthermore, from the questionnaire, it was deduced that smoking is strongly associated with certain life-style like alcohol consumption. To the question "Do you consume alcohol?" in the questionnaire, all tests subjects answered 'yes'. It therefore, goes to imply that reduction in TAC may not have been induced solely by cigarette smoke but due to other confounding factors like alcohol consumption which has been shown to negatively affect the level of antioxidant, as it is a pro-oxidant generator also.

In this research, SOD level was found to be lowered in cigarette smoking subjects when compared with the nonsmoking control subjects. This could be due to the inactivation by hydrogen peroxide which is increased in smokers. Study by Eizadi et al., (2014)[18];Haziel et al., (2015)[19] showed significantly higher SOD enzyme activity in the blood (i.e., in erythrocytes) and saliva of smokers with periodontitis, compared to nonsmokers with periodontitis and healthy controls. Sedigheh et al., (2012)[20], collaborated this report that cigarette smoke leads to an elevation in salivary superoxide dismutase activity. This is in contrast to our finding. However, another study have shown significantly lower activity of SOD among smokers with chronic periodontitis in blood (Garg et al., 2006)[21]. Furthermore, Hallivell and Gutteridge (1989)[22], result revealed an alteration in mean SOD levels in cigarette smokers as it was decreased in test subjects compared to control group. These studies suggest that increased oxidative stress induced by smoking would have resulted in the depletion and inactivation of SOD caused by increased production of hydrogen peroxide. However, the increased level of SOD found in the studies that affirms that there was an increased SOD activity in blood could be as a result of SOD activity increase, directly after oxidative stress. This elevation of the SOD level in blood and saliva occurs as a protective defense mechanism to scavenge the excessive superoxide radical produced by smoking-induced oxidative stress they claimed.

Furthermore, a contrasting result obtained by Bray and Cockle (1974)[5], suggested that free radicals, particularly hydrogen peroxide are generated by the direct interaction between smoke and tissues that will lead to a significant change in the level of antioxidant enzyme SOD, thereby lowering it. The present study observed a decrease in the enzymatic activity in smokers than nonsmokers which might have been caused by higher levels of hydrogen peroxide formation. Similar trend was also observed in the findings of Naga-Sirisha and Manohar (2013)[23], in which they observed that an initial rate of hydrogen peroxide removal is directly proportional to its concentration.

In the studies undertaken by Altuntaş et al., (2002)[24]; Fabrizia et al., (2006)[25]; Nagaraj et al., (2014)[22]; Adunmo et al., (2015)[27]; Huseyin et al., (2015)[28], serum MDA were significantly higher in smokers when compared to controls. These studies collaborated the finding in this research. However, Miller et al., (1997)[29] contrasted the study by concluding that there was no sufficient evidence to highlight the presence of lipid peroxidation in smokers.

Furthermore, Interval of smoking was compared within the smoking group, it was deduced that year of smoking significantly affected the parameters studied in this research. As the years of smoking increased, the effect on TAC, SOD and MDA, also increased. Altuntaş et al., (2002)[24]; Adunmo et al., (2015)[27], noted that MDA levels were significantly higher in current smokers compared with nonsmokers and that total antioxidant status were significantly lower in current smokers compared with control and that it correlated with duration of smoking. These studies are in agreement with my findings that duration of smoking significantly affected the TAC, SOD and MDA in smoking subjects, as TAC and SOD were decreased, MDA was increased. However, it may not be completely out of place to hypothesize that other factors (like nutrition) could have exacerbated this outcome.

Nagaraj et al., (2014)[26]; Adunmo et al., (2015)[27] concluded that the oxidative stress level was elevated in accordance with the intensity of smoking. The above studies are in contrast with the findings of this study which found no correlation between effects of smoking and the number of sticks smoked by an individual. This finding was however in agreement with the work of Altuntaş et al., (2002)[24], which found no

relationship between lipid peroxidation and the number of cigarettes smoked by an individual. Furthermore, Richard (2007)[30], concluded that the effects noted on the parameters studied (LDL, MDA) was accounted for primarily by the number of years of smoking as opposed to the number of cigarettes smoked per day. For example, within smokers it was noted that the number of years smoking accounted for the greatest variability in MDA. The number of cigarettes smoked per day did not explain a large portion of the variability in any of the dependent variables. This could however be due to the fact that all smokers in this study agreed that they are also passive smokers. For a smoker who claims to smoke less than 5 sticks of cigarettes/day. It is possible that such smoker inhaled more smokes from other smokers during the time such person spent in the place. This assumption is based on the question on questionnaire that asked 'if participants always stay where people smoke?' to which all test group subjects replied 'yes'. In as much as active smoking could induce oxidative stress, so also can passive smoking. Some researcher argued that passive smoking has more adverse effects on the body than active smoking but this claims could not be upheld or refuted by this research as it is beyond the scope of the work. Therefore, passive smoking could be the reason there was no significant effect on the parameters with reference to rate of smoking as those that smoked less compensated by being heavy passive smokers while the heavy smokers are the source of the smoke, the less smokers compensated by passively inhaling the smoke.

CONCLUSION

From this study, it was observed that tobacco smoking could weaken the body antioxidant status, increase the index of lipid peroxidation and significantly affect heavy metal pool concentration of the body. It was also observed that these effects tends to increase as years of smoking increases while the effects of rate of smoking possibly cuts across board as those that smoke less, passively inhale the smoke from other smokers which could make a smoker that smokes fewer sticks of cigarettes to have the same effect as a heavy smoker.

It is probably based on the foregoing that, World Health Organisation and other international agencies has consistently warned against smoking (both active and passive smoking). The research still reiterates the same warning that smoking is bad for the general health of individuals.

Recommendation

It is recommended that smoking should be ban completely as its risks outweighs its gains (which are; for excitements and pleasures). More volunteers are encouraged to join the fight against tobacco menace in the society as many smokers still hold to the belief that cigarettes are not as hazardous as being claimed. To reduce the risk of passive smoking, strict penalties should be handed over to individuals that sell or smoke in public places.

Fabrizia *et al.,* (2006)[31], found out Baseline free malondialdehyde concentrations were significantly higher in smokers than in non-smokers and normalised after 30-day supplementation, it is therefore further recommended that smokers eat foods that are rich in fruits and vegetables to aid in curbing down the negative effects of smoking on health by providing the body enough antioxidant to mop up the high concentration of free radicals and pro-oxidants generated by smoking. This also should be borrowed by passive smokers too. This is because, fruits and vegetables contain mmany phytonutrient compounds, including antioxidants, vitamins, trace elements and fibre. By interacting with biomolecules, they can protect against ROS damage and improve antioxidant status and endothelial functions. Many governments and health organisations encourage people, including smokers, to increase their daily intake of fruit (two to four servings) and vegetables (three to five servings)[31].

Furthermore, more research may need to be conducted in this area; research that will follow up smokers with the aim of deducing how often smokers around Nnewi metropolis come down with cancers so as to enlighten the public the more. Also roles of smoking on the epigenetic inheritance could be another area of research that would throw more light on the molecular effects of smoking on the body with special emphasis on chronic disease development and tumourgenesis.

The limitation of the study

The limitation of this study is that the smoking status was recorded by the self-report of the study participants. However, the estimation by serum cotinine assay would be more reliable for the evaluation of the smoking status of an individual.

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