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EFFECT OF EXPOSURE TO SOOT ON TROPONIN I AND LIPID PROFILE LEVELS

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ABSTRACT
Exposure to soot, to some extent can lead to cardiovascular disease. This study was undertaken to determine serum Troponin I and lipid profile levels of cooks exposed to soot. 50 subjects (cooks) exposed to soot (test group) and 50 people not exposed to soot were recruited. Ethical approval was obtained from the Ethics committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University and informed consents of the subjects were obtained. Troponin I was assayed using Enzyme-linked immunosorbent assay (ELISA). Total Cholesterol levels were determined using cholesterol enzymatic end-point method, Low density lipoprotein cholesterol (LDL-C)levels were determined using clearance method while Spectrophotometric and Enzymatic Methods were employed for the High Density Lipoprotein Cholesterol (HDL-C) and Triglyceride while the BMI (Body mass index) was calculated using weight (kg)/height^2 (m^2). The results showed that the mean values of the serum troponin I level of cooks exposed to soot (test group) was significantly higher when compared with the control (4.29 ± 3.01) ($P<0.05$). The mean values of total cholesterol, LDL-C, Triglycerides and the BMI (10.29±3.37), (3.37±1.36), (1.43±0.52), (26.53±4.98) respectively were significantly higher in test group when compared with the control (8.92±6.11), (0.98±0.69), (0.95±0.51), (22.62±5.58) ($p<0.05$) while there was no significant decrease in HDL-C level in test group (2.74±0.68) when compared with the control (3.03±0.73) ($p>0.05$). This study showed an increased level of Troponin I, total cholesterol, Triglycerides, low density lipoprotein cholesterol and reduced level of High Density Lipoprotein among cooks exposed to soot, this suggests a predisposition to cardiovascular disorder.

KEYWORDS: Soot, Lipid profile, Troponin I, Cardiovascular disease.

INTRODUCTION
Soot is an impure carbon resulting from incomplete combustion of hydrocarbons and can be gotten from the burning wood, coal and exhaust fumes. It is a powder-like form of amorphous carbon. It contains polycyclic aromatic hydrocarbons known as mutagens.[1] This powdery brown or black dust sticks to the inside of chimneys (sometimes escaping into the air) and carries a few risks factors such as lung hazard and respiratory tract disease. Wood smoke contains sulphur oxide, nitrogen oxide, carbon monoxide, and potentially carcinogens such as dioxin, formaldehyde, and beno(a)pyrene a cause of human cancer.[2]

Soot, as an air borne contaminant in the environment has different sources, they include soot from coal burning, internal combustion engines, power plain boilers, hog-fuel boilers, ship boilers, central steam heat boiler, waste incineration, local field burning, forest fires, fire places, furnaces, etc. external sources such as smoking of plant matter, cooking, oil lamps, candles, halogen bulbs with settled dust, fire places, defective furnaces, etc.

Also, occupational exposures to soot from diesel exhaust have been linked with acute short-term symptoms such as headache, dizziness, light-headedness, nausea, coughing, difficult or labored breathing, tightness of chest, and irritation of the eyes and nose and throat. Long-term exposures could lead to chronic, more serious health problems such as cardiovascular disease, cardiopulmonary disease, and

lung cancer[3] Soot has been theorized to be the second largest cause of global warming.[4, 5]

Soot reduces high density lipoprotein and is directly responsible for about 20% of all deaths from heart disease, stroke and cancer. Soot has been quoted to cause coronary heart disease.[6] In several human experimental studies using a well validated exposure chamber setup diesel exhaust has been linked to acute vascular dysfunction and increased thrombus (blood clot) formation.

Moreover, high cholesterol levels have been associated to cardiovascular disease. Cholesterol is a lipid that is the principal sterol of all higher animals, distributed in body tissues, especially the brain and spinal cord, and in animal fats oil.[7] Total cholesterol is defined as the sum of High density lipoprotein (HDL), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL). High cholesterol levels are dangerous, and are cause of heart disease and other ailments.[8,9] Longstanding elevated serum cholesterol contributes to the elevated formation of Atherosclerosis.[10] Over a period of decades, chronically elevated serum cholesterol contributes to the formation of atheromatous plagues in the arteries. This can lead to progressive stenosis (narrowing) or even complete occlusion (blockage) of the involved arteries. Alternatively smaller plagues may rupture and cause a clot to form and obstruct blood flow. A sudden occlusion of a coronary artery results in a myocardial infarction or heart attack. An occlusion of the artery supplying the brain causes a stroke.

Troponin is the inhibitory or contractile regulating protein complex of striated muscle. It is located periodically along the thin filament of the muscle and consists of three distinct proteins: troponin I, troponin C, and troponin T. The troponin I subunit exists in three separate isoforms; two in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. The cardiac isoform (cTnl) is about 40% dissimilar. Antibodies made against this cardiac isoform are immunologically different from antibodies made against the other two skeletal isoforms, and the unique isoform and tissue specificity of cardiac troponin I is the basis for its use as an aid in the diagnosis of acute myocardial infarction (AMI). The cardiac troponins exhibit myocardial tissue specificity and high sensitivity.

Therefore, this research was therefore designed to find out the effect of exposure to soot on lipid profile and Troponin I.

MATERIALS AND METHOD

Study area

The study was carried out in Nnewi, Nnewi North Local Government area of Anambra State, South Eastern, Nigeria. Nnewi is a commercial city with a large auto spare parts market and the residents are mainly business men and women as well as Health workers (Doctors, Medical Laboratory Scientists, Nurses, Imaging Scientists, Pharmacists, Physiotherapists etc.) who work at the Nnamdi Azikiwe University Teaching Hospital and other private and mission hospitals located in the town.

Study design

The study was a case-control study designed to assess the Troponin I and lipid profile levels of subjects exposed to soot.

Study Population

This study was carried out amongst cooks (bean cake fryers, and palm fruit cooks) exposed to soot in Nnewi, Anambra state. Hundred subjects were recruited as follows;

- Fifty (50) participants exposed to soot.
- Fifty (50) participants not exposed to soot as controls.

Ethical Consideration

The approval for this study was obtained from Ethics committee of Faculty of Health Science and Technology Nnamdi Azikiwe University and informed consent was obtained from each participant before the commencement of the study.

Inclusion criteria

Apparently healthy Male and female subjects exposed to soot as well as apparently healthy subjects not exposed to soot as controls.

Exclusion criteria

Smokers, hypertensive subjects on antihypertensive drugs, obese subjects, and subjects that refused to give their consent were excluded from the study.

Procedure for sample collection

Five milliliters (5ml) of blood was collected from the vein of each participant into a plain tube and allowed to clot. The serum was separated for the analysis of Troponin I and Lipid profile.

Laboratory method and procedure

All the reagents were commercially purchased and manufacturers' standard operating procedures (SOP) were strictly followed. Spectrophotometer 21D USA was used for the analysis.

Estimation of Troponin I level

The desired numbers of coated wells were secured in the holder. Hundred microliter (100µl) of standard, specimens and controls were dispensed into appropriate wells. Hundred microliter (100µl) of enzyme conjugate reagent was dispensed into each well. The wells were thoroughly mixed for 30seconds. It was then incubated at room temperature for 90 minutes. Microtitre wells were rinsed and flicked 5

times with distilled water. Hundred microliter (100µl) of TMB reagent was dispensed into each well and gently mixed for 5 seconds and incubated at room temperature for 20 minutes. The reaction was stopped by adding 100µl of stop solution to each well and mixed for 30 seconds. Absorbance was read at 450nm with a microtitre plate reader within 15 minutes.

Estimation of total cholesterol (TC)
Total cholesterol was determined using the method as described by Roeschlau *et al.*[11] This is essentially an enzymatic end point method.

Procedure: About 10µl of distilled water, cholesterol standard and serum were pipetted into the tubes labelled reagent blank, standard and sample respectively and 1000µl of reagent pipetted into each of the tubes. The tubes were mixed and incubated for 5minutes at 37°C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank within 60minutes at 500nm wavelength.

Calculation
TC concentration (mmol/l) =

$$\frac{A_{sample} \text{ x concentration of standard (5.09mmol/l)}}{A_{standard}}$$

Estimation of triglycerides
The enzymatic method as described by Tietz[12] was adopted in the estimation of triglycerides.

Procedure for triglycerides estimation
About 10µl of distilled water, triglyceride standard and serum were pipetted into the tubes labelled blank, standard and sample respectively. And 1000µl of reagent pipetted into each of the tubes which were mixed and incubated at 37°C for 5minutes. The absorbance of sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 60minutes at 500nm wavelength.

Calculation
Triglyceride concentration (mmol/l) =

$$\frac{A_{sample} \text{ x } C_{Standard} \text{ (2.19mmol/l)}}{A_{standard}}$$

Estimation of high density lipoprotein cholesterol (HDL- C)
The estimation of HDL was performed using the method as described by Burstein *et al.*[13] This is principally a combination of phosphotungstate precipitation and enzymatic method.

Procedure for HDL estimation
Stage 1: precipitation
About 0.2ml of the subject's sample was pipetted into the respective tubes and 0.5ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly mixed and allowed to stand for 10minutes. Centrifugation was done at 4000rpm for 10minutes and the supernatant was then carefully collected.

Stage 2: Colorimetry
About 50µl of HDL-cholesterol standard, sample supernatant and distilled water was pipetted into the tubes labelled standard, sample and reagent blank respectively and 1.0ml of reagent (B) pipetted into each of the tubes. The tubes were thoroughly mixed and incubated for 10minutes at 37°C. The absorbance of the standard and sample was measured at 500nm against the blank.

Calculations
Concentration of HDL cholesterol (mmol/l)=

$$\frac{A_{sample} \text{ x } C_{Standard} \text{ (1.36 mmol/l)}}{A_{standard}}$$

Estimation of low density lipoprotein cholesterol (LDL-C)
The method of Assman *et al.*[14] was adopted. This is a combination of polyvinyl sulphate precipitation and enzymatic method.

Procedure of LDL estimation
Stage 1: precipitation
About 0.2ml of subjects' sample was pipetted into the respective tubes and 0.2ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly mixed and allowed to stand for 15minutes at room temperature. Centrifugation was done at 4000rpm for 15minutes and the supernatant was then carefully collected.

Stage 2: colorimetry
About 20µl of distilled water, cholesterol standard and sample supernatant was pipetted into the tubes labelled reagent blank, standard and sample respectively and 1.0ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly mixed and incubated for 10minutes at 37°C. The absorbance of the standard and sample was measured at 500nm against the blank.

Calculations
The cholesterol concentration in the supernatant ($C_{Supernatant}$) in mmol/l was calculated using the following general formula.

$$\frac{A_{Sample} \text{ x } C_{standard} \text{ (5.18mmol/l)}}{A_{Standard}} = C_{Supernatant}$$

The LDL cholesterol (mmol/l) in the sample was calculated as follows.
LDL cholesterol concentration = Total cholesterol – cholesterol in supernatant.

Statistical analysis
Statistical package for social sciences (SPSS) (version 20.0 for windows, SPSS Inc. Chicago, USA) was used

to analyze the data. Data were expressed as Mean ± SD. The differences in parameters studied between the test and control groups were evaluated using student t-test. Statistical significance was set at p-value ≤ 0.05

RESULTS

Table 1 shows that the mean values of serum total cholesterol, LDL-C, and Triglyceride of the subjects exposed to soot were significantly higher when compared to the normal control group (P<0.05) while there was no significant difference on the mean values of serum High Density Lipoprotein of the subject exposed to soot when compared to the controls (P>0.05).

Table 2 shows that mean values of Troponin I and BMI were significantly higher in subjects exposed to soot when compared compared to the controls (P<0.05)

Table 1: Comparison of the mean values of serum lipid profile of test and control group.

Parameter	Test group N=50	Control group N=50	t-value	P-value
Cholesterol (mmol/L)	10.29±3.37	8.92±6.11	1.313	0.000*
LDL (mmol/L)	3.37±1.36	0.98±0.69	10.496	0.000*
TG (mmol/L)	1.43±0.52	0.95±0.51	-4.333	0.000*
HDL (mmol/L)	2.74±0.68	3.03±0.73	1.954	0.054

*Key: * Statistically significant at p<0.05`*

Table 2: Comparison of the mean values of serum troponin I and body mass index (BMI) of test and control group.

Parameters	Test group N=50	Control group N=50	t-value	p-value
Troponin I	4.29 ± 3.01	6.31 ± 8.45	1.054	0.014*
BMI (kg/m^2)	22.16 ± 7.16	26.56 ± 4.83	2.390	0.021*

- *Significant at P<0.05*

DISCUSSION

Lipid profile, also called Lipid Panel, Coronary Risk Panel or Complete Cholesterol test is mainly used to assess the risks of developing cardiovascular diseases and also to monitor management of the afflicted.[15] Lipid profile can also be used to assess the effects of environmental contaminants on the health and well-being of persons exposed to such pollutants.[16]

This study revealed that the mean serum concentrations of total cholesterol (TC), triglyceride and low density lipoprotein cholesterol were significantly increased (p<0.05) in subjects exposed to soot when compared to normal control subjects. This is consistent with the findings of Egwurugwu *et al.* (2013)[16] that discovered an increased level of total cholesterol, triglyceride and low density cholesterol among subjects exposed to soot as a result of gas flaring compared to the control subjects. This also correlates with a previous study[17] which findings showed a higher level of serum cholesterol and low density lipoprotein cholesterol (LDL-C) in experimental subjects when compared to the control. Increased serum cholesterol and Low Density Lipoprotein cholesterol levels indicated in the result may be due to the harmful effects of the constituents of soot. LDL-cholesterol is referred to as "bad cholesterol" because it carries cholesterol and phospholipids from the liver to the peripheral tissues and organs like the heart. It is responsible for the deposition of cholesterol on the walls of arteries causing atherosclerosis. The observed increase in serum LDL-cholesterol in the test group subjects may result from the impairment in the receptor-mediated endocytosis which prevents the binding of LDL to specific receptors that could lead to its degradation and release of cholesterol.[18] LDL receptors are present in all cells but most abundant in hepatic cells and adrenal cortex. The liver has a major role in the control of plasma levels of LDL cholesterol because most of the LDL receptors are present in the liver which also synthesizes cholesterol and removes cholesterol from lipoprotein remnants.[19] Hyperlipidemia constitutes a major etiopathological factor for atherosclerosis.[20] It has been established that total plasma cholesterol and LDL-Cholesterol are the best markers of plasma lipemia for the evaluation of cardiovascular diseases (CVD).[21] Similarly, the increase in total cholesterol in the test group may have resulted from the damage to the hepatic cells by the toxic pollutants in soot.

High Density Lipoprotein was non-significantly higher in the control than the exposed subjects. This is also in line with the findings of Egwurugwu *et al* (2013).[16] HDL is called "good cholesterol" because it carries cholesterol and phospholipids from tissues and organs back to the liver for degradation and elimination. Thus, it prevents the deposition of cholesterol on the walls of arteries by carrying cholesterol away from arteries to liver. High level of HDL is a good indicator of a healthy heart because it reduces the blood cholesterol level.[22, 23]

Studies have demonstrated a correlation between environmental pollution and the development of cardiovascular disease (CVD).[24] Our findings support the fact that subjects exposed to soot are more

predisposed to cardiovascular risk because according to[25] those with higher level of HDL-C tend to have fewer problems with cardiovascular diseases while those with low HDL-C level have increase rate for heart diseases.

This study showed a significant high mean level of Troponin I on cooks using firewood exposed to soot. This is in consonance with a previous finding of[10] which states that long term exposure to urban air pollution containing soot increases the risk of coronary heart disease, thereby increasing the level of troponin in the blood. This may be because Troponin I is found in heart muscle cells, hence when the cells of the heart muscle are injured by the accumulation of the inhaled soot, it will cause troponin to be released into the general circulation, thus the reason for the high troponin I on cooks using firewood exposed to soot compared to control.

CONCLUSION

The findings from this present study show that cardiovascular risk factors like increased LDL, increased total cholesterol and reduced HDL were significantly higher in subjects exposed to soot when compared to the control subjects. Troponin I which is a marker of cardiac muscle damage was also increased in subjects exposed to soot. Therefore, this research has shown that continuous exposure and inhalation of soot tend to have an adverse effect on cardiovascular health.

RECOMMENDATIONS

It is recommended that lipid profile be checked frequently in people exposed to soot. Cooks should also be encouraged to use protective covering such as nose masks as well as ensure good ventilation when cooking in order to reduce soot inhalation. These may help in the prevention of cardiovascular disorders and other pathological conditions associated with alterations in lipid profile in people exposed to soot.

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