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Research Article

Evaluation of Interleukin 4, High Sensitivity C-Reactive Protein and Activity of Alanine Transaminase Among Cooks in Nnewi

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Abstract



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Soot arises from burning wood, oils, tires, and other hydrocarbon or organic compounds. Soot is widespread and has a massive detrimental effect on human health, climate, and air quality. The serum levels of Interleukin 4, High sensitivity C-reactive protein, and alanine transaminase activity were evaluated. This cross-sectional study recruited 90 participants consisting of 45 participants exposed to soot (test group) and 45 participants not exposed to soot (control group). The Faculty of Health Sciences and Technology Ethics Committee at Nnamdi Azikiwe University granted ethical approval, and participants' informed consent was acquired. Interleukin 4 and high sensitivity C-reactive protein levels were determined using the Sandwich ELISA method while the activity of alanine transaminase was determined using the spectrophotometric method. Questionnaires were used to obtain the socio-demographic data of the participants and the body mass index (BMI) was calculated using weight (kg)/height² (m²). Independent t-test and Pearson's correlation coefficient were used for statistical analysis and significance was set at p<0.05. The results showed that the mean serum activity of alanine transaminase (9.30 ± 0.28) was significantly lower in the participants exposed to soot compared with the control (9.48 ± 0.37) (p<0.05). However, there was no significant difference in the mean serum levels of Interleukin 4 and high sensitivity C-reactive protein of participants exposed to soot compared with the control (p>0.05). Hence, exposure to soot through the use of firewood did not predispose cooks to systemic inflammation, or cardiovascular and hepatic dysfunctions.

Keywords: Soot, alanine transaminase, Interleukin 4, high sensitivity C-reactive protein, inflammation

INTRODUCTION

Soot is the byproduct of incomplete combustion of organic compounds ¹. Soot refers to carbonaceous particles produced by the incomplete or pyrolytic burning of hydrocarbon fuels ². Both human-induced (such as traffic, household heating, and industrial) and naturally occurring (such as wildfire) combustion processes emit soot, or its associated substances black carbon (BC) and elemental carbon (EC) ³. Around the world, soot is recognized as a major air pollutant with Africa ranking high among the highest emitters ¹. Global warming is caused by soot emissions into the atmosphere ⁴. It is believed that long-lived greenhouse gases (GHGs) and soot particulate matter (PM), which is mostly caused by incomplete engine combustion, are the two main climate change forcing agents ⁵. The tiny size of soot particles—which are also found in PM10 and PM2.5 dust—allows them to pass through the body's protective barrier and enter not just the lungs but occasionally the skin as well ⁶. Soot particles easily penetrate the body

tissues causing respiratory diseases, like asthma, skin disorders, reproductive problems, and cardiovascular diseases ^{7,1}. The primary organic substance in soot, polycyclic aromatic hydrocarbons (PAHs), has also been linked to mutagenic and carcinogenic consequences ². Several past studies have described soot as a carcinogen and linked it to different kinds of cancer ^{8,9,10}. PM2.5 is known to cause oxidative stress and inflammation in the respiratory tract, which aggravates long-term chronic diseases like cancer and asthma as well as respiratory and cardiovascular problems ¹¹. Many chronic and acute disorders, including chronic respiratory conditions, heart disease, hypertension, and ischemic stroke, are strongly correlated with long-term exposure to particulate matter (PM) ¹². Also, ambient PM2.5 exposure may be a substantial risk factor for the progression of non-alcoholic fatty liver disease (NAFLD) ¹³, which has been linked to an increase in the prevalence and mortality of liver cancer. Research claimed that there is a higher death rate from ischemic heart disease among chimney sweeps as well as a higher incidence of

myocardial infarction¹⁴. Also, by starting and fostering the formation of atherosclerosis, the primary cause of most cardiovascular disorders, exposure to soot particulate matter increases the chance of developing cardiovascular disease¹⁵. In a Swedish retrospective cohort research, working as a chimney sweep was linked to a higher chance of developing liver cancer¹⁶. It was observed in a study that PM_{2.5} caused hepatic fibrosis in a mouse model and had a direct negative impact on the liver's health¹⁷. Long-term exposure to ambient particulate matter has been linked to an increased risk of metabolic dysfunction-associated fatty liver disease (NAFLD), according to a study conducted in southwest China¹⁸. Also, according to a study in the Niger Delta region of Nigeria, the serum activities of liver enzymes (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) were significantly increased in women exposed to air pollution caused by gas flaring¹⁹.

Interleukin 4 (IL-4) is a pleiotropic cytokine that regulates immunological responses linked to lymphocyte development and survival factors²⁰. As a pleiotropic cytokine, interleukin-4 (IL-4) is primarily recognized for its function in type 2 immunity²¹, which guarantees a number of host-protective functions, including wound healing and helminth parasite resistance²². During allergic reactions, type 2 T helper (Th2) cells secrete the important cytokine interleukin-4 (IL-4), which directs the immune system's response²³. The approximately 10 kb-sized IL4 gene, which codes for interleukin 4(IL-4), has four exons and is located on chromosome 5q31.1²⁴. IL-4 induces the immunoglobulin (Ig) class switching to IgG1 and IgE in B cells, differentiates naive CD4 T cells into Th2 cells in T cells, and triggers alternative macrophage activation in macrophages²⁵. C reactive protein, a homopentameric acute phase inflammatory protein was first discovered in 1930 when Tillet and Francis studied sera of patients with the acute stage of pneumococcus infection. It was named c reactive protein due to its reaction with the capsular(c)-polysaccharide of pneumococcus²⁶. Hepatocytes produce the acute phase protein known as C reactive protein (CRP) in response to pro-inflammatory cytokines during inflammatory and infectious processes²⁷. CRP levels are elevated in a variety of inflammatory disorders, and its serum concentration is commonly measured to gauge the severity of systemic inflammation. High-sensitivity CRP assays (hs-CRP) have been developed recently for sensitive CRP quantification. These assays are particularly valuable because they may indicate the presence of low-grade inflammatory lesions as they can detect little fluctuations in serum CRP levels within the range of normal limits²⁸. Hs-CRP is employed as a predictive marker of the likelihood of developing heart disease and stroke²⁹. Also, hsCRP testing is used to determine the prognosis of coronary stent thrombosis, as well as cardiac failure, atrial fibrillation, hypertension, and valve dysfunction³⁰. The liver's gluconeogenesis and cellular nitrogen metabolism are both aided by the enzyme alanine transaminase (ALT), which catalyzes the conversion of alanine and α -ketoglutarate to pyruvate and glutamate³¹. The coenzyme pyridoxal 5'-phosphate, often known as vitamin B6, is required for this reaction³². Since ALT is mostly found in the cytoplasm of hepatocytes, an increase in its concentration in the serum indicates that the membranes of the liver cells had been damaged³³. Many people are exposed to soot daily through their occupation without any knowledge of what such exposures could mean to their health. This research evaluated the effect of soot exposure on cooks to validate the possible health effects.

MATERIALS AND METHODS

All the reagents were commercially obtained and the manufacturer's Standard operation procedures (SOP) were strictly adhered to. This study was carried out in Nnewi, Nnewi North local government area of Anambra State, in the South-East region of Nigeria.

Study Participants

G* Power software version 3.0.10 (Universitat Dusseldorf, Germany) was used to determine the sample size. Using an alpha of 0.05, a power of 0.80, and a medium effect size of 0.4, the power to identify an appropriate sample size was calculated. Based on these, the estimated sample size of 80 can detect differences of 0.4 at a 0.05 level of significance with 80% accuracy. A total sample size of 90 was employed for this study to account for potential attrition. Ninety participants within the age range of 12 – 60 years were enlisted and the participants were divided into two groups as follows; Group one (1) comprising of forty-five (45) participants who were exposed to soot (the test group). Group two (2) comprising of forty-five (45) participants who were not exposed to soot (the control group). A questionnaire to determine the socio-demographic information of the participants and frequency of exposure to soot was used. The ethical approval for this research was obtained from the ethics committee of the Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus with the protocol number: FHST/REC/023/00252. Consent for the study was sought from and signed by the participants before the study. Participants residing outside Nnewi, those who were smokers or had chronic diseases, those outside the age range of 12-60, and those who were not willing to be part of the study were excluded. Five milliliters (5ml) of venous blood was aseptically drawn from each participant's ante-cubital vein using a plastic syringe and dispensed in a plain tube. Following centrifugation at 4000rpm for 5 minutes, the serum was obtained for the assessment of interleukin 4, High sensitivity C reactive protein, and activity of alanine transaminase. The samples were kept at a temperature of -20°C before the biochemical examination.

Statistical analysis

Statistical Package for Social Science (SPSS) (version 26.0) for Windows, SPSS Inc. Chicago, USA, was used to analyze the data. Data was expressed as Mean \pm standard deviation (SD). The differences in parameters studied between the test and the control groups were evaluated using an independent t-test and Pearson's correlation coefficient to determine the association between the serum levels of Interleukin 4, High sensitivity C-reactive protein, and the activity of alanine transaminase with BMI, Systolic blood pressure and Diastolic blood pressure. Statistical significance was set at p-value < 0.05.

RESULTS

Table 1. The mean values of the BMI and blood pressure of participants exposed to soot (Test Group) and participants not exposed to soot (Control Group) (Mean \pm SD).

The mean value of BMI (24.80 \pm 4.12) was significantly higher in participants exposed to soot when compared to participants not exposed to soot (22.18 \pm 2.72) (p<0.05). However, there was no significant difference in the mean value of blood pressure of participants exposed to soot compared to participants not exposed to soot (p>0.05).

Table 1. The mean values of the BMI and blood pressure of participants exposed to soot (Test Group) and participants not exposed to soot (Control Group) (Mean ± SD).

Body mass index (BMI) and blood pressure	Test Group (n=45)	Control Group (n=45)	t - Value	p - Value
BMI(kg/m ²)	24.80 ± 4.12	22.18 ± 2.72	3.561	0.005
SBP(mmHg)	119.33 ± 5.62	119.33 ± 5.62	1.374	0.326
DBP(mmHg)	77.84 ± 5.274	77.47 ± 5.11	0.345	0.668

*Statistically significant at P<0.05

BMI= Body Mass Index, SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure

Table 2. The mean serum levels of Interleukin 4, High Sensitivity C-Reactive Protein and Activity of Alanine Transaminase of participants exposed and not exposed to soot (Mean ± SD).

The mean serum activity of alanine transaminase (9.30 ± 0.28) was significantly lower in the participants exposed to soot

when compared to participants not exposed to soot (9.48 ± 0.37) (p<0.05). However, there was no significant difference in the mean serum levels of Interleukin 4 and high sensitivity C-reactive protein of participants exposed to soot compared to participants not exposed to soot (p>0.05).

Table 2: The mean serum levels of Interleukin 4, High Sensitivity C-Reactive Protein and Alanine Transaminase of participants exposed and not exposed to soot (Mean ± SD).

Parameters	Test Group (n=45)	Control Group (n=45)	t - Value	p - Value
IL-4(pg/ml)	5.77 ± 1.10	5.83 ± 3.06	-0.121	0.904
Hs-CRP(mg/l)	3.56 ± 0.85	3.48 ± 1.20	0.349	0.728
ALT(IU/L)	9.30 ± 0.28	9.48 ± 0.37	-2.534	0.013

*Statistically significant at P<0.05

IL-4= Interleukin 4, Hs-CRP= High Sensitivity C-Reactive Protein, ALT= Alanine Transaminase

Table 3. Correlation of the serum levels of Interleukin 4, High sensitivity C-reactive protein, and Activity of Alanine Transaminase with the BMI, Systolic blood pressure, and Diastolic blood pressure of participants exposed to soot.

There was no correlation observed between the serum levels of Interleukin 4 and high sensitivity C-reactive protein and activity of alanine transaminase and the BMI, systolic blood pressure and diastolic blood pressure in participants exposed to soot.

Parameters		BMI	SBP	DBP
IL - 4(pg/ml)	r - Value	-0.116	-0.042	-0.157
	P - Value	0.448	0.786	0.302
Hs - CRP(mg/l)	r - Value	-0.052	-0.073	-0.157
	P - Value	0.735	0.636	0.423
ALT(IU/L)	r - Value	0.278	0.051	0.109
	P - Value	0.064	0.737	0.478

*Statistically significant at P<0.05

IL-4= Interleukin 4, Hs-CRP= High Sensitivity C-Reactive Protein, ALT= Alanine Transaminase

DISCUSSION

The majority of chefs are unaware of how exposure to soot or particulate matter (PM) might negatively impact their health. Unfortunately, most homes and kitchens in Nigeria, particularly in Nnewi, lack chimneys or pipes that discharge kitchen smoke into the atmosphere, which increases the concentration of soot and makes it easier for cooks to inhale³⁴. There was no significant difference in the blood pressure of cooks exposed to soot compared with the control (p>0.05). This was expected since every participant was in good health. However, a significantly higher difference was seen in the mean value of BMI of cooks exposed to soot compared to the control. This could be because the participants were not height and weight-matched. A non-significant difference was

observed in the mean serum level of interleukin 4 in participants exposed to soot compared to the control (p>0.05). This is in contrast with the findings of³⁵ which found a significant difference in IL-4 levels in chronic obstructive pulmonary disease (COPD) patients exposed to air pollution compared to control. Additionally,³⁶ observed a significant difference in the level of Interleukin 4 in Allergic Asthma (AA) patients exposed to allergic substances compared to the control. The difference in the results could be a result of the difference in the study design used for the studies.

A nonsignificant difference in the mean serum level of high sensitivity C-reactive protein (Hs-CRP) in cooks exposed to soot compared to control (p<0.05) was observed in this research study. This result is consistent with the study by³⁷,

which reported no significant difference in the level of C-reactive protein in chimney sweeps exposed to polycyclic aromatic hydrocarbons (PAH) through soot. Additionally, a non-significant association between C-reactive protein and long-term air pollution was seen in a German study on C-reactive protein and long-term air pollution³⁸. However, this study result varies from that of³⁹ which found the level of environmental carbon monoxide to be significantly associated with high sensitivity C-reactive protein in Peritoneal dialysis patients. An older Chinese adult cohort study by⁴⁰ also found a significant association of evaluated air pollutants with elevations in high-sensitivity C-reactive protein. Additionally,⁴¹ reported a significant difference in the mean serum level of high-sensitivity C-reactive protein (hs-CRP) in vehicle inspectors exposed to particulate matter compared to the control. The contrasts observed in the works could be because of the difference in the study design, method of analysis, statistical approach, confounding variables, and number of participants involved in the studies.

A significantly lower difference in the activity of alanine transaminase was seen in participants exposed to soot compared to the control. The statistical significance of alanine transaminase in this study did not infer clinical significance since the mean serum activity in the participants remained within the reference range. This result differs from the findings of⁴² where there was a significantly higher difference in the mean serum activity of ALT in participants exposed to air pollution in areas near oil drilling sites since birth compared with control. In a study to determine the serum activities of liver enzymes in cooks exposed to soot,³⁴ found the serum activity of alanine transaminase to be significantly higher in the test group compared to control. Additionally,⁴³ in a Taiwanese population study saw an association between long-term exposure to particulate matter and an increase in the activities of liver enzymes, including alanine transaminase. In an animal study to investigate the health impacts of chronic exposure to urban air on the liver, heart, and serum risk biomarkers,⁴⁴ reported a significant increase in the activities of liver enzymes in mice exposed to urban air compared to control.

This research observed no association between the levels of Interleukin 4, High sensitivity C-reactive protein, and activity of alanine transaminase and the body mass index, systolic blood pressure, and diastolic blood pressure of cooks exposed to soot. This agrees with the work of³⁴ on the effect of exposure to soot on the activities of liver enzymes, which observed no relationship between the activity of alanine transaminase and the BMI, systolic blood pressure, and diastolic blood pressure. No statistically significant correlation was seen between BMI and interleukin 4 (IL-4)⁴⁵. Also, a study to evaluate serum high-sensitivity C-reactive protein levels, as a marker of inflammation, in a large sample of the Iranian population without a history of cardiovascular or inflammatory disease and cancer, found no significant independent correlation between hs-CRP and blood pressure⁴⁶.

CONCLUSION

The levels of Interleukin 4 and high sensitivity C-reactive protein did not change significantly, according to this study. Although it did not necessarily imply any clinical importance, a significantly lower difference in alanine transaminase activity was also discovered. Consequently, in this study, it is possible that soot exposure does not increase the risk of developing systemic inflammation, cardiovascular disease, or liver disease.

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Conflicts of Interest:

None declared

Author contributions:

ACI, JCI, and PCO

conceived and designed the research proposal. CEO and ON performed sample

collection, experiments, and data analysis. ACI, ECA, and AJC contributed to

the final version of the manuscript. All authors have read and approved the

final manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical approval:

The study was approved by the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria FHST/REC/023/00252

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