DOI: https://dx.doi.org/10.4314/gjpas.v29i1.7

GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 29, 2023: 55-62
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# EVALUATION OF SERUM COTININE, TOTAL PROTEIN, THIOCYANATE, NEURON SPECIFIC ENOLASE AND URIC ACID LEVELS OF CIGARETTE SMOKERS IN CALABAR METROPOLIS, NIGERIA: A CROSS SECTIONAL STUDY

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(Received 15 February 2023; Revision Accepted 13 March 2023)

## ABSTRACT

Cigarette smoke contains harmful chemicals with deleterious health effects leading to oxidative stress and neuronal damage. This study investigated the levels of serum cotinine (COT), thiocyanate (thioc), uric acid (UA), neuron specific enolase (NSE), and the association between these variables in cigarette smokers. This cross sectional study enrolled 60 cigarette smokers and 45 non-smokers as participants. Serum thiocyanate and UA were estimated by colorimetric methods. Serum cotinine and NSE were determined by ELISA methods. Height and weight were measured and BMI computed. Data were analyzed using Student's t-test, ANOVA, and Pearson's correlation at P<0.05. Serum cotinine, thioc and NSE concentrations were significantly higher while BMI and UA levels were significantly lower (P<0.05) in smokers than in the controls. Age was not significantly different between the two groups (P>0.05). Age, COT, UA, thioc and NSE vary significantly (P<0.05) between the light, moderate and heavy cigarette smokers. From the least significant difference post hoc analyses, the mean age and UA level of light smoker were significantly higher (P<0.05) compared with the moderate smokers, while COT level of moderate smokers was significantly higher (P<0.05) compared with light smokers. Neuron specific enolase level of moderate cigarette smokers was higher (P>0.05) compared with the light smokers. The mean age of light smokers was significantly higher (P<0.05) compared with the heavy smokers, while the COT, thioc and NSE levels of light smokers were significantly lower (P<0.05) compared with the heavy smokers. Serum UA, thioc and NSE levels of moderate smokers were significantly lower (P<0.05), than those of the heavy smokers. Significant negative correlations were observed between age and COT (r = -0.554, P<0.001), age and thioc (r = -0.421, P= 0.001), age and NSE (r = -0.346, P=0.001), age against smoking pack years (r = -0.623, P<0.001) respectively. Significant positive correlations were observed between serum thioc and NSE (r = 0.324, P=0.012), COT against smoking pack years (r = 0.399, P=0.002), and NSE against smoking pack years (r = 0.311, P=0.015), correspondingly. This study has shown that high levels of cotinine and thiocyanate are associated with increased levels of neuron specific enolase, smoking pack years and decreased uric acid in smokers.

**KEYWORDS:** Smoking, Cotinine, Thiocyanate, Uric acid, neuron specific enolase.

## INTRODUCTION

Cigarette smoking remains one of the greatest public health problems worldwide, resulting in a single most preventable cause of many tobacco-linked diseases and deaths. It is a major cause of preventable high burden of lung cancer, chronic obstructive pulmonary disease (COPD), ischemic heart diseases, stroke, diabetes, and hypertension (Loretan, et al., 2022; Perez-Warnisher, et al., 2018). Tobacco was responsible for 8.71 million deaths and 229.77 million disability-

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adjusted life years (DALYs) for all-cause disease globally in 2019, (He, et al., 2022), and is the third leading risk factor for attributable disability-adjusted life-years (DALYs) among level 2 risks defined by the Global Burden of Disease Study, (Zhang, et al., 2022). It has been projected that by 2030, tobacco smoking will kill over eight million people each year (Todorovic, et al., 2022). Severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) emergence in the last two years exemplifies a grave threat to global health and the pandemic may inspire public perception of the health risks of tobacco use (Szymański, et al., 2022; Baker, et al., 2022). Cigarette smoke contains over 4,000 different compounds both particle and gaseous phases including carcinogens such as nicotine (non-carcinogenic), Hydrogen cyanide, formaldehyde, Lead, Cadmium, Arsenic, Ammonia, Radioactive elements, such as polonium-210, Carbon monoxide, Tobacco-specific nitrosamines (TSNAs), Polycyclic aromatic hydrocarbons (PAHs), and Benzene (Soleimani, et al., 2022). Hydrogen Cyanide in the smoke formed during the incomplete combustion of nitrogen-containing components of cigarette is quickly transformed through a variety of chemical reactions, normally involving sulfur donors, to form more stable chemical species. Depending on the nature of the sulfur donor, cyanide may be transformed into free thiocyanate (the main metabolite and detoxification product of cyanide transformation), 2-amino-2-thiazoline-4-carboxylic acid or protein-bound thiocyanate (PB-SCN) adducts (Youso et al., 2012).

Nicotine is the key addictive component of tobacco which is metabolized to cotinine, a stable product with a half-life of 18 hours to 24 hours. Cotinine can be used as the biomarker for cigarette smoke exposure. Nicotine is not a direct cause of most tobacco-related diseases, but it is highly addictive [Hukkanan et al., 2005]. The key metabolites of nicotine which have been fully defined, cotinine (70%) and nicotine N-oxide (4%) are formed via the two main routes for nicotine metabolism, the oxidation of the 5'-carbon and N-oxidation respectively. These occur in two steps, the first step is cytochrome P450 (P450, CYP) 2A6–catalyzed 5′-oxidation to an iminium ion, and the second step is oxidation of the iminium ion to cotinine (Murphy, 2021). Nicotine N-oxide is largely excreted in the urine without additional metabolism, while cotinine is extensively metabolized further (Kasprzyk et al., 2022; Tan, et al., 2021; Jacob, et al., 1988).

The main health effects of cigarette smoke include, cancer, non-cancerous lung diseases, atherosclerotic diseases of the blood vessels and heart, and toxicity to the human reproductive system. Uric acid (UA) is the end product of purine nucleotide metabolism in the human body. Hyperuricemia is an abnormally high level of UA in the blood and may result in arthritis and gout (Haj Mouhamed et al., 2011). Hyperuricemia promotes the occurrence and development of cardiovascular diseases by regulating molecular signals, such as inflammatory response, oxidative stress, insulin resistance/diabetes, endoplasmic reticulum stress, and endothelial dysfunction. Smoking associated reactive oxygen species contribute to vascular oxidative stress and endothelial dysfunction, which are associated with the risk of arteriosclerosis and the release of neuron specific enolase (Puddu, et al., 2012). Neuron specific enolase (NSE) is an enzyme of the glycolytic pathway and is closely related to the differentiated state of mature nerve cells, primarily localized in the cytoplasm. Since NSE cannot be secreted by cells, an increase of NSE in serum is a marker for neuronal damage (Streitbürger, et al., 2012). Tobacco has known immunomodulatory effects, which suggests that it might affect peripheral nerve regeneration and functional recovery following injury (Rodriguez-Fontan, et al., 2020).

Cigarette smoke is loaded with a large number of harmful chemicals some of which are neurotoxins. Uric acid has been implicated in neuro-degeneration, secondary arteriolosclerosis, and nerve damage. Studies on assessment of biomarkers of cigarette smoke such as thiocyanate and cotinine are rife, however studies relating these markers with uric acid and marker of nerve damage neuron specific enolase in smokers are scanty in our locality. Also, uric acid has a dual role associated with high and low levels, thus, serving as a double edge sword, its actual role in cigarette smokers is still indefinable. This study assessed uric acid, neuron specific enolase levels in relation to serum cotinine, thiocyanate levels and smoking pack years indices of exposure to cigarette smoke in smokers in Calabar.

## MATERIALS AND METHODS

**Study design:** This cross sectional study was conducted in Calabar Metropolis, Southern Nigeria. The smokers were further subcategorized into three groups based on the duration of exposure to active smoking and the number of cigarettes sticks smoked per day (smoking pack years) thus; light smokers (smoking pack years, 12.0-30.0, n = 22), moderate smokers (smoking pack years 31.0-40.0, n = 17), and heavy smokers (smoking pack years 41.0-53.1, n = 21). Socio-demographic data, family history, medical history and anthropometric data were obtained from each participant using a well-structured questionnaire.

**Study setting:** The population consists of cigarette smokers in Calabar Metropolis, comprising of residents of Calabar South and Municipal Local Government Areas, Southern Nigeria. Cigarette smokers aged 20-55 years and age-matched apparently healthy non-smokers residing in the same geographic location who served as controls were enrolled in the study between July 2021 and March 2022.

**Participants and inclusion criteria:** The study participants included 60 cigarette smokers and 45 apparently healthy non-smokers who served as controls, residing in the same geographic location. Smokers who gave informed consents and having no other forms of diseases were included in the study

after adjustment to potential confounders' factors (factors of artherosclerosis such as serum cholesterol, high-density lipoproteins [HDL] and low-density lipoproteins (LDL), alcohol drinking, creatinine, and body mass index [BMI]) and smokers who did not give consent and those who had other form of diseases at the time of the study were excluded.

**Data collection:** A standard venepuncture method was used to obtain 6 mL of blood from all the participants after an overnight fast. Three milliliters of blood was dispensed into lithium heparin sample bottles for uric acid and thiocyanate estimation and 3mls into plain bottles for the determination of neuron specific enolase. The samples were allowed to clot after one hour and then centrifuged at 3000 rpm for 5 minutes at room temperature. The sera for estimation of neuron specific enolase, serum cotinine were separated immediately into aliquots using sterile Pasteur pipettes and stored at −20 °C in the UCTH laboratory until analysis. Patients' information on general health, history of past diseases and addictions were collected using an interviewer questionnaire method. The information collected from the patients included socio-demographic characteristics (such as age, gender, marital status, education, habits such as smoking, consumption of alcohol, medication) and bio-clinical information (such as disease duration of smoking, number of cigarette smoked per day). A medical weighing scale was used to measure the weight of each participant to the nearest 0.1 kg. Height was measured using a measuring tape on a vertical rod to the nearest 0.1 cm. Body mass index was computed as the ratio of weight (kg) to height ($m^2$). Body mass index of less than 18.5 $kg/m^2$ was considered underweight. Normal weight was a BMI of 18.50-24.99 kg/m2, the overweight had a BMI of 25.00-29.99 kg/m2 and the obese had a BMI ≥30.00 kg/m2 (Ko, et al., 2020).

**Laboratory methods**

Determination of cotinine: Serum cotinine concentrations were determined by a solid phase competitive Enzyme Linked Immunosorbent Assay (ELISA) method using a kit obtained from Calbiotech, Inc., El Cajon, California, USA. The samples and cotinine enzyme conjugate were added to the wells coated with anti-cotinine antibodies. Cotinine in the sample competes with a cotinine-enzyme, (Horse Radish Peroxidase) conjugate for binding site. The sample mixture was incubated for 60 minutes in the dark. Unbound cotinine and cotinine-enzyme conjugate was washed off by washing step. Upon the addition of the enzyme substrate and incubation for 30 minutes at room temperature in the dark a colour develops and the intensity of the colour is inversely proportional to the concentration of cotinine in the sample. A standard curve was prepared relating colour intensity to the concentration of cotinine, from the standard concentration curve, corresponding absorbance of samples and controls were deduced.

Determination of Uric acid: Uric acid was estimated using Enzymatic Colorimetric method using RANDOX kit obtained from Randox Laboratories Ltd. Crumlin, Country Antrim, U.K. Principle: Uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase enzyme oxidizes 3,5-Dichloro-2-hydroxybenzinesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound (Prencipe et al., 1978).

**Estimation of Neuron specific enolase (NSE)**

Neuron specific enolase was determined by ELISA method using a kit obtained from Calbiotecth Inc, USA. It is based on a solid phase direct sandwich ELISA method. The diluted samples and the conjugate reagents (anti-NSE, Biotin and horse-raddish peroxidase) were added to the wells which have been pre-coated with streptavidin. Neuron specific enolase in the sample was sandwiched between two specific antibodies to NSE. The unbound protein and HRP were washed off. Upon the addition of TMB substrate, the intensity of the color developed was in direct proportion to the concentration of NSE in the samples. It produced a standard curve that relates the intensity of the color to the concentration of NSE.

Determination of Thiocyanate: Serum thiocyanate was measured using potassium thiocyanate-iron (III) coupled reaction by colorimetric method. Thiocyanate reacts with iron (III) ions in solution to form an intense red coloured complex ion. A standard graph produced was used to deduce to concentration of thiocyanate in the sample. (Newman & Price, 1999)

Smoking pack years was calculated using the formula

Smoking Pack Years = (Cigarettes per day/Pack size) × Years, pack size is the standard 20 cigarettes.

**2.8 Study size:** Sample size was determined according to the method of Sullivan (Sullivan, 2020), using the formula

$$\frac{(Z\alpha + Z_{\beta})^2 . \dot{P}(1-\dot{P})}{(p0 - p1)^2}$$

The power of 0.84 was calculated at beta error of 80%. The sample size of 60 smokers was arrived at, while 45apparently healthy non-smokers who served as controls were selected for the study.

**Quantitative variables:** BMI, cotinine, Uric acid, thiocyanate and neuron specific enolase

**Statistical analyses**

Results were presented as mean ± standard deviation. Data were analyzed using the statistical package for social sciences (SPSS version 23.0, IBM, USA). Student's t test was used in comparing differences between groups. One way analysis of variance (ANOVA) was used to test the variations within and among group means and Fisher's least significant difference (LSD) post-hoc test was used for the comparison of multiple group means. Pearson's correlation was used to determine the associations between variables. The confidence interval was set to 95%. The significance level of the tests was set at α=0.05.

**RESULTS**

**Participants**: A total of 105 participants comprising of 60 cigarette smokers and 45 non-smokers who served as controls were enrolled in the study.

**Outcome data:** In this study 36.67% (n=22) were light smokers, moderate smokers were 28.33% (n=17), while heavy smokers were 35.00% (n=21) of the smokers respectively. The controls were 45.

**Main results:** The Comparison of Age, body mass index, serum cotinine, uric acid, thiocyanate and neuron specific enolase in cigarette smokers and non-smokers and neuron specific enolase cigarette smokers and control was shown in Table 1. Serum cotinine, thiocyanate and neuron specific enolase concentrations were significantly higher while body mass index and uric acid levels were significantly lower (P<0.05), in smokers when compared with the controls. Age was not significantly different between the two groups (P>0.05). The comparison of age, body mass index, serum cotinine, uric acid, thiocyanate and neuron specific enolase in light, moderate and heavy cigarette smokers was shown in Table 2. Age, serum cotinine, uric acid, thiocyanate and neuron specific enolase varied significantly (P<0.05) between the light, moderate and heavy cigarette. From the Fisher's least significant difference (LSD) post hoc analyses, the mean age and uric acid level of light smoker were significantly higher (P<0.05) than those of the moderate smokers, while serum cotinine level of moderate smokers was significantly higher (P<0.05) than that of the light smokers. Serum thiocyanate of light smokers was higher than that of moderate smokers, while neuron specific enolase level moderate cigarette smokers was higher (P=>0.05) than that of the light smokers. The mean age of light smokers was significantly higher (P=<0.05) compared with moderate smokers, while the serum cotinine, thiocyanate and neuron specific enolase levels of light smokers were significantly lower (P=<0.05) compared with moderate smokers. Serum uric acid, thiocyanate and neuron specific enolase levels of moderate smokers were significantly lower (P=<0.05), than those of the heavy smokers. Correlation plots between various parameters studied are depicted in Table 3. Significant negative correlations were observed between age and cotinine (r = -0.554; P<0.001), age and thiocyanate (r = -0.421, P = 0.001), age and neuron specific enolase (r = -0.346, P = 0.001), age against smoking pack years (r = -0.623, P<0.001) respectively. Significant positive correlations were observed between serum thiocyanate and neuron specific enolase (r = 0.324, P = 0.012), cotinine against smoking pack years (r = 0.399, P = 0.002) and neuron specific enolase against smoking pack years (r = 0.311, P = 0.015), correspondingly

Table 1, Comparison of Age, body mass index, serum cotinine, uric acid, thiocyanate and neuron specific enolase in cigarette smokers and non-smokers

| Parameters | Cigarette smokers (n=60) | Non-smokers (n=45) | Cal. T | P-Value |
|---|---|---|---|---|
| Age (years) | 28.62 ± 6.29 | 27.51 ± 6.85 | 0.857 | 0.393 |
| BMI (kg/m$^2$) | 22.16 ± 2.25 | 24.02 ± 2.62 | 3.885 | <0.001 |
| Cotinine (ng/ml) | 94.99 ± 45.25 | 3.11 ± 1.39 | 13.601 | <0.001 |
| Uric acid (mg/dl) | 3.50 ± 1.20 | 4.37±1.663 | 3.087 | 0.003 |
| Thiocyanate (umol/l) | 26.67±13.12 | 16.35±4.37 | 5.066 | <0.001 |
| Neuron specific enolase (ng/mL) | 91.79± 31.56 | 8.26 ± 2.84 | 17.691 | 0.001 |

Table 2, Comparison of Age, body mass index, serum cotinine, uric acid, thiocyanate and neuron specific enolase in light, moderate and heavy cigarette smokers

| Parameters | Light smokers (SPY=13.0-30.0) (n=22) | Moderate smokers (SPY 31.0-40.0) (n=17) | Heavy smokers (SPY 41.0-53.1) (n=21) | Cal. F | P-Value |
|---|---|---|---|---|---|
| Age(years) | 33.23±7.09[a] | 27.71±2.64 | 24.52±4.11 | 15.789 | <0.001 |
| BMI(Kg/m$^2$) | 21.89±2.19 | 22.21±2.27 | 22.41±2.38 | 0.282 | 0.756 |
| Cotinine (ng/ml) | 63.99±34.89 | 118.73±39.49[b] | 108.26 ± 42.35 | 11.354 | <0001 |
| Uric acid (mg/dl) | 3.75±1.93 | 2.84±1.12 | 3.78±1.32[c] | 3.918 | 0.025 |
| Thiocyanate (umol/l) | 25.31±13.82 | 18.81±6.85 | 34.47±12.30[d] | 8.674 | 0.001 |
| Neuron specific enolase (ng/mL) | 81.63±9.04 | 85.76±22.74 | 107.34±45.16[e] | 4.471 | 0.016 |

Key: Result presented as mean ± SD, *, significant at P<0.05; a=significant difference between light and moderate and light and heavy smokers, b=significant difference between light and moderate and light and heavy smokers, c=significant difference between light and moderate and moderate and heavy smokers, d= significant difference between light and heavy and moderate heavy smokers, e= significant difference between light and heavy smokers, moderate and heavy smokers, BMI= body mass index, SPY= smoking pack years

Table 4, Correlation between the various parameters in smokers

| Correlation Parameters | r | P-value |
|---|---|---|
| Age vs Cotinine | -0.554 | <0.001 |
| Age vs Thiocyanate | -0.421 | 0.001 |
| Age vs NSE | -0.346 | 0.007 |
| Thiocyanate vs NSE | 0.324 | 0.012 |
| Age vs SPY | -0.623 | <0.001 |
| Cotinine vs SPY | 0.399 | 0.002 |
| NSE vs SPY | 0.311 | 0.015 |

Key: NSE=neuron specific enolase, SPY=smoking pack years

## DISCUSSION

This study investigated uric acid and neuron specific enolase levels in relation to serum cotinine, thiocyanate levels and smoking pack years, (the biological markers of exposure to cigarette smoke) in cigarette smoker. Chemicals found in tobacco smoke include nicotine (the addictive drug that produces the effect people are looking for), Carbon monoxide, Nitrosamines, Formaldehyde, Hydrogen cyanide, Lead, cadmium, Ammonia, Radioactive elements, such as uranium, Benzene, Arsenic, Polycyclic Aromatic Hydrocarbons (PAHs), Several of these substances are carcinogenic, while others can cause lung disease, heart disease, or other serious health problems (Cheng et al., 2022) The various harmful health effects of cigarette smoke, coupled with the considerable prevalence of cigarette smoking, makes smoking a leading global cause of death and accounting for enormous economic burden. The reduced body mass index of smokers compared to non-smokers may be attributable to the harmful effect of the components of cigarette smoke including cyanides, Lead, Cadmium, formaldehyde, acrolein, benzene, and certain N-nitrosamines, phenol, polyaromatic hydrocarbons (PAHs), which may be enzyme poisons affecting appetite and metabolic alterations. This may result in breakdown of protein from the somatic compartment to release amino acids necessary for energy and formation of proteins like immunoglobulins to detoxify and eliminate these toxic substances. An account of weight loss in smokers had also been given by Andersson and Arner (2001), who demonstrated nicotine-induced increases in plasma adrenalin and noradrenaline levels, mediated by both beta-adrenoceptors and local nicotinic cholinergic receptors abundant on fat cells as the mechanisms for catecholamine's lipolytic effects with consequent loss of body weight. Adrenalin released in response to nicotine raises blood pressure by constricting the blood vessels, resulting in hypertension and other cardiovascular events in smokers. Also, Jitnarin et al., (2014), demonstrated that smoking is associated with lower weights and BMI. However, Wang (2015),

reported that the effects of cigarette smoking on body weight remain inconclusive, he observed smoking to have a positive effect on underweight and healthy weight, while having a negative effect on overweight and obesity.

The significantly lower level of uric acid in smokers compared to the controls in the present study may be due to uric acid depletion during free radical scavenging and oxidative stress limiting functions. This observation is similar to that of Tibullo, et al., (2016), who reported that apart from numerous ROS, epoxides, peroxynitrate, and nitric oxides are present in the gas phase, while hydroxyl radicals, semiquinone, peroxides, hydrogen peroxide, and other organic compounds, which are involved in redox cycling with the release of the superoxide anion occur in the tar phase of cigarette smoke. Similarly, Kang and Ha, (2014) and Sun, et al., (2017), reported that UA is a potent scavenger of singlet oxygen, peroxyl radicals (RO◦2), and hydroxyl radicals and protects the cell from oxidative damage by chelating metal ions, decreases oxidative stress–induced malondialdehyde and protein carbonyl contents, increases superoxide dismutase (SOD) activity and inhibits radicals generated by the breakdown of peroxynitrite (ONOO−). Tobacco smoke is a complex blend of thousands of different chemical species including metals, ROS/RNS, aldehydes, quinones, ketones, existing in gas and tar phases, which significantly contributes to enhanced oxidative stress. Thus, UA is considered a highly potent free radical scavenger, accounting for more than half of the antioxidant activity of plasma. However, UA, also demonstrate pro-oxidant capacity, can decrease nitric oxide (NO) production, induce lipid peroxidation, and interact with peroxynitrite to generate free radicals. Decreases UA in smokers as seen in this therefore reflects a reduction in the physiological roles of UA and the ensuing consequences. Low levels of UA as observed in smokers from this study could be damaging to the neurons because it impairs antioxidant capacity in the neuronal cells. Nerve tissues require significant amounts of nutrients and oxygen via blood flow, smoking constricts blood vessels making them smaller, resulting in less blood and nutrients being delivered to the peripheral nerves. Normal blood vessels have an inner lining, the endothelium that keeps blood flowing smoothly by producing local Nitric oxide (NO). NO aids the relaxation of smooth muscles in the walls of blood vessels and avert cells from sticking to the walls. A disturbance of this mechanism in cigarette smokers via NO bioavailability reduction, leads to impairments of vascular tone and hemostasis and endothelial dysfunction with the release of neuron specific enolase. The significantly higher neuron specific enolase in smokers compared to non-smokers may be due to the harmful constituents of cigarette smoke such as peroxynitrite (ONOO−), which neutralizes NO, resulting in reduced NO bioavailability and generation of more free radicals through (ONOO−) breakdown. Also, UA pro-oxidant activity decreases NO production, leading to vasoconstriction in different tissues, hypoxia and endothelial dysfunction and nerve damage in smokers with the release of neuron specific enolase. This observation is similar to those of Lee et al., (2021) and Janaszak-Jasiecka et al., (2021) who reported an association between endothelial dysfunction driven by hypoxia and the influence of oxygen deficiency on NO bioavailability. The higher serum cotinine, and thiocyanate concentrations in smokers compared to the controls may suggest that both are metabolic products of the constituents of cigarette and may serve as biological markers of exposure to cigarette smoke. Age decreased with increase in smoking pack years, suggesting that the younger experimenting smokers smoke more than the older smokers. There were significant negative correlations between age and cotinine, age and thiocyanate, age and neuron specific enolase, age against smoking pack years, suggesting that the older smokers smoke less compared to the younger ones with decrease in the markers of cigarette smoke exposure and nerve damage in the older smokers cohort. The significant positive correlations between serum thiocyanate and neuron specific enolase, cotinine against smoking pack years, and neuron specific enolase against smoking pack years correspondingly, may suggest a positive association between markers of exposure to cigarette smoke and nerve damage.

**Limitation of the study:** Participants were not screened to rule out other sources of NSE such as small cell lung carcinoma (SCLC) and non-small cell lung cancer (NSCLC).

**CONCLUSION:** This study has shown that smokers have reduced body mass index, uric acid and higher cotinine, thiocyanate and neuron specific enolase levels compared to non-smokers. Both serum thiocyanate and smoking pack years correlated positively with the levels of neuron specific enolase. Neuron specific enolase correlated positively with cotinine levels. Therefore, cigarette smoking may be associated with nerve damage. The older smokers smoke less compared to younger smokers. Cotinine and thiocyanate levels correlated positively with levels of neuron specific enolase, smoking pack years and negatively with uric acid in smokers.

**ACKNOWLEDGEMENTS:** The authors thank the staff of University of Calabar Teaching Hospital, Calabar for their support during sample storage.

**Conflicting Interest:** None declared

**Ethical consideration:** This study was carried out in accordance with the ethical principles for Medical Research involving human subjects as outlined in the Helsinki Declaration in 1975 and subsequent revisions. The study protocol was approved by the Health Research Ethics Committee, Ministry of Health, Cross River State (REC.NO.CRSMOH/RP/REC/2021/156). A written informed consent was obtained from each of the study participants, after explaining the purpose of the study. The confidentiality of patient's information was

preserved at all steps. The rights to withdraw from participation in the study at any point in time were respected.

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