

Research Article

Assessment of Serum Cotinine, C-Reactive Protein Levels and Body Mass Index in Smokers in Calabar, Nigeria

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Abstract

Cigarette smoking has been linked to health challenges of global concern. This study determined Body Mass Index (BMI), serum cotinine and C-reactive protein levels of smokers. Forty-five smokers and forty-five aged-matched non-smokers were recruited into the study. Informed consents were obtained from the participants. Ethical consideration was granted by Cross River State Ministry of Health. Blood samples were collected by standard phlebotomy. Smokers were categorized based on Smoking Pack Years (SPY) into light, moderate and heavy smokers. Serum cotinine and C-reactive protein levels were determined by spectrophotometry. Height and weights were measured and BMI calculated. The data were analyzed using SPSS version 23.0. Student's t-test, ANOVA, Pearson's correlation were utilized for comparison, results were considered significant at $p < 0.05$. There was no significant difference ($p = 0.103$) between the mean age of smokers and non-smokers. Mean BMI of smokers was significantly lower ($p = 0.015$) than that of non-smokers. Mean Serum cotinine and C-reactive protein of smokers were significantly higher ($p = 0.001$) than those of non-smokers. Mean age, serum cotinine and C-reactive protein levels vary significantly ($p < 0.05$) among the smokers categorized based on SPY. BMI did not vary significantly ($p = 0.269$) among the groups. A correlation between age and serum cotinine was negative ($r = -0.359$, $p = 0.016$), and that between age and SPY was negative ($r = -0.348$, $p = 0.019$). Correlations between cotinine and C-reactive protein and between cotinine and SPY were positive ($r = 0.928$, $p = 0.001$) and ($r = 0.947$, $p = 0.001$) respectively. A correlation between C-reactive protein and SPY was positive ($r = 0.957$, $p = 0.001$). The study had shown that C-reactive protein increases with cotinine levels and smoking pack years in smokers.

Keywords: Cigarette; Smokers; C-RP; Cotinine; Pack-years; Inflammation; Tissue; Damage

Introduction

Smoking is an act of burning a substance and the resultant smoke breathed in; to be absorbed in the blood stream. A report on trends in prevalence of tobacco smoking had shown that the prevalence of tobacco use is steadily increasing in developing countries including Nigeria despite the broadly known health, economic, and social consequences associated with tobacco use [1]. Tobacco is the only ratified drug that kills a reasonable number of its users when used exactly as intended by the manufacturers. It has been Tobacco kills more than 8 million people each year. More than 7 million of those deaths are the result of direct tobacco use [2].

The amounts of the chemical constituents in cigarette smoke are influenced by many different factors. The soil in which the tobacco plant is grown and the fertilizers used to promote growth of the plant may influence the levels of the metals and nitrogen-containing compounds [3]. Tobacco smoke is composed of a deadly blend of more than 7,357 chemicals [4,5]. Hundreds of these are toxic and more than 70 cause cancer [6]. A number of these chemicals are poisons; with the

ability to damage body tissues. The chemicals in tobacco smoke reach the lungs quickly following inhalation, the toxicants being absorbed and carried in the blood to every organ in the body. Chemicals in tobacco smoke cause inflammation and damage to the cells lining every body organ [7]. The body fights endlessly to heal the damage as one keeps smoking but the damage cannot heal.

The toxic mix in cigarette smoke may cause systemic inflammation. Serum C-reactive protein in smokers may serve as a marker of presence systemic of inflammation. Serum C-RP, an acute phase protein, is a marker of systemic inflammation formed principally by the liver and to a less extent by adipocytes in reaction to inflammatory signals [8]. Inflammatory response induces increased secretion of proinflammatory cytokines including IL-6 and TNF- α from polymorphonuclear neutrophil, which bind to their receptors on hepatocyte surface and increase C-reactive protein production [9]. C-reactive protein has been recognized as a potential biomarker for pathologies involving inflammation having the most vigorous implication for clinical events. It is widely used as a biomarker for most clinical events because of its biological stability and ease of determination without much analytical variations [10]. As one of the first reactants released into plasma, its measurement has been considered a classic indicator of the acute phase response in inflammation [11]. It serves the vital roles of activating the complement system, prompting expression of adhesion molecule, enhancing phagocytosis and macrophage and leukocyte activation and opsonization [12].

Among the major components of cigarette smoke are nicotine, tar, carbon monoxide and certain other poisonous substances like hydrogen cyanide, nitrogen oxide and ammonia [13,14]. Nicotine, the major addictive component of tobacco is metabolized to cotinine,

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a stable metabolite with a half-life of 18 hours to 24 hours, which can be used as the biomarker for cigarette smoke exposure. The powerful addicting elements of tobacco products affect multiple types of nicotine receptors in the brain and heart, thus, changing the way the brain works and making the smoker to crave more and more for nicotine [15,16]. Nicotine is not a direct cause of most tobacco-related diseases, but it is highly addictive [17]. 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. Nicotine N-oxide is largely excreted in the urine without additional metabolism, while cotinine is extensively metabolized further [18].

Nicotine reduces appetite and alters feeding styles typically affecting body weight and Body Mass Index (BMI) of smokers. Mobilization of free fatty acids through lipolysis from adipose tissue by nicotine may directly influence fat stores resulting in weight loss and modification of BMI in active smokers.

Studies have been carried out to determine the effect of tobacco use on systemic response but the relationship between C-reactive protein and cotinine and the influence of cotinine on metabolism and body weight are still debatable. Not much is known on this topic in our locality. Hence the study was, carried out to assess the relationship between serum C-reactive protein, serum cotinine levels, smoking pack years and BMI of male smokers in Calabar.

Materials and Methods

This study was conducted in Calabar, Cross River State, between November 2015 and August 2016. Ethical approval was obtained from the Research Ethical Committee of the Cross River State Ministry of Health and informed consent was obtained from the participants of the study. A standard venipuncture method was used to obtain five milliliters (5 ml) of blood from all the subjects. The blood was dispensed into plain containers and allowed to clot, then centrifuged at 3000 rpm for 5 minutes and the serum obtained was stored in plain containers frozen until analyses. A total of ninety (90) adult males aged 18 years to 50 years, comprising of forty five (45) smokers and forty five (45) age matched non-smokers who served as control subjects were enlisted for the study. Participants' information on cigarette smoking habits, medical history and socio-demographic indexes including age, gender, and occupation were obtained by administration of a structured questionnaire. smokers were further categorized into three groups based on the duration of exposure to active smoking and the number of cigarettes sticks smoked per day (smoking pack years) into light (smoking pack years, 12.30 to 28.10), moderate (smoking pack years, 28.70 to 47.12) and heavy (smoking pack years, 47.42 to 53.10) smokers. Height and weight of participants were measured and Body Mass Index (BMI) determined. Serum C-reactive protein levels were determined by immuno turbidimetric method using a kit obtained from Elitech Clinical Systems, France. Serum cotinine concentrations were determined by a solid phase Enzyme Linked Immunosorbent Assay (ELISA) using a kit obtained from Calbiotech, California, USA. Statistical analysis: The data generated were analyzed using SPSS version 20.0 statistical package, differences between groups were determined using Students t-test, variations among groups by ANOVA and relationship between parameters using Pearson's correlation, differences were considered significant at $P < 0.05$.

Results

Mean age of smokers (29.87 ± 6.73 years) was not significantly different ($p=0.103$) from that of non-smokers (27.51 ± 6.85 years). The mean body mass index of smokers (22.67 ± 2.52 kg/m²) was significantly lower ($p=0.015$) than that of non-smokers (24.02 ± 2.62 kg/m²). Mean serum cotinine and CRP of smokers (69.79 ± 59.38 ng/ml, 28.15 ± 14.37 mg/L) were significantly higher ($p < 0.05$) than those of non-smokers (3.93 ± 1.80 ng/ml, 4.39 ± 2.16 mg/L) respectively, Table 1. Mean age and BMI did not vary significantly ($p > 0.05$) among the smokers. However, the mean age of light smokers (32.26 ± 8.37 years) was the highest while that of heavy smokers was the least (27.70 ± 4.95), similarly, mean BMI of heavy smokers was the least (22.44 ± 3.20 kg/m²) and that of moderate smokers (22.76 ± 2.23 kg/m²) being the highest. Mean serum cotinine and CRP concentrations vary significantly ($p=0.001$) among smokers, with heavy smoker (139.83 ± 19.07 ng/ml and 42.69 ± 3.15 mg/L) being the highest while those of light smokers (14.49 ± 11.91 ng/ml and 13.92 ± 7.94 mg/L) respectively being the least, Table 2. Negative correlations were observed between age and serum cotinine concentration ($r=-0.359$, $p=0.016$) and between age and smoking pack years ($r=-0.348$,

Table 1: Age, BMI, serum cotinine and C-reactive protein concentrations of smokers and non-smokers.

Parameters	Smokers (n=45)	Non-smokers (n=45)	Cal. T	P-value
Age (years)	29.87 ± 6.73	27.51 ± 6.85	1.646	0.103
BMI (kg/m ²)	22.67 ± 2.52	24.02 ± 2.62	2.478	0.015
Cotinine (ng/ml)	69.79 ± 59.38	3.93 ± 1.80	7.437	0.001
CRP (mg/L)	28.15 ± 14.37	4.39 ± 2.16	10.973	0.001

BMI: Body Mass Index; COT: Serum Cotinine Concentration; CRP: C-Reactive Protein

Table 2: Age, BMI, serum cotinine and C-reactive protein concentrations of smokers categorized based on smoking pack-years into light, moderate and heavy smokers.

Parameters	Light smokers (Spy=12.30-28.10) n=19	Moderate smokers (spy=28.70-47.12) n=16	Heavy smokers (Spy=47.42-53.10) n=10	Cal. F	P-value
Age(years)	32.26 ± 8.37	28.38 ± 4.60	27.70 ± 4.95	2.237	0.119
BMI(kg/m ²)	22.72 ± 2.49	22.76 ± 2.23	22.44 ± 3.20	0.053	0.948
COT(ng/ml)	14.49 ± 11.91	91.67 ± 47.95	139.83 ± 19.07	59.813	0.001
CRP(mg/L)	13.92 ± 7.94	35.96 ± 7.83	42.69 ± 3.15	67.933	0.001

BMI: Body Mass Index; COT: Serum Cotinine Concentration; CRP: C-Reactive Protein Concentration; SPY: Smoking Pack-Years

Table 3: Correlation of age, serum cotinine, serum CRP and smoking pack years in smokers.

Indices	r	P-value
Age (yrs.)and COT(ng/ml)	-0.359	0.016
Age (yrs.) SPY(yrs.)	-0.348	0.019
COT(ng/ml) and CRP(mg/L)	0.928	0.001
COT (ng/ml) and SPY(yrs.)	0.947	0.001
CRP (mg/L) and SPY(yrs.)	0.957	0.001

r: Correlation Coefficient; COT: Serum Cotinine Concentration; CRP: C-Reactive Protein Concentration; SPY: Smoking Pack-Years

$p=0.019$) in smokers, in that order. Significant positive correlations were observed between serum cotinine concentration and CRP ($r=0.928$, $p=0.001$) and between serum cotinine concentration and smoking pack years ($r=0.947$, $p=0.001$). Correlation between CRP and smoking pack years was positive and significant (0.957 , $p=0.001$), Table 3.

Discussion

Cigarette smoking is a major avoidable cause of a continuum of medical conditions involving many organ systems. 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, Ammonia, Radioactive elements, such as uranium, Benzene, Arsenic, Polycyclic Aromatic Hydrocarbons (PAHs), Several of these substances are carcinogenic. A number of these can cause lung disease, heart disease, or other serious health problems. The various harmful health effects of cigarette smoke, coupled with the considerable prevalence of cigarette smoking, makes smoking a major worldwide cause of death, contributing so heavily to mortality and accounting for enormous economic burden.

The reduced body mass index of smokers relative to non-smokers may be attributable to the effect of the constituents of cigarette smoke like nicotine, cyanides and other poisons on appetite and nicotine-induced metabolic changes. Nicotine associated appetite loss 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 [19], who demonstrated nicotine-induced increases in plasma adrenalin and noradrenaline levels, mediated by both beta-adrenoceptor 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. Raised CRP in smokers compared to non-smokers, may be due to cigarette induced inflammatory processes mediated by cytokine stimulation of the hepatocytes. Similar observations have been reported in studies by [20-22].

CRP concentrations of smokers were observed to increase with smoking pack years and serum cotinine levels, demonstrated by positive correlations between CRP and serum cotinine and between CRP and smoking pack years. A similar observation have been reported by Shiels et al. [23], and Tibuakuu et al. [24] who reported increase serum cotinine with increased levels of CRP in smokers compared to never smoker.

The poisons in tobacco smoke result in inflammation of the delicate lining of your lungs. Years of smoking can damage the lungs so much that they no longer stretch and exchange air. CRP plays a central role in inflammation, an exceptionally vital process for wound healing, immune response to restrict bacteria and viruses spread, and other processes crucial for survival. Sustained inflammatory response may be fundamental to a range of health challenges associated with cigarette smoking. Ridker [25] had equally observed that excess inflammation may have adverse effects, particularly on the blood vessels that carry oxygen and nutrients to all the tissues of the body. The decrease in serum cotinine levels and smoking pack years with age, may suggest that as smokers advance in age they tend to smoke less, unlike the beginner smoker who may be trialing smoking and while being fully cut-up anew in addiction to nicotine may smoke more [26].

Conclusion

C-reactive protein and serum cotinine concentrations of smokers increase with smoking pack years. Smoking induced inflammatory tissue damage may underlie the pathological processes in smokers. There may be no safer level of exposure to tobacco smoke, but smoking associated adverse health conditions may increase with exposures.

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