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Evaluation of Serum Levels of LP-PLA2 and CA-242 in Adult Male Cigarette Smokers in Nnewi Metropolis

Manafa, P.O.[1], Nwankwo, N.B.[1], Ekuma-Okereke, O.[1], Chukwuma, G.O.[1], Ibe N.C.[1], Okocha, E.C.[2], Chukwuanukwu, R.C.[1], Nwene, K.E.[2], Ebugosi, R.S.[3], Manafa, V.I.[4]

1 *Department of Medical Laboratory Science, Faculty of Health Sciences & Technology, Nnamdi Azikiwe University, Nnewi Campus, Awka, Nigeria*

2 *Center for Clinical research in Nigeria*

3 *Department of Biochemistry, Tansian University, Anambra State, Nigeria*

4 *Pathology Department, Clinical Biochemsitry, East Kent Hospital University NHS Foundation Trust, United Kingdom*

Abstract

Background: Cigarette smoking is a behavioural lifestyle in which a substance is burned and the resulting smoke breathed into the body system. Thus, cigarette smoking is a known public health challenge given the number of tobacco-related diseases like hypertension, lung cancer, cardiovascular diseases (CVD) etc. leading to increased mortality in developed and developing countries. Notwithstanding that the effects of smoking are well documented, individuals who practice cigarette smoking are still on the increase most especially in the developing countries. Study **Design/Aim:** This was a cross-sectional study designed to evaluate the serum levels of Cancer Antigen-242 (CA-242) and Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) in adult male smokers in Nnewi Metropolis, as emerging inflammatory biomarkers. **Materials and methods:** A total of 135 subjects aged between 16-65 years were selected for this study. They were classified into 2 major groups (test and control); comprising of 85 cigarette smokers (55 and 30 as test subjects for the evaluation of CA-242 and Lp-PLA2) respectively and 50 non-cigarette smokers (35 and 15 as control subjects for CA-242 and Lp-PLA2 evaluations) respectively. A well-structured questionnaire was used for the collation of information from the participants. **Results:** the mean serum level of Lp-PLA2 was significantly elevated ($P<0.05$) in cigarette smokers (67.52±27.29) compared with the non-smokers (63.63±20.81). While the serum level of CA-242 among smokers (1.77±0.70) was of no significant difference ($P=0.711$) when compared with the non-smokers (1.81±0.20). More so, the mean serum levels of Lp-PLA2 correlated positively with the duration of smoking ($r=0.297$) and age ($r=0.085$) in male cigarette smokers. However, there were negative relationships when CA-242 were correlated with duration of smoking ($r = -0.156$) and age of smokers ($r=-0.155$). **Conclusion:** The increased level of Lp-PLA2 along with its positive correlation with other traditional markers like age and smoking duration suggests that Lp-PLA2 is a suitable biomarker to predict cardiac related diseases among cigarette smokers. This is because, Lp-PLA2 is a more specific cardiac predictor compared to the non-specific conventional biomarkers. We therefore suggest that Lp-PLA2 as an independent advanced predictor of cardiovascular disease be further evaluated using follow-up studies with better sample size in CVDs related cases.

Keywords: Smoking, Carbohydrate Antigen-242, Lipoprotein-Associated Phospholipase A2, Cardiovascular Diseases.

INTRODUCTION

The rate of heart related diseases (CVDs) has been on the increase globally, thereby becoming a leading cause of morbidity and mortality in the developed and developing countries and it is projected to remain so [1]. The management of heart related diseases is a global burden and it affects the quality of life of the concerned individuals. The incidence of heart related diseases may increase due to various risk factors such as tobacco use, hypertension, hyperlipidemia, obesity, lack of physical activity and diabetes [2]. To successfully prevent or manage cardiac related diseases, a lot of measures are required and this includes in part the ability to identify individuals who are at risk for future cardiovascular diseases (risk prediction). Conventionally, risk prediction has relied on assessment of risk factors such as tobacco use, hypertension, diabetes mellitus, hyperlipidemia, obesity, family history, etc. [1]. Conversely; we tend to ascertain the cardiovascular disease predictability potentials of emerging biomarkers like LP-PLA2 and CA-242 among cigarette smokers.

Biomarkers are regarded as a better tool for early detection of diseases and therefore can help early decision making regarding disease management and treatment where necessary. Commonly, the lipid profile family of total cholesterol, LDL-cholesterol, HDL-cholesterol, Lipoprotein and Triglycerides, are the most used parameters to assess cardiac related diseases [3]. However, the use of new and more specific

***Corresponding author:**
Ekuma-Okereke, Ogbonnia
Department of Medical Laboratory Science, Faculty of Health Sciences & Technology, Nnamdi Azikiwe University, Nnewi Campus, Awka, Nigeria
Email:
ogbonniaekuma@gmail.com

biomarkers to predict is required. This is because the well documented and randomly used conventional risk predictors do not often identify everyone who will eventually develop CVD [4], as supported by the Adult Treatment Panel III (ATP III) guidelines which proposed that cardiovascular risk is not fully exposed by traditional risk factor assessment, adding that "when major risk factors are present, they account for only half of the variability in cardiovascular risk in the various populations" [5]. These therefore necessitate the need for more reliable, specific and accurate biomarkers to predict cardiac related diseases.

More so, there are significant evidence that inflammation plays a pivotal role in the triggering and progression of cardiac related disease like atherosclerosis, proposing that biomarkers of inflammation may help in predicting an individual's risk for cardiac related diseases. High-sensitivity C-reactive protein (hs-CRP), an acute-phase reactant, has also been implicated as a useful inflammatory marker in the prediction of cardiac related diseases [6]. However, hs-CRP as cardiac marker is relatively nonspecific and its level may be increased in other non-cardiac related inflammatory signal [6]. To this effect, a group of researchers have substantiated the role of inflammation in atherogenesis as well as the risk predictive value of variety of other inflammatory markers. Among these, Lp-PLA2 has been shown to be a cardiovascular risk marker independent and additive to traditional risk factors [7,8].

Lipoprotein-associated Phospholipase A2 (Lp-PLA2), also known as Platelet activating factor, is an enzyme that specifically hydrolyzes oxidized phospholipids on oxidized LDL particles within the arterial intima [9]. Having been found to be specific to arterial inflammation, Lp-PLA2 is therefore considered as a promising biomarker in cardiovascular disease cases [7]. Also, it has been reported that the incidence of heart related disorders is very high among tobacco use individuals. Thus, evaluating the role of a new predictive marker that is more specific to cardiovascular diseases in this group of subjects could be quite useful for the society at large [8].

On the hand also, tumor markers are substances developed in or induced by tumor cells and secreted into body fluids where they exact their actions [5]. The malignant transformation of cells has been implicated in increased concentrations of tumor markers and thus can be associated with malignant diseases. It appears, however, that other proliferative processes, such as inflammatory and benign transformations are also able to induce the rise of tumor marker levels [5]. There are many molecular tumor markers for diagnosing and monitoring cancer patients. Especially, quantitative assay for serum levels of tumor markers; carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9) are frequently used in daily practice because of their relative specificities and usefulness to the common cancers [6].

Cancer Antigen-242 (CA-242) is a new tumour marker, based on monoclonal antibody C-242 (C-242 antibody), obtained after immunization of mice with a human colorectal adenocarcinoma cell line, COLO 205, the same carcinoma cell line against which the C-50 antibody was raised [10]. CA-242 is very well related, although not identical to the antigenic epitopes of tumour markers CA-19-9 and CA-50 [11,12]. Thus, this new marker has proved very promising and the sensitivity and specificity for pancreatic cancer have been similar to those of CA 19-9. CA-242 also, is of great significance to the early detection, treatment monitoring and prognosis assessment of pancreatic cancer [13], hence the desire to ascertain its organ dysfunction predictiveness in cigarette smokers.

MATERIALS AND METHODS

Study site:

The research was carried out in Nnewi metropolis. Nnewi is the second largest city in Anambra State of South Eastern Nigeria.

Study population: A total of 135 subjects aged between 18 to 65 years were randomly selected for this study. They were classified into 2 major groups (test and control); consisting of 85 cigarette smokers (55 and 30 as test subjects for the evaluation of CA-242 and LP-PLA2) respectively and 50 non-cigarette smokers (35 and 15 as control subjects for CA-242 and LP-PLA2 evaluations) respectively. A well structured questionnaire was used for the collation of information from the participants.

Inclusion criteria:

Adult male cigarette smokers and non-smokers within the age range of 18-65 were included for this study.

Exclusion criteria:

Subjects outside the age bracket of 18-65 and those with Diabetes Mellitus, auto-immune disorders, Human Immuno Deficiency Virus, cardiac and pancreatic disorders were excluded from the study.

Ethical consideration

The ethical approval for this research was obtained from the Ethics Committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus.

Sample collection

Five (5) ml of whole blood was aseptically collected from each participant using a plain specimen container. The serum obtained after centrifugation was stored at -20^{0}C until analyzed.

Methods of analysis:

The serum levels of CA-242 and LP-PLA2 were analyzed using Enzyme-Linked Immunosorbent Assay procedure as adopted by [14].

Statistical analysis

Data collected were subjected to statistical analysis using the Student's t-test and the Analysis of Variance (ANOVA). Values were deemed significant at $P<0.05$. Correlation of the parameters with duration of smoking and age was determined using the Pearson's correlation coefficient. The statistical analysis was conducted by using SPSS software (version 20.0).

RESULTS

Table 1: Mean and standard deviation of the serum levels of CA-242 and Lp-PLA2 in smokers and non-smokers

The mean serum level of Lp-PLA2 in adult male cigarette smokers (67.52±27.29) was significantly higher (P=0.024) compared with non-smokers (63.63±20.81). However, no significant difference was observed in the mean serum level of CA-242 in smokers (1.77±0.70) and non-smokers (1.81±0.20). (P=0.711).

Table 1: (Mean ± SD) of the Serum Levels of CA-242 and Lp-PLA2 in smokers and non-smokers.

Parameters	Smokers	Controls	T-test	P-value
CA-242 (U/ml)	1.77±0.70 (n=55)	1.81±0.20 (n=35)	-0.372	0.711
Lp-PLA2(µg/L)	67.52±27.29 (n=30)	63.63±20.81 (n=15)	2.538	0.024

Figures 1 and 2: Relationship between age and serum levels of CA-242 and Lp-PLA2 in smokers and non-smokers: there was a positive non-significant correlation between the serum levels of Lp-PLA2 and age in adult male cigarette smokers (r=0.085) (Figure 1) and a negative non-significant correlation between the serum levels of CA-242 and age in adult male cigarette smokers (r= -0.155) (Figure 2).

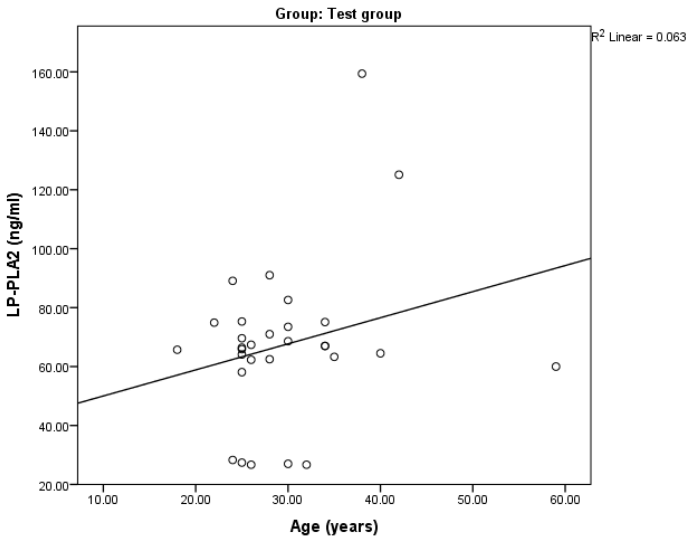
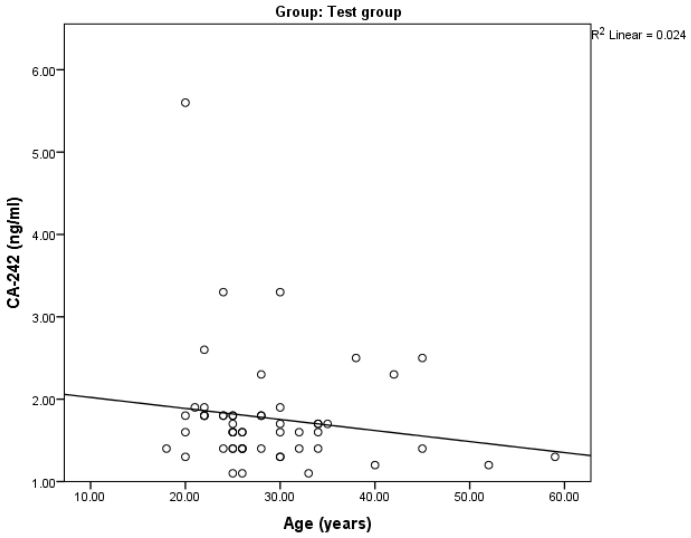


Figure 1: Relationship between serum level of Lp-PLA2 and age in smokers and non-smokers



Figures 2: Relationship between the serum level of CA-242 (U/ml) and age (years) in adult male cigarette smokers

Figures 3 and 4: Correlation studies between smoking duration and the serum levels of Lp-PLA2 with CA-242 (U/ml) in adult male cigarette smokers.

A non-significant positive correlation existed between the serum levels of Lp-PLA2 and duration of smoking in male cigarette smokers (r=0.297) (Figure 3). The serum levels of CA-242 had a non-significant negative correlation with duration of smoking in male cigarette smokers (r = -0.156) (Figure 4).

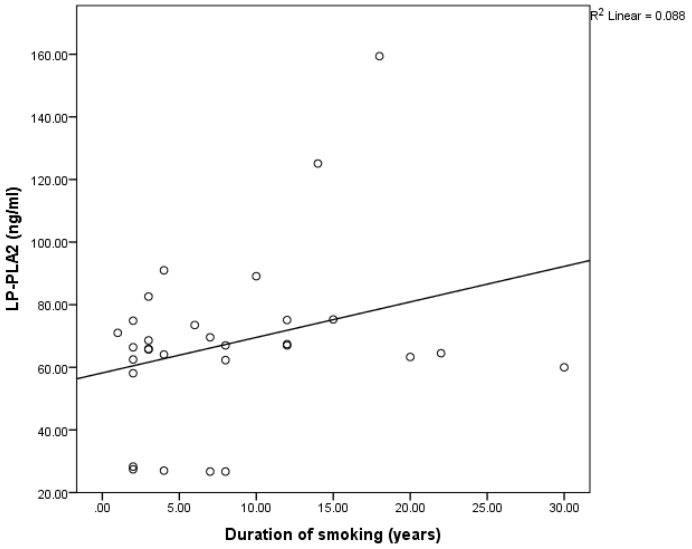


Figure 3: Correlation studies of the serum levels of Lp-PLA2 (µg/L) and duration of smoking (years) in adult male smokers

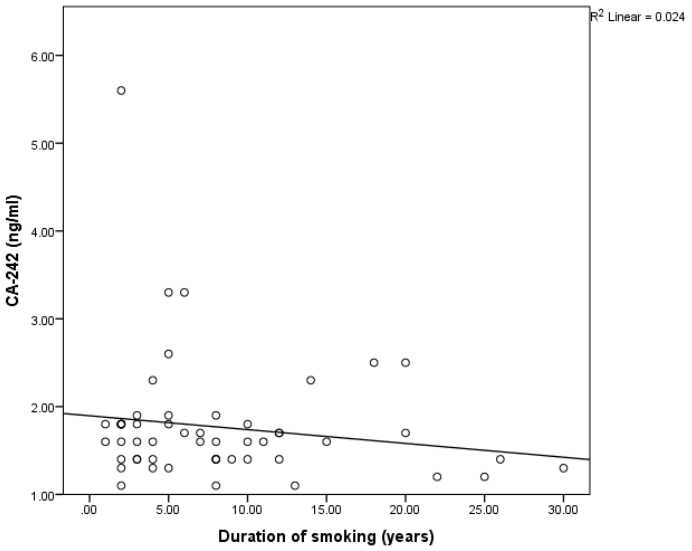


Figure 4: Correlation studies of the serum levels of CA-242 (U/ml) and duration of smoking (years) in adult male cigarette smokers

DISCUSSIONS

Cigarette smoking is the inhalation of smoke produced from the burning of cigarettes. It is a major cause of organ dysfunction and death in the developed and developing countries including Nigeria. Cigarette smoking has also been proposed to be a significant risk factor for the onset and progression of cardiovascular diseases (CVDs) [15]. Cardiovascular diseases have become major causes of morbidities and mortalities across the globe, most especially the developing countries. The incidence of cardiovascular diseases can be prevented or minimized to a large extent should the risk factors be identified early. The success of preventive measures depends largely in part on the precise identification of individuals who are at risk for future cardiovascular events. The lack of a specific biomarker which can predict cardiovascular risks has fueled the intense interest in identifying new biomarkers for early identification of risks for cardiovascular diseases [9]. Several studies have proposed Lp-PLA2 and CA-242 as emerging cardiovascular and tumor markers respectively, as evidently shown by their elevated levels in vascular inflammatory cases like atherosclerosis [7,9,13]. This study was conducted to identify the role of Lp-PLA2 and CA242 as a cardiac risk marker in cigarette smokers.

In our current study, the mean serum level of Lp-PLA2 was significantly higher in smokers compared with non-smokers. However, no

significant difference was observed in the mean serum level of CA-242 in both smokers and non-smokers. This could be attributed to oxidative stress because, induced oxidative stress plays a central role in accelerated cardiovascular aging and thus could be so in smokers. Also, this activity has been associated with increases low density lipoprotein which is actively related with Lp-PLA2. Over the years also, it has been established that cigarette smoking is an independent risk factor for atherosclerosis and coronary heart disease [16]. This is because, ingredients like nicotine and carbon monoxide (CO) contained in cigarettes can cause significant increase in oxidative stress, endothelial damage and organ dysfunctions as demonstrated by [17,18]. This has been evidently demonstrated by [19] whose findings revealed that cigarette smoking influenced plasma Lp-PLA2 and lysoPC expression in peripheral blood mononuclear cells (PBMC), through the induction of oxidative stress. More so, a positive correlation between Lp-PLA2 activity and circulating oxidized low-density lipoprotein (oxLDL) has been demonstrated in a hypercholesterolemic swine model of atherosclerosis [20]. Moreover, it has been suggested also, that oxLDL can up-regulate the expression of Lp-PLA2 in human monocyte-derived macrophages [21].

Cigarette smoking has also been reported to induce changes in lipoproteins (quantity and quality), markers of inflammation like Lipoprotein-Associated Phospholipase A2 (Lp-PLA2), endothelial dysfunction, arterial stiffness, chronic kidney disease (CKD), non-alcoholic fatty liver disease (NAFLD) and factors related to coagulation/fibrinolysis which are all closely interrelated and increases CVD risks [22]. It has also been suggested that the role of Lp-PLA2 in atherosclerosis may depend on the type of lipoprotein particle with which this enzyme is associated with [23]. For instance, [24,25] stated that the vast majority (80%) of plasma Lp-PLA2 mass binds to low-density lipoprotein (LDL) while a smaller amount is associated with high-density lipoprotein (HDL). Therefore, dyslipidemia promotes an increase in plasma Lp-PLA_2 activity [26]. As a result, smoking-related dyslipidaemia could be reason behind the significant elevation of serum Lp-PLA2 through the induction of low-grade inflammation of the arterial wall. This is because Lipoprotein-associated phospholipase A2 (Lp-PLA2), is an enzyme produced by the macrophages that hydrolyzes phospholipids of oxidized low-density lipoprotein (LDL), releasing oxidized fatty acids and lysophosphatidylcholine, which are potent proinflammatory and prooxidative molecules [27,28]. Given that atherosclerosis is an inflammatory disease which begins in the vascular wall, Lp-PLA2 may have a prominent role in its pathophysiology.

In this study also, no significant difference was observed in the mean serum level of CA-242 in smokers and non-smokers. This could be associated with the significant presence of endogenous estrone and estradiol in smokers. This is in accordance with the population-based study of 590 Rancho Bernardo, California men aged 30-79 years without a history of cardiovascular disease, where cigarette smokers were found to have higher mean of endogenous estrone, and estradiol levels compared to nonsmokers [29]. According to the American cancer society, it is known that women are less likely to develop pancreatic cancer and this is not fully explained by the differences in exposure to the known risk factors. The apparent negative correlation between ET use and pancreatic cancer risk is consistent with the inhibitory effect of estrogen on the growth of preneoplastic pancreatic lesions or transplanted pancreatic carcinoma in rats [30,31]. Therefore, the increase in the levels of estrogen in male cigarette smokers is thought to be the reason for the non-significant decrease in mean serum levels of CA 242 in adult male cigarette smokers. More so, in a study by [32], it was observed that serum CA19-9 values were higher among children than among adults. Most of the subjects used in our work were adults which could be another reason for decreased mean serum of level of CA 242 in adult male smokers.

Furthermore, a positive correlation existed between the serum levels of LP-PLA2 and duration of smoking (years). Same pattern was also observed between the serum levels of LP-PLA2 and age in adult male cigarette smokers. Conversely, the serum levels of CA-242 correlated negatively with age and duration of smoking in adult male cigarette smokers. The possible explanation for this positive correlation observed between the serum levels of Lp-PLA2 and age could be because aging is associated with a progressive decline in numerous physiological processes, leading to an increased risk of health complications and disease. Age is a well-known traditional risk factor, which is generally considered to be non-modifiable for obvious reasons [33]. The physiologic changes of human cardiac aging mainly include left ventricular hypertrophy, diastolic dysfunction, valvular degeneration, increased cardiac fibrosis, increased prevalence of atrial fibrillation, and decreased maximal exercise capacity [34]. Also, cigarette smoking has a remarkable effect on the heart and arterial system, leading to an increase in CVD including atherosclerosis, hypertension, myocardial infarction, and stroke [35]. Aging cardiovascular tissues are exemplified by pathological alterations including hypertrophy, altered left ventricular (LV) diastolic function, and diminished LV systolic reverse capacity, increased arterial stiffness, and impaired endothelial [36]. Atherosclerosis is classified as a disease of aging, such that increasing age is an independent risk factor for its development [37].

A reduction in cardiac output due to decline in function with age stimulates the myocardium compensated by undergoing cardiac hypertrophy; although this may provide short-term enhancement of cardiac output, the long-term effect of hypertrophy diminishes cardiac function [38]. Intrinsic aging of the heart also makes the heart more susceptible to stress and contributes to increased cardiovascular mortality and morbidity in the elderly [39]. With age, cardiomyocytes are more susceptible to stress, including oxidative stress [40]. Smoking therefore, may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through weakening of the antioxidant defense systems [41]. Therefore the increase in oxidative stress due to the increase in reactive oxygen species (ROS) production with age could result in the overall enhancement in the rate of cardiomyocyte death with age as seen in our current study.

CONCLUSION

The increased level of Lp-PLA2 along with its positive correlation with other traditional markers like age and smoking duration suggests that Lp-PLA2 is a suitable biomarker to predict cardiac related diseases among cigarette smokers. This is because, Lp-PLA2 is a more specific cardiac predictor compared to the non-specific conventional biomarkers. We therefore suggest that Lp-PLA2 as an independent advanced predictor of cardiovascular disease be further evaluated using follow-up studies with better sample size in CVDs related cases.

Conflicts of interest

The authors vehemently declare that there is no conflict of interest herein. Therefore, this original research that has neither been published nor being considered for publication elsewhere.

Support

No support in the form of grants or funding was utilized in this research. The study was solely funded by the researchers.

Research constraints

The unavailability of funding was a major drawback in this research as the sample size was reduced to the researchers' capability.

Author's contribution

M.P.O., C.G.O., I.N.C., designed research, M.P.O., N.N.B., E.E.O., performed research, C.R.C, O.E.C., M.V.I., analyzed data, N.N.B., N.K.E., E.O.O., E.R.S., M.V.I., wrote paper.

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