Novel Cardiovascular Risk Markers in Nigerian Cigarette Smokers

Abstract

Background: Cardiovascular disease (CVD) is the leading cause of mortality and morbidity worldwide. While the effect of cigarette smoking on conventional markers that account for <50% of CVD s has been well studied, there are only a few studies on the effect of cigarette smoking on novel cardiovascular (CV) risk markers. **Objective:** To evaluate the effect of cigarette smoking on the novel CV markers such as homocysteine (HCY), lipoprotein (a) (Lp(a)), and C-reactive protein (CRP). **Materials and Methods:** One hundred and forty smokers, 12 ex-smokers, and 84 controls were recruited for the study. A structured questionnaire was used to obtain information on their clinical history, daily cigarette consumption, and duration of smoking. The smokers were further grouped according to the amount of cigarette consumption: light (<5 sticks/day), moderate (6–10 sticks/day), and heavy (>10 sticks/day) and duration of smoking: short (5–10 years), medium (11–20 years), and long (>20 years). HCY was determined by enzyme-linked immunosorbent assay method, and Lp(a) and CRP were determined spectrophotometrically. **Results:** HCY, Lp(a), and CRP were significantly elevated in smokers when compared with control ($P < 0.05$) and they correlated with daily cigarette consumption and duration of smoking. Ex-smokers also exhibited a significant increase in HCY, Lp(a), and CRP level ($P < 0.05$) when compared with the control, but were significantly lower than the current smokers. **Conclusion:** There is a linear relationship between the intensity and duration of cigarette smoking and serum levels of all three novel risk CV markers. These findings suggest that these markers may be an important mechanism by which smoking promotes atherosclerosis.

Keywords: *Cardiovascular diseases, cigarette smoking, C-reactive protein, homocysteine, lipoprotein (a)*

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Introduction

Despite recent advances in the management of dyslipidemia, hypertension, and diabetes mellitus, cardiovascular disease (CVD) remains the leading cause of death worldwide.[1] Although most cardiovascular (CV) events can be attributed to one or more of the aforementioned major risk factors, there remains a significant proportion of the population who will experience an event in the absence of traditional risk factors. It has been estimated that up to 50% of all myocardial infarctions and strokes occur in men and women with low-density lipoprotein (LDL) levels below the recommended goals.[2] Identification of these emerging risk factors also allows for further risk stratification of patients who may be at an intermediate risk of disease and thus may encourage more aggressive therapy.[3-5] Some of these novel risk factors include apolipoprotein B, apolipoprotein A-I, triglycerides and triglyceride-rich lipoprotein remnants, lipoprotein (a) (Lp(a)), homocysteine (HCY), and high-sensitivity C-reactive protein (CRP). Among the CV risk factors, cigarette smoking is responsible for six of the eight leading causes of deaths in the world. According to the World health Organization, the use of tobacco products is increasing worldwide and this epidemic is gradually shifting to the developing world.[6] The relationship between cigarettes smoking and many conventional CV risk factors has attracted much attention. Cigarette smoking has been associated with elevated levels of cholesterol, lower level of high-density lipoprotein cholesterol, and platelet aggregation.[7,8] However, there is still a paucity of data on the effect of cigarette smoking on emerging risk factors for CVD. To the best of our knowledge, this will be the first attempt in Nigeria to evaluate the impact of cigarette smoking on these emerging factors. We, therefore, examined the effect of cigarette smoking on selected emerging risk factor for CVD which includes CRP, HCY, and Lp(a).

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Materials and Methods

This was a population-based case–control study carried out in the three urban areas in northeastern part of Nigeria. The survey was conducted from November 2011 to May 2012. The participants include adult males who were living in the location of the study. The following assumptions were used to calculate the sample size for the smokers. The acceptable margin of error was 5%, with a standard deviation of 1.96 at 95% confidence interval, and a prevalence rate of 9.0% obtained from the 2008 Nigeria Demographic and Health Survey (2008).[9] The sample size of 125 was calculated using a Cochran's formula. We anticipated a participation rate of 80%, and the final sample size was increased to 150.

The participants were informed about the study by trained field workers, who also lived in the same areas. Those who gave verbal or written consent were included in the study. The inclusion criteria included written consent. Participants with clinical conditions such as diabetes, hypertension, tuberculosis, and alcoholics and those on medication known to affect biomarkers of CV, renal, and hepatic diseases were excluded from the study.

The cases were current or former smokers, and the current smokers must have been smoking for at least 5 years and ex-smokers must have ceased from smoking for at least 5 years; the definition of ex-smokers has been varying depending on the type of clinical research and survey instruments utilized in data collection. The Centers for Disease Control in National Health Interview Survey of 2007 defines ex-smoker as someone who has smoked >100 cigarettes in their lifetime.[10] Furthermore, the United Kingdom General Household Survey conducted in 2006 defines ex-smoker as someone who has smoked >100 cigarettes in their lifetime and does not currently smoke.[11] In our study, the rationale for using 5-year cut-off for ex-smokers was based on the following: (1) clinical studies have found that the risk of stroke can fall to that of a nonsmoker after 2–5 of quitting and the risk of coronary heart disease can fall to that of a nonsmoker's after 15 years;[12] (2) large-scale studies such as the Third National Health and Nutrition Examination Survey,[13] the Northwick Park Heart Study,[14] and the MONICA Study[15] have also reported that smoking is associated with a broad range of alterations in systemic immune and inflammation markers and that inflammatory markers returned to baseline levels 5 years after smoking cessation.

The controls were never smokers and must not have been living with a smoker to prevent involuntary smoking. All the individuals were not taking alcohol and had no history of CVD, diabetes mellitus, and other systemic and metabolic diseases. They also had no history of substance abuse, and no history of the use of the following medications: corticosteroids, nonsteroidal anti-inflammatory medication; aspirin, statins, and testosterones replacement.

The data were obtained using a well-structured questionnaire designed for the study. Demographic information, anthropometric measurement, smoking, and other lifestyle factors of each participant were collected. Clinical examination of the participants that included vital signs was done by a medical practitioner to rule out unstable clinical state.

Thereafter, the blood sample from each of the participant was collected after an overnight fast including a light, fat-free diet before the day of collection. The venipuncture was done in the cubital fossa, tourniquet was used, but was released just before collecting the blood sample to avoid artificial increase in concentration of serum lipids and protein. The fasting blood sample of 15 ml was aseptically collected from each participant and poured into lithium heparin and plain bottles respectively. It was left undisturbed for an hour. The blood sample was then centrifuged at ×4000 g for 10 min and the serum/plasma collected was stored at −20°C. The analysis of biochemical parameters was done in 2 weeks. HCY measurement was done by enzyme-linked immunosorbent assay method kits (Diazyme Laboratories, Gregg court, Poway, USA). The CRP and Lp(a) were determined spectrophotometrically with kits (Agappe diagnostics, Switzerland).

The smokers were grouped according to the intensity of smoking (number of cigarette smoked per day):[16] light smokers (1–5 sticks of cigarette daily), moderate smokers (6–10 sticks of cigarette daily), and heavy smoker >10 sticks of cigarette daily) and also according to the duration of smoking: short-term (5–10 years of smoking) smokers, medium-term (11–20 years of smoking) smokers, and long-term (>20 years of Smoking) smokers.

The data obtained were analyzed using the SPSS statistical package version 20 (SPPS Inc., Chicago, IL, USA). Frequency statistics were generated to examine the characteristics of the smokers and nonsmokers. The Student's *t*-test and analysis of variance were used to compare the means and standard deviation of the continuous variables for both the smokers and nonsmokers. Chi-square test was used to test for statistical significance, which was set at a $P < 0.05$.

The permission to conduct research was obtained from the State Ethical Committee before the commencement of the study.

Results

A total of 234 adult males participated in the study, 140 were current cigarette smokers, and 12 were ex-smokers. Eighty-four age-matched apparently healthy non-smokers were also recruited from the same areas to serve as control [Table 1]. The age of individuals ranges from 20 to 65 years.

Plasma CRP, HCY, and Lp(a) concentrations were all significantly elevated ($P < 0.05$) in smokers and ex-smokers

when compared with control. However, plasma CRP, HCY, and Lp(a) were significantly reduced ($P < 0.05$) in ex-smokers when compared with current smokers [Table 2].

Plasma CRP, Lp(a), and HCY concentrations were all significantly elevated ($P < 0.05$) in smokers with increase in the number of cigarettes smoked daily [Table 3]. In the same vein, plasma CRP, HCY, and Lp(a) concentrations were significantly elevated ($P < 0.05$) with increase in the duration of smoking compared to the controls [Table 4].

Discussions

The results of this study showed that HCY, Lp(a), and CRP level were increased by cigarette smoking that depends on the dose and duration of smoking of the respondents.

In this study, the mean serum level of HCY was three times higher in smokers when compared with nonsmokers, while the mean level in former smoker was two times higher than in the nonsmoker. The level of HCY was also found to increase with the level of cigarette consumption and the

duration of smoking of the respondents. This finding is consistent with earlier studies.[17-20] HCY is an independent biochemical marker of CVD. The increased level of plasma HCY level suggests that cigarette smoking may cause the inactivation of methionine synthase, the enzyme required for HCY remethylation. Although the mechanism is unclear, smoking may directly inactivate enzymes of HCY remethylation such as methionine synthase.[21] Smoking is accompanied by changes in plasma thiol redox status, possibly due to a higher formation of reactive oxygen species.[22] Several studies have shown that HCY impairs the production of nitric oxide, an endogenous vasodilator.[23] This may contribute to the impaired endothelium-dependent vasodilation, which could further enhance the development of vascular injury.

The mean serum level of Lp(a) was five times higher in current smokers when compared to the nonsmokers, while the mean level of Lp(a) in former smokers was three times higher than in the nonsmokers. The mean serum level of

Table 1: Participants' characteristics

Characteristics	Smokers (*n*=140)	Ex-smokers (*n*=12)	Nonsmokers (*n*=80)
Body mass index (kg/m^2)	21.54±0.44	23.54±0.84	23.94±0.31
Systolic BP (mmHg)	118.21±0.69	114.75±2.29	114.31±0.84
Diastolic BP (mmHg)	76.09±0.63	73.50±1.93	74.60±0.86

BP: Blood pressure

Table 2: Selected emerging risk factors of cardiovascular disease of smokers and ex-smokers

Subjects	Plasma C-reactive protein concentration (mg/l)	Plasma lipoprotein-a concentration (mg/dl)	Plasma homocysteine concentration (µmol/L)
Control (*n*=84)	1.91±0.08[a]	9.79±0.67[a]	7.88±0.41[a]
Smokers (*n*=140)	3.72±0.07[b]	44.91±1.40[b]	20.54±0.65[b]
Ex-smokers (*n*=12)	2.42±0.11[c]	29.75±1.00[c]	14.25±1.14[c]

Values with different superscripts are significantly different at *P*<0.05, values are mean±SEM. SEM: Standard error of mean

Table 3: Effect of level of cigarette smoking on plasma concentrations of emerging biomarkers of cardiovascular risk

Subjects	Plasma C-reactive protein concentration (mg/l)	Plasma lipoprotein-a concentration (mg/dl)	Plasma homocysteine concentration (µmol/L)
Control (*n*=84)	1.91±0.08[a]	9.79±0.67[a]	7.88±0.41[a]
Light smokers (*n*=26)	3.20±0.12[b]	28.62±1.43[b]	16.23±1.18[b]
Moderate smokers (*n*=66)	3.56±0.08[c]	42.67±1.70[c]	19.33±0.71[c]
Heavy smokers (*n*=48)	4.23±0.11[d]	56.83±2.16[d]	24.54±1.06[d]

Values with different superscripts are significantly different at *P*<0.05, values are mean±SEM. SEM: Standard error of mean

Table 4: Effects of duration of cigarette smoking on plasma concentrations of emerging biomarkers of cardiovascular risk

Duration	Plasma C-reactive protein concentration (mg/l)	Plasma lipoprotein (a) concentration (mg/dl)	Plasma homocysteine concentration (µmol/L)
Control (*n*=84)	1.91±0.08[a]	9.79±0.67[a]	7.88±0.41[a]
5-10 years (*n*=53)	3.34±0.08[b]	34.62±1.45[b]	17.92±0.85[b]
11-20 years (*n*=48)	3.79±0.11[c]	50.25±2.31[c]	20.13±1.07[b]
>20 years (*n*=39)	4.67±0.12[d]	59.38±2.47[d]	25.67±1.23[c]

Values with different superscripts are significantly different at *P*<0.05, values are mean±SEM. SEM: Standard error of mean

Lp(a) also increased with level of cigarette consumption and duration of smoking of the respondents. Our findings are consistent with previous studies that investigated the association between smoking and lipid profile parameters.[24-26]

Lp(a) was first described by Berg in 1963 and has been reported as the lipoprotein with the strongest atherogenic effect.[27] Circulating Lp(a) levels are primarily influenced by genetic factors without significant dietary or environmental effects.[28] Thus, the mechanism of association between Lp(a) and cigarette smoking remain unclear. Although the specific function of Lp(a) is yet unknown, studies have shown a direct relationship between elevated plasma lipoprotein with an increased risk of atherosclerotic diseases such as coronary heart disease, arterial occlusive disease, and cerebral stroke.

The inhibition of transforming growth factor 1 (TGF-1) activation is another mechanism via which Lp(a) contributes to the development of atherosclerotic vasculopathy. TGF-1 is subject to proteolytic activation by plasmin, and its active form leads to an inhibition of the proliferation and migration of smooth muscle cells, which plays a central role in the formation and progression of atherosclerotic vascular diseases.[27] If TGF-1 fails to be activated, due to Lp(a) accumulation in the vascular wall, it is associated with an increased proliferation and migration of the smooth vascular muscle cells and the formation of atherosclerotic lesions.[27] The striking structural similarity with plasminogen suggests a function for Lp(a) in thrombogenesis. According to Cooper *et al.*,[29] Lp(a) strongly contributes to coronary heart disease when LDL-C and lipoprotein are both elevated. Thus, the elevated Lp(a) in smokers suggests that cigarette smoking promotes thrombogenesis.

Elevated levels of serum CRP that was both dependent on the number of cigarette smoked per day and duration of smoking suggest that inflammation plays a major role in atherosclerosis seen in cigarette smokers. This finding is similar to what was reported by other previous studies.[30]

Vascular inflammation plays a major role in the pathogenesis of atherosclerosis and mediates various stages of atherosclerotic plaque development, from lipids, streak formation, to plaque rupture and destabilization that precedes the clinical syndromes of CVD s.[2] Elevated levels of serum CRP that was both dependent on a number of cigarette smoked per day and duration of smoking suggest that inflammation plays a major role in atherosclerosis seen in cigarette smokers.

Elevated levels of CRP, HCY, Lp(a) suggest a strong direct relation between coronary heart disease and cigarette smoking. The elevated plasma levels of HCY, lipoprotein-a and CRP in ex-smokers but significantly reduced when compared with current smokers suggest that ex-smokers though still predisposed to CVD, are at a vantage position of a reduced risk compared to the current smokers.

The limitation of the study is the sampling technique which is subjected to selection bias, misclassification of the former smokers because of recall bias by the respondents as well as the inability to obtain information on nutritional parameters, especially plasma folate and vitamin B12 levels, which are factors known to influence plasma HCY levels. The strength of the study is sample size of the study.

In conclusion, from this study, we found elevated HCY, Lp(a), and CRP levels in smokers that are dependent on dose and duration of smoking. Our study also demonstrated a linear relationship between cigarette smoking and elevated levels of all three novel risk CV factors. These findings thus suggest that inflammation, elevated Lp(a), CRP, and hyperhomocysteinemia are the mechanisms through which cigarette smoking promotes atherosclerosis.

Conflicts of interest

There are no conflicts of interest.

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