



Research Article

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Levels of cholesteryl-ester transfer protein in female bean cake fryers in Nnewi metropolis

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Abstract

Background: Bean cake is prepared from peeled beans deep fried in oil. High heat and firewood are employed to obtain the product. Females may be exposed to occupational health hazard emanating from the constituents of firewood smoke. **Objective:** This work was a prospective cross-sectional study aimed at assessing the cardiac functions in female bean cake fryers using cholesteryl ester transfer protein (CETP) as a biomarker. **Materials and methods:** A total of 80 subjects; comprising 40 female bean cake fryers exposed to firewood smoke for a minimum of 2 years as the test group and 40 female non-bean cake fryers as control all within the age range of 18 to 65. The socio-demographic data were obtained using well-structured questionnaire. The serum levels of CETP was determined using Enzyme Linked Immunosorbent Assay (ELISA) technique. **Result:** There was no significant difference in the mean serum levels of CETP ($p = 0.515$) of the test subjects compared with the control. There was a weak negative correlation between the levels of CETP ($r = -0.142$, $p = 0.438$) with age in this group. No significant correlation was observed between the levels of CETP ($r = -0.054$, $p = 0.770$) with duration of exposure in the test subjects. **Conclusion:** The non-significant difference of Cholesteryl-ester transfer protein (CETP) amongst female bean cake fryers suggests that bean cake fryers are not predisposed to cardiac disorder. However, we recommend that Cholesteryl-ester transfer protein should not be used as a sole parameter to assess cardiac function.

Keywords: Cardiac function, Cholesteryl ester transfer protein, Bean Cake, Smoke.

INTRODUCTION

Bean cake is also known as "Akara". It is a dish made from peeled beans formed into a ball and fried in oil. It is found in West African and Brazilian cuisine. The food was taken to other parts of the world by slaves from West Africa countries. It is a street food and can be found in various forms in Nigeria, Ghana, Togo, Benin, Mali, Gambia, and Sierra Leone [1]. Bean cakes are made with cooked and mashed black-eyed bean peas seasoned with salt, chopped onion and pepper moulded into the shape of a round scone and deep fried in oil which could be palm oil, groundnut oil, olive oil, soya oil etc. in the front of the customers [2].

The trade is done on the streets and the female make use of firewood. High heat is needed to produce the bean cake. Firewood smoke has been associated with anthropogenic pollution, evidenced by the soot found in prehistoric caves [3]. Many mechanisms like; disruption of cell adhesion and loss of epithelial cadherin in polarized airway epithelial [4] have been proposed as some of the basis for the pathological consequences of smoke inhalation, including increased permeability and epithelial damage [5]. The continuous inhalation of smoke poses a health challenge to the female that are involved in such trade [6,7].

Smoke from firewood constitutes a mixture of gas, liquid and solid; it contains carbon monoxide, fine particles and soot. Since the trade is done in an open-air space, the carbon monoxide constituent is reduced. The main hazardous substance is the fine particles [8]. The fine particles are a combination of liquid and solid which mix up together to cause health effects when inhaled over a period of time [9]. Fine particles can induced cytotoxicity [10,11] which may be involved in tissue damage in the lungs and in other organs, whereas the carcinogenic risk primarily is linked to genotoxicity [12].

There is also the presence of organic compounds in the wood that can also cause a harmful effect. Such compounds include; benzene, formaldehyde, acetaldehyde, acrolein, polycyclic aromatic hydrocarbon (PAH) [13,14]. The most abundant PAHs in wood smoke emissions are naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene [15]. With regards to carcinogenicity,

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benzo (a) pyrene (B (a) P) and fluoranthene seem to be the most important compounds in wood smoke emissions [16]. The severity of the effect or harm caused by firewood depends on the amount of time you spend around it [17].

The formation of soot is very complex and Bockhorn has given a well-adapted soot formation pathway, via polycyclic aromatic clusters, particle inception, surface growth and coagulation [18]. Carbon aggregates of soot may be emitted during incomplete combustion in conventional wood stoves and masonry heaters [19], from open fireplaces and firewood [20,21]. Soot also contains some percent of hydrogen, originating from the primary aromatic compounds.

Cholesteryl ester transfer protein (CETP) is also called plasma lipid transfer protein. The activities of plasma lipid transfer protein have a major effect on the composition, size, and concentration of the lipoproteins [22]. It is involved in the transfer of lipid and it facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins. It collects triglycerides from very low-density lipoprotein (VLDL) or low-density lipoprotein (LDL) and exchanges them for cholesteryl esters from high-density lipoprotein (HDL) and vice versa [23,24]. Cholesteryl ester transfer protein is a transport protein as it is involved in the exchanges of the lipoproteins. CETP expression is increased in atherosclerosis [25].

Statement of research problem

Female bean cake fryers are exposed to occupational hazards from smoke and heat emanating from firewood. Smoke emitted from firewood is a serious public health concern [26]. Studies have shown that long time exposures to such can affect the health of people [9,27]. There is evidence that complication of smoke inhalation injury range from increases in airway pressure to the destruction of the small airways. There is no regulatory policy or guidelines on this activity which may result in health complications. This study attempted to evaluate some environmental health risk in female bean cake fryers in Nnewi metropolis.

Justification of research problem

Smoke and heat from bean cake frying have posed some health challenges [28]. In addition to these chronic health effects, inhalation exposures can also have significant acute impacts. For example, the incidence of heart failure exacerbation and the number of hospital visits for asthma were increased in counties in North Carolina that experienced higher concentrations of air pollution from a wildfire in the eastern part of the state [29]. Animal and human in vitro cell studies link polycyclic aromatic hydrocarbon (PAH) exposure to the generation of oxidative stress, DNA damage and inflammation via activation of the aryl hydrocarbon receptor in the metabolism and secretion of the PAHs by cytochrome P450 (CYP) enzymes [30]. Epidemiological studies have shown an association between urinary PAH levels and Type 2 Diabetes Mellitus development [31].

This study sought to assess the cardiac, pancreatic and thyroid function of female bean cake fryers in Nnewi metropolis. The study may provide evidence-based data with the potentials to help in sensitizing the general public on occupational hazards of the trade. Information provided in this study may serve as a valuable guide in policy formulations in public health. The aim of the study was to evaluate the cardiac function in female bean cake fryers using the levels of cholesteryl ester transfer protein as biomarker.

MATERIALS AND METHODS

Research design

This was a prospective cross-sectional study designed to evaluate the levels of cholesteryl ester transfer protein (CETP) in female bean cake fryers exposed to firewood smoke for a minimum of 2years and control

in Nnewi metropolis. A total of 80 subjects were recruited using purposive sampling technique. This comprised 40 female bean cake fryers as test subjects and 40 female non-bean cake fryers as control subject within the age range of 18 to 65. Information on socio-demographic, medical history, lifestyle, occupation and duration of trade was obtained using a questionnaire. Informed consent was sought and obtained from all participants prior to the study. After which, females bean cake fryers and healthy females that are not bean cake fryers were included whereas females with underlying cardiac diseases such as atherosclerosis, subject with diabetic mellitus, pancreatic disorders, thyroid dysfunction, thyrotoxicosis, autoimmune disorders and other chronic diseases and subjects outside the age bracket of 18 to 65years were excluded from the study. This study was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria.

Ethical approval

Ethical approval for this research was obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Sample collection

Five (5) ml of venous blood was collected aseptically from each of the participants and dispensed into plain containers. The samples were allowed to clot and centrifuged at 5000 r.p.m for 5 minutes. Serum was separated and stored at -20°C prior to analysis.

Laboratory analysis

The samples were analyzed in the Chemical Pathology Laboratory of Nnamdi Azikiwe Teaching Hospital, Nnewi, Anambra state, Nigeria.

Determination of cholesteryl ester transfer protein (CETP)

CETP levels were evaluated using the method as described by Cole *et al.* [32]. This procedure is essentially an Enzyme-Linked Immunosorbent Assay (ELISA).

Principle

This is an enzyme-linked immunosorbent assay that follows a double-antibody sandwich one-step process to assay cholesteryl ester transfer protein (CETP) in human serum, blood plasma, saliva, urine, and other biological fluids. The standard, test sample and HRP-labeled cholesteryl ester transfer protein (CETP) antibodies are added to wells which are pre-coated with cholesteryl ester transfer protein (CETP) antibody. After incubation and washing to remove the uncombined enzyme, chromogen solution A and B are added. The colour of the liquid will change into blue and at the effect of an acid, the colour finally becomes yellow. The colour change is measured spectrophotometrically at a wavelength of 450nm. The concentration of cholesteryl ester transfer protein (CETP) in the samples is then determined by comparing the O.D (optical density) of the samples to the standard curve.

Procedure for CETP determination

- All reagents and samples were brought to room temperature before starting the assay procedure.
- The standard, blank and sample wells were set.
- Using a micropipette, 50µl of standard, 10µl of samples were added to their assigned wells, and 40µl of sample diluent was added to sample wells.
- Then 100µl of HRP-conjugate reagent was added to all the wells.

- The microplate wells were covered and incubated for 60 minutes at 37°C.
- The content of the wells were discarded by aspiration.
- Then 400µl of Wash solution was added to the microplate wells and aspiration was done. This process was repeated for additional 4 times for a total of 5 washes.
- After the last wash, the remaining Wash solution was removed by decantation. The microplate was inverted and blotted against clean absorbent paper.
- To the content of the microplate wells 50µl of chromogen solution A was added followed by 50µl of chromogen solution B to all the wells.
- It was mixed gently and incubated for 15 minutes at 37°C.
- The reaction was stopped by adding 50µl of Stop solution to each well. The colour in the wells changed from blue to yellow.
- The optical density (O.D.) was measured at 450nm using a microtiter plate reader and result was read within 15 minutes of adding the Stop solution.

Statistical analysis

The data collected was analyzed statistically using the Student's t-test and the analysis of variance (ANOVA). Values were deemed significant if $p < 0.05$. Correlations of parameters were elucidated using the Pearson's correlation coefficient.

RESULTS

Table 1: Mean levels of cholesteryl ester transfer protein (CETP) in test and control groups

Parameters	Test subjects	Control subjects	T-test	p-value
CETP	2.77±1.56	2.55±1.48	0.654	0.515

No significant difference was observed in the mean value of CETP ($p = 0.515$), GAD 65 ($p = 0.163$), TSH ($p = 0.364$) and T4 ($p = 0.136$) in the test subjects compared with the control. However, the mean value of T3 was significantly higher in the test subjects compared with the control ($p = 0.03$).

Table 2: Correlation studies of the mean levels of CETP with age in the test group

Parameters	r	p-value
Age vs CETP	-0.142	0.438

There was a non-significant negative correlation between the levels of CETP ($r = -0.142$) with age in this group.

Table 3: Correlation studies of the levels of CETP with duration of exposure in test group

Parameters	r	p-value
Duration vs CETP	-0.054	0.770

No significant correlation was observed in the levels of CETP ($r = -0.054$) with duration of exposure in the test subjects.

DISCUSSION

Firewood is employed in the frying of bean cake. The impact from the constituents of firewood has raised major health concerns [33]. The constituents are known and have been recorded to alter the functionality of the biological system [34-36]. In this study the levels of cholesteryl ester transfer protein (CETP) were evaluated in female bean cake fryers in Nnewi metropolis.

The findings of this study revealed that no significant difference was observed in the mean value of CETP in the test subjects compared with the control. One of the hazardous constituents of firewood smoke is fine particle; exposure to fine particle results in small increase in cardiovascular mortality [37], though the result of this study showed no significant difference in the mean level of CETP, which could be due to other factors. The effects of fine particle may involve pro-thrombotic mechanism such as elevation of fibrinogen, increased platelet aggregation and alteration of arterial vasoconstriction [38,39]. The mechanism of action includes; free radical production, oxidative stress, cytokine release, inflammation, stimulation of endotoxin-mediated damage, stimulation of capsaicin receptors, autonomic nervous system activity [40-42]. Studies with animal have shown that exposure to fine particle has a casual relationship with atherosclerosis [43,44].

Meanwhile this study revealed no significant change in the level of CEPT this could be as a result of other factors like duration spend with firwood smoke, the open space in which the trade is conducted, the

number of participants recruited for the study. Dullart *et al.* reported higher CETP activity in Insulin-dependent diabetes mellitus (IDDM) men that smoke compare to non-smokers. This is contrary to the result of this study [45]. CETP level in plasma is associated with HDL-cholesterol level [46] i.e. inversely related, this is anti-protective against cardiovascular disease. Skoczynska *et al.* reported no significant difference in CETP activity between smokers and non-smoker in hypothyroid subjects [47]. Arian *et al.* reported no statistical association between CETP gene polymorphism and the smoking status [48]. Though Smoking disturbs lipoprotein metabolism by increasing insulin resistance and lipid intolerance, and is implicated in the production of low density lipoprotein (LDL) [49]. Decreased CETP level or activity is associated with hypothyroidism [47].

There was a weak negative correlation between the levels of CETP ($r = -0.142$, $p = 0.438$) with age in this group. CETP level have a positive correlation with development of atherogenic disturbances [50]. Balcan *et al.* [51] reported a negative and statistically significant relationship between the time of starting to cook with fossil fuel in smaller ages and existence of both obstructive and restrictive pulmonary functions. In another study it has been established that a positive association exists between the risks of cardiovascular and Type 1 diabetes [52,53]. These suggest that both smoking and exposure to smoke are associated with an increased risk of cardio-metabolic risk factors and metabolic syndrome in adolescents. Previous studies [54-56] have confirmed that the relationship between smoking, low physical activity levels, lipid profile is worse in youths with type 1 diabetes mellitus compared to

adult smokers. Ertunc *et al.* reported that the age of menopause has an inverse relationship in female passive smokers [57].

In this study, no significant correlation was observed between the levels of CETP ($r = -0.054$, $p = 0.770$) with duration of exposure. Balcan *et al.* reported no statistically significant correlation between hours per day, and weeks per month spend exposed to biomass smoke and altered pulmonary functions [51]. Skoczynska *et al.* [47] reported a linear negative correlation exists between TSH and CETP; both parameters have no correlation with duration of exposure in the test subjects [47]. Hanna *et al.* reported no correlation of creatinine levels with duration in cigarette smokers [58].

CONCLUSION

The non-significant difference of Cholesteryl-ester transfer protein (CETP) amongst female bean cake fryers suggests that bean cake fryers are not predisposed to cardiac disorder. However, we recommend that Cholesteryl-ester transfer protein should not be used as a sole parameter to assess cardiac function. More so, larger sample size with prolonged duration of bean cake frying be evaluated to validate our research finding.

Conflict of interest

There is no conflict of interest.

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