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The Status of Cancer Antigen 15-3 and Lipoprotein-Associated Phospholipase A2 in Menopausal Women in Nnewi Metropolis

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Abstract *Background:* Menopause is the time of life when menstrual cycles cease. Menopausal women are constantly exposed to biochemical and metabolic changes as some hormonal levels drop. These may subsequently give rise to some pathologic conditions. *Aim:* This study was aimed at assessing the risk of breast cancer and cardiovascular disease in menopausal women in Nnewi metropolis, Anambra State, Nigeria using Cancer Antigen (CA) 15-3 and Lipoprotein Associated Phospholipase A2 (Lp-PLA2) as markers. *Materials and Methods:* A total of 135 (between 18 and 65 years) subjects were recruited for this study comprising 50 menopausal and 40 pre-menopausal subjects for the determination of CA 15-3; 30 menopausal and 15 pre-menopausal women for the measurement of Lp-PLA2 using the Enzyme Linked Immuno-sorbent Assay (ELISA) technique. *Results:* The mean serum levels of CA 15-3 and Lp-PLA2 were significantly higher ($P<0.05$) in menopausal subjects compared with the pre-menopausal group. However, non-significant positive correlation existed between the mean serum level of Lp-PLA2 and age in menopausal women ($r= 0.084$, $p=0.660$) whereas, the mean serum level of CA 15-3 significantly correlated positively with age in menopausal women ($r= 0.690$, $p<0.001$). *Conclusion:* The significantly elevated serum levels of Lp-PLA2 and CA 15-3 in menopausal subjects compared with the premenopausal group in addition with the strong positive correlation between CA 15-3 and age in menopausal women suggests increased breast cancer and cardiovascular risks in menopausal women especially in advanced age.

Keywords breast cancer, cardiovascular diseases, menopause, Carbohydrate Antigen 15-3, Lipoprotein-Associated Phospholipase A_2

Introduction

Menopause is defined as a physical event in which there is at least twelve months of amenorrhea caused by depletion of ovarian function [1]. It is the permanent cessation of menstruation at the end of reproductive life due to

loss of ovarian follicular activity [1]. Menopause does not occur suddenly. A phase called perimenopause usually begins a few years before the last menstrual cycle. The average age of women at menopause today is around 51 years (although it can occur as early as 40 to as late as the early 60s) [2]. Early menopause tends to occur among women who have never had children and women who smoke [3]. Since women now have a life expectancy of more than 80 years, most of them can expect to live some 30-40 years of their life in the postmenopausal state [4]. The major consequences of menopause are related primarily to estrogen deficiency [4]. Menopause is not a disease however, many conditions are associated with lower levels of the female hormone estrogen, including heart disease and osteoporosis among other problems. Fortunately, effective treatments are available for these conditions [5,6].

Menopause often involves troublesome symptoms, including vasomotor symptoms, vaginal dryness, decreased libido, insomnia, fatigue, and joint pain [7]. Some symptoms, however, may be improved with treatment. Menopause is not a disease but the symptoms and their severities which are mainly subjective can be very challenging. Hot flashes, for example are associated with decreased quality of life [8].

Breast cancer is a life threatening malignancy which is most common cancer among women and the second leading cause of cancer death in women today [9]. Breast cancer is a cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, Fluid coming out from the nipple, a newly inverted nipple, or a red or scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin [10]. Mutations that can lead to breast cancer have been experimentally linked to estrogen exposure [11]. Numerous serum tumor markers have been described for breast cancer, including members of the MUC1 family of mucin glycoproteins (e.g., CA 15-3, BR 27.29 and MCA, CA 549), Carcino Embryonic Antigen (CEA), oncoproteins (e.g. HER-2/c-erbB-2) and cytokines (e.g., tissue polypeptide antigen and tissue polypeptide-specific antigen) [12].

Cancer antigen (CA) 15-3 is a high molecular weight (> 400kD) glycoprotein which belongs to a subgroup of polymorphic epithelial mucins (PEM) [13]. These mucins are normally found in the luminal secretion of glandular cells and do not circulate in the blood. When these cells become malignant and their basal membranes permeable, PEMs are detectable in serum using the CA 15-3 assay. CA15-3 has been proven to be the most sensitive and specific tumor marker for breast cancer [14]. Although it is also elevated in other benign and malignant diseases such as colorectal cancer, lung cancer, cirrhosis, hepatitis, and benign breast disease, it is the most relevant tumor marker in breast cancer in combination with the carcinoembryonic antigen (CEA) [15].

Cardiovascular disease (CVD) is a class of diseases that involve the heart or blood vessels. Cardiovascular disease includes coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack) [16]. CVD is associated with many risk factors which include age, gender, tobacco use, physical inactivity, excessive alcohol consumption, unhealthy diet, obesity, genetic predisposition and family history of cardiovascular disease, raised blood pressure (hypertension), raised blood sugar (diabetes mellitus), raised blood cholesterol (hyperlipidemia), undiagnosed celiac disease, psychosocial factors, poverty and low educational status, and air pollution [17]. Estrogen is thought to contribute to premenopausal women's tendency to have lower systolic blood pressure, higher levels of HDL cholesterol, and lower triglyceride levels than men [18]. Thus, estrogen withdrawal could confer risks of CVD in menopausal women.

Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) is a recently described and potentially useful plasma biomarker associated with cardiovascular disease [19]. Lp-PLA2 is an enzyme produced in atherosclerotic plaque by inflammatory cells, linked to LDL, HDL and VLDL. The binding of Lp-PLA2 to a specific lipoprotein fraction renders it more atherogenic. Lp-PLA2 has been demonstrated by increasing evidence as a novel "ideal" marker for CVD as of its high specificity for vascular inflammation and low biologic variability [20]. The enzyme, originally named platelet-activating factor acetylhydrolase (PAF-AH), has two prominent biological activities. First, it inactivates the prominent proinflammatory mediator PAF-AH. Second, Lp-PLA2 hydrolyzes oxidatively modified polyunsaturated fatty acids producing lysophosphatidylcholine (LysoPC) and oxidized nonesterified fatty acids (OxNEFA). OxNEFA have potent monocyte chemotactic activity and LysoPC upregulates inflammatory mediators, including cytokines, and adhesion molecules [20]. Lp-PLA2 is secreted by monocytes, macrophages, T lymphocytes

and mast cells and catalyzes the hydrolysis of oxidized LDL (OX-LDL) [21]. There has been a growing interest in Lp-PLA2 because of its key role in lipid metabolism and in initiating inflammation [22].

Statement of Problem

Variety of physiological changes occurs in women as they progress into menopause. These changes sometimes affect the level of some substances in the body which in turn may increase or decrease the risk of developing certain diseases and hence the need to monitor such changes. After menopause, lipid and lipoprotein metabolism changes and menopausal women are at greater risk of cardiovascular disease compared to fertile women [23]. These changes cause menopausal women to be exposed to more oxidative stress than fertile women hence, the need to carry out a study on Lp-PLA2 and CA 15-3 in menopausal women. This can help in the diagnostic, therapeutic and monitoring purposes and therefore contribute to the reduction of morbidity and mortality posed by CVDs and cancer.

Justification of Study

Cancer Antigen (CA 15-3) and Lipoprotein-Associated phospholipase A2 (Lp-PLA2) are biomarkers which are used in the diagnosis of cancer (such as breast cancer) [14] and cardiovascular diseases [19] respectively. Women at the post-menopausal stage are at higher risk of developing complications leading to these diseases in relationship to women at pre-menopausal stage [13]. The classic prognostic markers in breast cancer such as axillary lymph node status, tumor size, histological grade, and receptor expression, require tissue sampling, are costly and cannot by themselves predict the risk of development of distant metastasis and outcome in patients with breast cancer [15]. Tumor markers that can accurately predict overall survival, which can identify the group of patients needing close follow up and those who will benefit most from adjuvant therapy, are needed. Serum CA 15-3 has been the most frequently investigated tumor marker in breast cancer [13] while Lp-PLA2 has been demonstrated to be a suitable marker to predict CVD thus, may be useful in evaluating the risk of developing breast cancer and cardiovascular diseases in menopausal women.

Aim of study

This study is aimed at assessing the risk of breast cancer and cardiovascular disease in menopausal women using CA 15-3 and Lp-PLA2 as biomarkers.

Specific objectives

1. To evaluate the levels of CA 15-3 and Lp-PLA2 in menopausal and premenopausal women.
2. To correlate the levels of these parameters with age in pre-menopausal women.
3. To correlate the levels of these parameters with age in menopausal women

Materials and Methods

Study Area

This study was conducted in Nnewi, Anambra state, South East of Nigeria.

Study Design

This is a case-control study designed to evaluate the serum levels of CA 15-3 and Lp-PLA2 in menopausal women in Nnewi, Anambra State, Nigeria. Subjects within the age range of 18 to 65 were recruited by convenient random sampling. Written consents were obtained from participants and questionnaires were administered. A total of 135 subjects were recruited for the study. These comprised of 50 menopausal and 40 pre-menopausal women for the evaluation of CA 15-3; 30 menopausal and 15 pre-menopausal of the total participants were used for the evaluation of Lp-PLA2.

Inclusion and Exclusion Criteria

Pre-menopausal and menopausal women within the age bracket of 18-65. Women outside the age bracket of 18 to 65 years; subjects with confirmed history of breast cancer and or cardiovascular diseases and individuals with chronic diseases such as Human Immunodeficiency Virus and Sickle Cell Disease.

Informed Consent

Consent of the subjects was sought and obtained prior to study.

Ethical Approval

The ethical approval for this research was obtained from Ethics Committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University (ERC/FHST/NAU/2018/164).

Collection of Samples

Five (5) ml of venous blood was aseptically collected from each of the subjects and dispensed into plain tubes. The samples were allowed to clot after which centrifugation was performed at 5,000 rpm for 5 minutes and serum separated for the evaluation of CA 15-3 and Lp-PLA2 levels.

Estimation of cancer antigen 15-3 and Lipoprotein-associated phospholipase A2

The levels of cancer antigen 15-3 and Lipoprotein-associated phospholipase A2 were estimated by Sandwich Enzyme linked immunosorbent assay (ELISA) technique as described by [24].

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), Version 20.0. The data generated were presented as mean ± standard deviation. Differences among groups were assessed with a one-way analysis of variance (ANOVA), while differences between groups were tested with student T-test. Associations between continuous variables were described by Pearson's correlation coefficients. Significance was accepted at $P<0.05$.

Results

In Table 1, there was a significant increase in the mean serum levels of CA 15-3 and Lp-PLA2 in menopausal women compared with the pre-menopausal group ($P<0.05$).

Figure 1: A strong positive correlation was observed between the levels of CA 15-3 and age in menopausal women ($r= 0.690$).

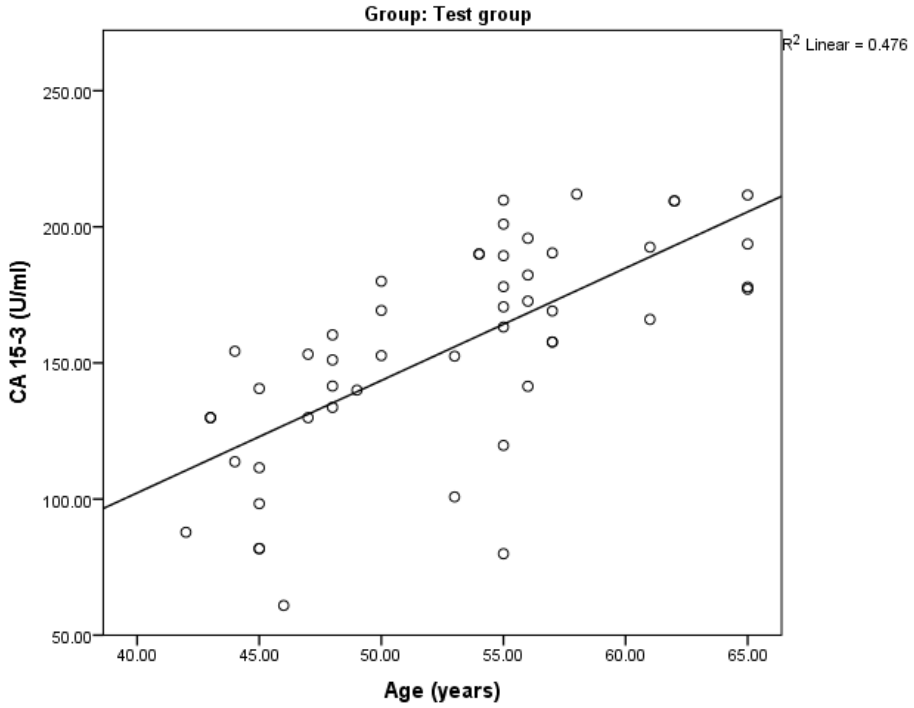
Figure 2: No correlation existed between the levels of Lp-PLA2 and age in menopausal women ($r= 0.084$).

Figure 3: A weak positive correlation was observed between the levels of CA 15-3 with age in premenopausal women (control group) ($r= 0.109$)

Figure 4 There was a weak positive correlation between the levels of Lp-PLA2 with age in premenopausal women ($r= 0.220$).

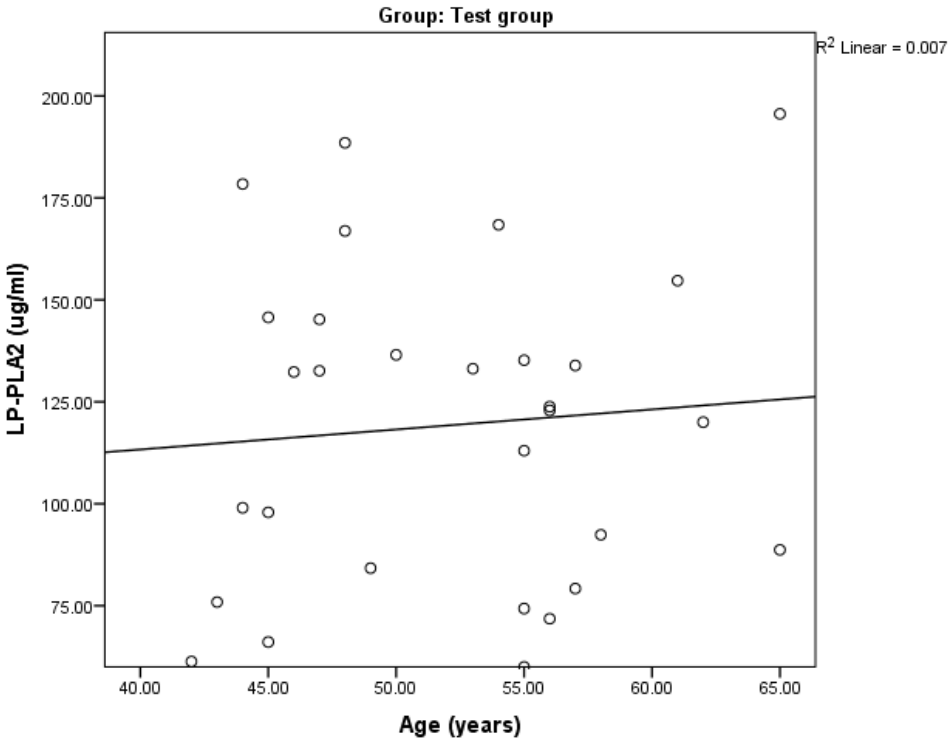
Table 1: Levels of CA-15-3 and Lp-PLA2 in postmenopausal and premenopausal subjects (Mean ± SD)

Parameters	Test group	Control group	t-test	p-value
Age (years)	52.84 ± 6.53	26.85 ± 6.49	18.814	<0.001
CA 15-3 (U/ml)	124.28 ± 38.12 (n= 50)	74.70 ± 38.41 (n=40)	6.478	<0.001
LP-PLA2 (µg/L)	119.25 ± 38.96 (n=30)	66.35 ± 31.77 (n=15)	4.549	<0.001



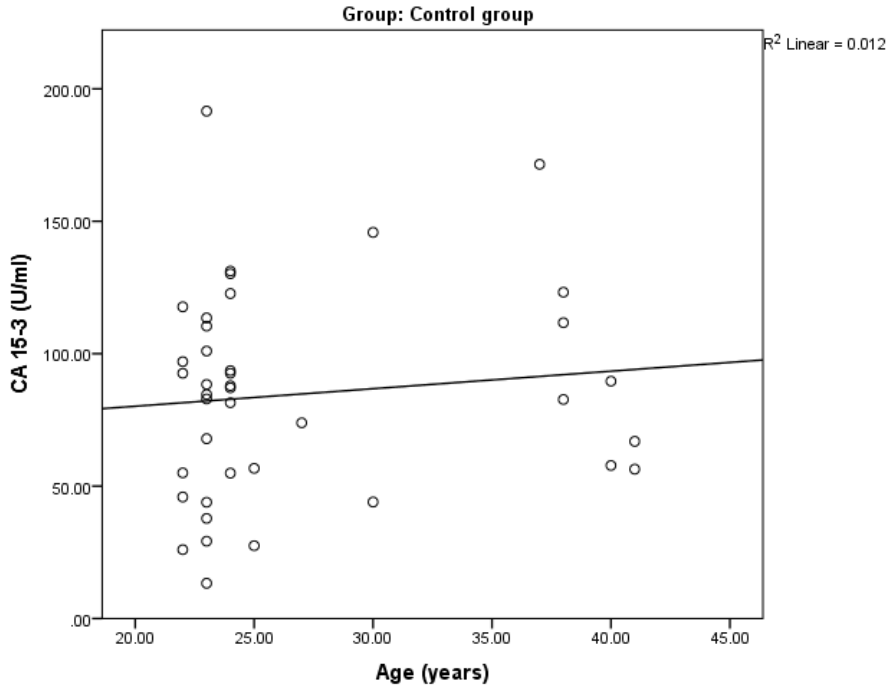
r= 0.690

Figure 1: Correlation of the levels of CA-15-3 (U/ml) with age in menopausal women



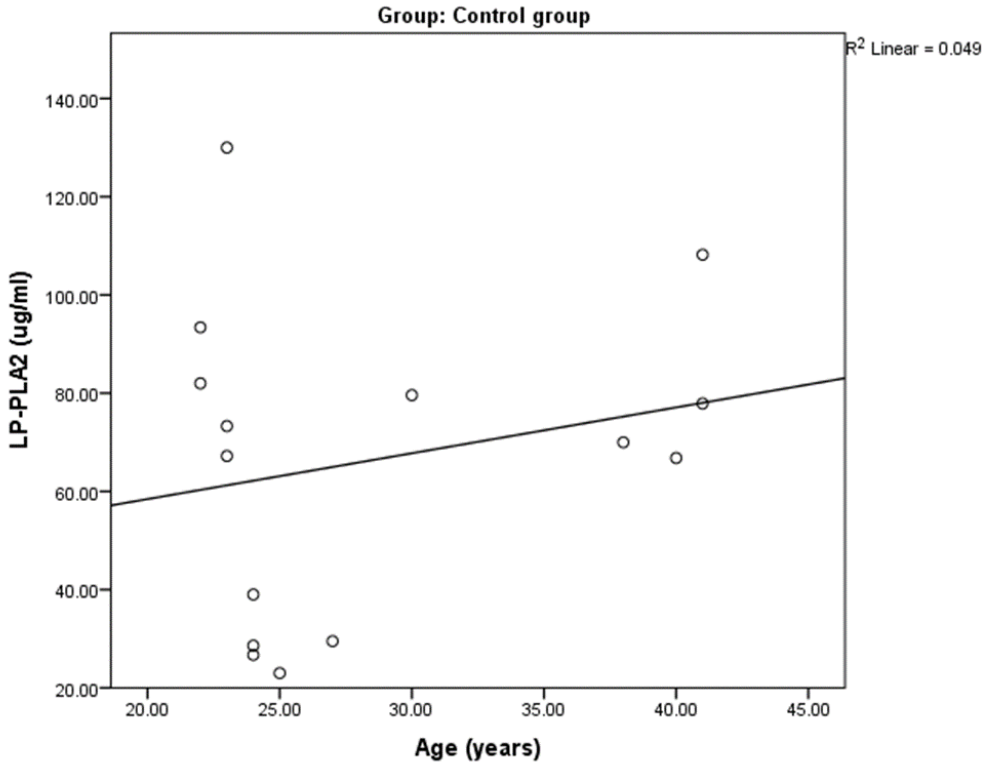
r= 0.084

Figure 2: Correlation of the levels of Lp-PLA2 (µg/L) with age in menopausal women



r= 0.109, p=0.503

Figure 3: Correlation of the levels of CA-15-3 (U/ml) with age in premenopausal women



r= 0.220, p=0.430

Figure 4: Correlation of the levels of LP-PLA2 (µg/L) with age (years) in premenopausal women

Discussion

Menopause describes the period following the final menses. Variety of physiological changes occur in women as they progress into menopause. These changes sometimes affect the level of some biomolecules in the body which in turn may increase or decrease the risk of developing certain diseases. In the present study, the levels of CA 15-3 and Lp-PLA2 were evaluated in menopausal and premenopausal subjects.

A significant increase was observed in the mean serum levels of Lp-PLA2 and CA 15-3 in menopausal subjects compared to the pre-menopausal group. The possible explanation for this increase can be attributed to the fact that estrogen withdrawal which occurs during menopause has a detrimental effect on cardiovascular function. Studies have shown that menopause compounds many traditional cardiovascular disease (CVD) risk factors, including changes in body fat distribution, reduced glucose tolerance, increased plasma lipids, blood pressure, sympathetic tone, endothelial dysfunction and vascular inflammation [26-28]. The findings in this study agree with the work of [28] who observed a significant increase in the levels of Lp-PLA2 in menopausal women compared with the pre-menopausal group. Furthermore, [29] reported an increased incidence rate of cardiovascular disease in menopausal women than pre-menopausal group. The effect of the hormonal changes associated with menopause on the serum lipid levels is known to play important roles in most cardiac related disorders associated with menopause [30]. In a study by [31], it was concluded that menopause is associated with altered serum lipid profile and thus an independent risk factor for developing cardiovascular diseases.

In line with this present study, [32] reported an increased serum level of CA 15-3 in menopausal women than pre-menopausal subjects. Similarly, [33] observed higher serum levels of CA 15-3 in menopausal suspected breast cancer patients than in their pre-menopausal control. The reason for this increase is not clearly understood, however it can be attributed to age, late menopause and postmenopausal obesity. Age has been known to be the major risk factor for most diseases including cancer. A study by [34] reported that in women, incidence rates of breast cancer rise sharply with age until ages 45 to 50, when the rise becomes less steep. [34] further stated that the change in slope probably reflects the impact of hormonal change (menopause) that occurs about this time and around 75 to 80 years. The curve actually flattens and then decreases. However, in a data published by [35], the association of age with serum levels for CA 15-3, was stated to be conflicting. Older women who are overweight or obese have been reported to have higher risk of developing breast cancer. A study by [36], concluded that menopausal obesity increases the risk of breast cancer. Similarly, [37] found elevated risk of breast cancer associated with increasing body mass index in younger postmenopausal women. [38] observed that height and obesity are independent risk factors for breast cancer in menopausal women.

In the menopausal subjects, a significant positive correlation was observed between the serum level of CA 15-3 and age ($r= 0.690$, $p<0.001$). However, a non-significant positive correlation existed between the serum level of Lp-PLA2 and age ($r= 0.084$, $p=0.660$) as was seen in the mean serum levels of CA 15-3 ($r= 0.109$) and Lp-PLA2 ($r= 0.220$) when compared with age in the pre-menopausal subjects. The reason for this could be attributed to the fact that with aging comes deteriorative changes in metabolic and cardiovascular function. [39] reported that age is the most important risk factor in developing cardiovascular or heart diseases, with approximately a tripling of risk with each decade of life. Multiple explanations are proposed to explain why age increases the risk of cardiovascular/heart diseases. One of them relates to serum cholesterol level. [40] reported that in most populations, the serum total cholesterol level increases as age increases. A study by [41], stated that aging is also associated with changes in the mechanical and structural properties of the vascular wall, which leads to the loss of arterial elasticity and reduced arterial compliance and may subsequently lead to coronary artery disease.

The findings of the present study, are in contrast with the data published by [42] who observed a positive correlation of Lp-PLA2 levels with age. Furthermore, the work of [43] found no significant correlation of CA 15-3 levels with age and further proposed that CA 15-3 may have a tendency to be relatively higher at a young age. However, [44] observed high levels of CA 15-3 in older people that could not be associated with a greater prevalence of cancer.

Inflammation has been postulated to play an essential role in the development of atherosclerosis which is known to be an underlying pathology responsible for coronary heart disease (CHD) [45]. Postmenopausal estrogen deficiency, hypertriglyceridemia, dyslipoproteinemia and advanced age have been described as risk factors for atherosclerosis

[46] and have been associated with increased coronary heart disease (CHD) risk in women [47]. The incidences of coronary heart disease have been observed to be increased in postmenopausal women until they become similar to the corresponding rates in men of similar age [48]. Lp-PLA2 and other human A2 phospholipase (such as secretory phospholipase A2) have been stated to propagate inflammation by producing precursors of arachidonic acid from membrane glycerophospholipids [49]. [50] confirmed the role of Lp-PLA2 as a significant biomarker of vascular inflammation. In addition, many epidemiological studies and meta-analyses [51,52] have reported that Lp-PLA2 has high specificity for vascular inflammation and has been proposed to be a risk marker of CVD. Also, [52] stated that Lp-PLA2, by virtue of its proinflammatory activity and close association with lipoproteins, has been shown to be associated with the presence of advanced lesions leading to plaque instability and clinical events.

Coronary artery disease (CAD) known to be a major cause of death worldwide, has been stated to be generally associated with older age [53]. Up to the age of 50 years, the prevalence of coronary artery disease (CAD) among women is lower than among men, but the incidence rises significantly after the menopause [31]. In a study by [54], LP-PLA2 was observed to be a marker of oxidative stress and inflammation rather than an independent risk factor of acute coronary syndrome (ACS). [55] reported that the risk factors for ACS in young patients are different from those in older patients. Young patients with ACS are less likely to have traditional risk factors for cardiovascular disease (CVD), such as hypertension, dyslipidaemia and diabetes mellitus (DM), than older patients. A recent study showed that in young patients (age <50 years) with myocardial infarction, 36% had none or only one traditional risk factor for ACS and would have been classified as low risk according to these traditional risk factors assessment [56]. The study by [54] also reported that Lp-PLA2 is positively associated with Oxidized-Low Density Lipoprotein level, which has been reported to be an independent risk factor for ACS and further indicated that the link between Lp-PLA2 and the risk of ACS may be dependent on ox-LDL levels.

CA 15-3 has been proven to be the most sensitive and specific tumor marker for breast cancer in that it offers a greater sensitivity than Carcinoembryonic antigen (CEA) and a higher specificity than Tissue plasminogen activator (TPA) (57). However, it has been reported that the combination of elevated values of CA 15-3 and CEA is more sensitive than CA 15-3 alone [58]. In a data published by [35], the association of age with serum levels of CA 15-3, was shown to be conflicting. Similarly, [58] found no correlation between the levels of CA 15-3 and age. However, risk of breast cancer has been reported to increase with age. [59] observed that reduced risk of breast cancer due to prolonged period of lactation was higher in young women than in older women. In addition, lack of physical activity (sedentary lifestyle) which is known to be more in older women has been stated to be associated with higher risk of breast cancer in women [60].

Conclusion

The significantly elevated serum levels of Lp-PLA2 and CA 15-3 in menopausal subjects compared with the premenopausal group in addition with the strong positive correlation between CA 15-3 and age in menopausal women suggests increased breast cancer and cardiovascular risks in menopausal women especially in advanced age. However, with the small and non-proportional sample size used in this current study, similar studies with large-scale sample size are recommended to further validate our findings.

Disclosure

The authors declare no conflicts of interest. 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.

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