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Diagnostic Utility of Lipoprotein associated Phospholipase A_2, Soluble Suppression of Tumorigenesis-2, and Heart Type Fatty Acid Binding Protein in Female Chronic Heart Failure

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Abstract Chronic Heart failure (CHF) is an increasing global problem with a current worldwide prevalence of more than 64 million cases. The aim of this study was to determine the diagnostic use of LP-PLA2, sST2 and H-FABP in female CHF. This was a hospital-based case-control study and data were collected from a total of 100 females (aged 30-80) years consisting of 50 subjects with CHF (test) and 50 apparently healthy (control) subjects who were recruited into the study through a convenient sampling technique. Five milliliter of blood was collected from each participant and their serum troponin I, sST2, H-FABP, and LP-PLA2 were measured using ELISA technique while their Lipid profile was analyzed using BS-120 chemistry auto analyzer. ROC-curve was carried out to assess the overall diagnostic performance of the novel biomarkers, and comparing their performance with cardiac troponin I and Lipid profile. Level of significance was taken at $P<0.05$. The serum levels of sST2, H-FABP, and Lp-PLA2 were significantly higher in CHF when compared with control group ($p<0.05$). Lp-PLA2 had a sensitivity of 99%, specificity of 95%, H-FABP had a sensitivity of 95%, specificity of 100%, and sST2 had a sensitivity of 80%, specificity of 92%, which were all higher when compared with that of Troponin I and lipid profile. In conclusion, Lp-PLA2, H-FABP, and sST2 were higher in CHF individuals, Suggesting the possibility of continual myocardial damage, inflammation, and/or endothelial dysfunction. The markers also showed higher sensitivity and specificity, indicating their possible usage as a better diagnostic markers in the assessment of CHF.

Keywords Chronic Heart failure, LP-PLA2, sST2, H-FABP

Introduction

One of the major growing clinical heart disease condition is chronic heart failure which according to the European Society of Cardiology, is defined as a clinical syndrome characterized by typical symptoms (e.g., dyspnea, ankle swelling, fatigue) that may be accompanied by signs (e.g., elevated jugular venous pressure, pulmonary crackles, peripheral edema) caused by a structural and/or functional cardiac abnormality, leading to a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress [1]. Chronic heart failure is an increasing global problem, with a current worldwide prevalence of more than 64 million cases [2-4].

Research has shown that about 50% of patients with CHF are women, although sex-related differences in the biological mechanisms, epidemiology, etiology, pathogenesis, risk factors, quality of care, and prognosis within CHF are poorly recognized and understood [5]. Despite the identification of conventional risk factors which has offered an improved primary prevention of heart failure over the decades, chronic heart failure is still associated with significant morbidity, mortality, and health care costs [6]. The discovery and measurement of a broad range of novel biomarkers associated with chronic heart failure risks such as lipoprotein-associated phospholipase A_2 (Lp-PLA_2), heart type fatty acid binding protein (H-FABP), and soluble suppression of tumorigenesis-2 (sST2) may have a diagnostic and/or prognostic usability independent of the previous conventional risk factors [7].

Lp-PLA2 is a member of the PLA2 superfamily, characterized by the specific ability to hydrolyze the sn-2 position of phospholipids within oxidized LDL particles to yield oxidized fatty acids and lysophosphatidylcholine (lysoPC), a molecule with a range of potentially atherogenic effects [8]. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a novel biomarker of vascular-specific inflammation that provides information about atherosclerotic plaque inflammation and stability. Inflammation which is an important pathophysiological factor in CHF, appears to contribute in different ways to each type of HF, predicting poor prognosis independently of left ventricular ejection fraction (LVEF) [9]. Growing evidence also points to a vital role of inflammation in the disease progression [10, 11].

Heart type fatty acid binding protein (H-FABP), also known as mammary-derived growth inhibitor, is a protein that in humans is encoded by the FABP3 gene, which is located on chromosome 1, with its specific location being 1p33-p32 [12]. It is a low molecular weight cytoplasmic protein with a molecular weight of 15 kDa, consisting of 132 amino acids, and is present abundantly in the cytosol of cardiomyocytes (0.5 mg/g), constituting about 5–15 % of the cytosolic protein pool [13]. H-FABP is rapidly released from the cytosol into the blood stream as early as 30 minutes of myocardial injury [14, 15]. It is highly specific, as it is predominantly expressed in cardiac muscle than the skeletal muscle, liver, and kidney. Hence it could be a potential marker for early diagnosis of myocardial infarction, has the potential for usage as a prognostic indicator and has been shown to be associated with chronic heart failure patients [16, 17].

Suppression of Tumorigenesis-2 (ST2) is a member of the Toll interleukin 1 (IL-1) receptor super-family. It has two important isoforms, these isoforms are membrane-bound ST2 (ST2L or mST2) and soluble ST2 (sST2) [18]. While binding of ST2L to IL33 has a cardioprotective effect (mechanical stimulation, decreased cardiac damage, prevention of apoptosis, lowered inflammatory effect, hypertrophy, and fibrosis), binding of sST2 to IL33 causes these positive effects to disappear, implying that sST2 competes with ST2L to bind with interleukin-33 (IL-33), which is involved in ameliorating myocardial hypertrophy and fibrosis in response to cardiovascular stretch [19-22]. Suppression of Tumorigenesis-2 (ST2) has been considered as an important prognostic biomarker because of its involvement in the pathogenesis of CVD, its stability, and easy measurement in clinical samples [23]. sST2 may be released by vascular endothelial and myocardial cells in response to cardiomyocyte biochemical strain. Endothelial dysfunction has been considered as a component underlying chronic heart failure pathophysiology since it plays an important role in HF progression, worsens the vasoconstriction, and increases myocardial damage [24, 25]. sST2 has been introduced as a marker associated with chronic heart failure, pro-inflammatory status, endothelial dysfunction, myocardial fibrosis and adverse remodeling with prognostic capability, it has also been found to have a low biological variability and a low index of individuality (0.25), favorable characteristics that may be used for guiding therapy and monitoring HF patients [26, 27].

There is several available literature on the epidemiology, etiology, and pathophysiology/pathogenesis of heart failure in female, and the clinical utility of brain natriuretic peptides and cardiac troponin I in the diagnosis and risk stratification of heart failure in female, but to the best of our knowledge, there is scarcity of literature on the diagnostic usability of Lp-PLA2, H-FABP, and sST2 in female with chronic heart failure. Therefore, this work focuses on determining the diagnostic utility of lipoprotein-associated phospholipase A_2 (Lp-PLA2), soluble suppression of tumorigenesis-2 (sST2), and heart type fatty acid binding protein (H-FABP) in female chronic heart failure**.**

Materials and Methods

This was a hospital based case-control study designed to determine the diagnostic utility of Lp-PLA2, sST2, and H-FABP in female subjects seeking care for chronic heart failure (CHF) at University of Nigeria Teaching Hospital (UNTH), Enugu State, Southeast Nigeria. A total of 100 participants were recruited for this study through a convenient sampling technique of which test participants were made up of 50 females with chronic heart failure and 50 age-matched apparently healthy female subjects as control. Five milliliter of venous whole blood was collected aseptically from all subjects into well labeled plain tubes and serum obtained by centrifugation at 3000 rpm for 10 minutes was separated, aliquot, frozen and stored at −20°C prior to analysis. The subjects' biochemical parameters like Troponin I, $LP\text{-}PLA_{2,}$ sST2, and H-FABP were assayed by enzyme linked immunosorbent assay (Elabscience Biotechnology ELISA kit method) using a microplate reader, model MR96A (Mindray, China). Lipid profile was analyzed using BS-120 auto chemistry analyzer (Mindray, China). Data were entered in Microsoft Excel 2016 and were processed through Statistical package for the Social Sciences (SPSS) statistical software, version 26.0. Continuous variables were expressed as mean ± standard deviation. Independent student T test was carried out to check for any statistical difference between the study groups. ROC-curve was carried out to assess the overall diagnostic performance of the novel biomarkers and to compare their performance with cardiac troponin I and lipid profile. Level of significance was taken at P<0.05.

Results

Table 1 shows the demographic and anthropometric characteristics of the study participants. There was no statistically significant difference in the mean value of age in CHF female subjects (57.91 ± 12.66 years) when compared with the control subjects (56.73 ± 14.11 years) (P=0.058). Body mass index of the CHF subjects (40.00 ± 24.64 kg/m^2) was significantly higher when compared with the control subjects (22.40 ± 4.21 kg/m^2) (P=0.001). Again, the mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in the CHF subjects (141.02 ± 17.86 mmHg and 90.67 ± 20.78 mmHg respectively, P=0.001) when compared with the controls subjects (118.45 ± 15.57 mmHg and 69.30 ± 12.21 mmHg) respectively, (P= 0.001).

Table 1: Demographic and anthropometric characteristics of the study participants

Parameters	Female CHF n=50	Female Control n=50	P-value
Age (year)	57.91 ± 12.66	56.73 ± 14.11	0.058
BMI (kg/m^2)	40.00 ± 24.64	22.40 ± 4.21	0.001*
SBP (mmHg)	141.02 ± 17.86	118.45 ± 15.57	0.001*
DBP (mmHg)	90.67 ± 20.78	69.30 ± 12.21	0.001*

Key: Value is significant when p≤0.05; *=significant; CHF = Chronic heart failure; n = number of subjects in the group; SBP=Systolic blood pressure; DBP= Diastolic blood pressure; BMI= body mass index:

Table 2 shows the biochemical characteristics of the study groups. There was no statistically significant difference in the mean level of HDL-C and troponin I in CHF subjects (1.11 ± 0.30mmol/l, and 1.79 ± 1.30 ng/ml respectively) when compared with the control (1.24 ± 0.32mmol/l and 1.73 ± 1.40 ng/ml respectively), p>0.05. The mean levels of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL-C) was significantly higher in the test group (5.28 ± 0.94mmol/l, 1.58 ± 0.59mmol/l, and 2.89 ± 0.63mmol/l), respectively when compared with the control group (3.64 ± 0.97mmol/l, 1.22 ± 0.68mmol/l, and 1.88 ± 0.57mmol/l), respectively P<0.05. Also, Lp-PLA2, H-FABP, and sST2 were significantly higher in the test group (16.18 ± 6.03 ng/ml, 9.02 ± 2.87 ng/ml, and 17.22 ± 7.33 ng/ml respectively) when compared with the control group (4.57 ± 3.43 ng/ml, 1.76 ± 1.47 ng/ml, and 6.04 ± 4.30 ng/ml respectively). p<0.05.

Table 2: Biochemical characteristics of the study groups

Parameters	Female CHF n=50	Female Control n=50	P-value
TC (mmol/l)	5.28 ± 0.94	3.63 ± 0.97	0.001*
TG (mmol/l)	1.58 ± 0.59	1.22 ± 0.68	0.011*
LDL-C (mmol/l)	2.89 ± 0.63	1.88 ± 0.57	0.001*
HDL-C (mmol/l)	1.11 ± 0.30	1.24 ± 0.32	0.058
Troponin I (ng/ml)	1.79 ± 1.30	1.73 ± 1.40	0.298
Lp-PLA2 (ng/ml)	16.18 ± 6.03	4.57 ± 3.43	0.001*
H-FABP (ng/ml)	9.02 ± 2.87	1.76 ± 1.47	0.001*
sST2 (ng/ml)	17.22 ± 7.33	6.04 ± 4.30	0.001*

Key: Value is significant when p≤0.05; *=significant; CHF = Chronic heart failure; n = number of subjects in the group; TC = Total Cholesterol; TG = Triglyceride; LDL-C = Low-density Lipoprotein; HDL-C = High-density Lipoprotein; Lp-PLA$_2$ = Lipoprotein associated phospholipase A2; H-FABP = Heart-type fatty acid binding protein; sST2 = soluble suppression of tumorigenesis-2

Table 3 shows the area under the ROC-curve for Lipid profile. Troponin 1, Lp-PLA2, H-FABP, and sST2, and their asymptotic significance (p-value) at 95% Confidence interval. TC: Area = 0.779, p = 0.001 & 95%CI = 0.711-0.837; TG: Area = 0.665, p = 0.005, & 95% CI = 0.592-0.735; HDL-C: Area = 0.302, p = 0.001, & 95% CI = 0.625-0.764; LDL-C Area = 0.764, p = 0.001, & 95% CI = 0.695-0.824; At 95% CI of 0.448-0.696, troponin I had a non-statistically significant area of 0.572 (p=0.254). Lp-PLA2 had a significant area of 0.946 (p=0.001) at 95% CI of 0.894-0.994. The area of H-FABP (0.922) was also significant (p=0.001) at 95% CI of 0.886-0.986. Again, sST2 under the curve had a significant area (0.904, p=0.001) at 95% CI of 0.845-0.963.

Table 3: Area under the ROC curve for Lp-PLA2, H-FABP, sST2, and Troponin I

Parameters	Area	P-value	95% CI
TC (mmol/L)	0.779	0.001*	0.711-0.837
TG (mmol/L)	0.665	0.001*	0.592-0.735
HDL (mmol/L)	0.302	0.001*	0.625-0.764
LDL (mmol/L)	0.764	0.001*	0.695-0.824
Troponin I (ng/ml)	0.572	0.254	0.448-0.696
Lp-PLA2 (ng/ml)	0.946	0.001*	0.894-0.994
H-FABP (ng/ml)	0.922	0.001*	0.886-0.986
sST2 (ng/ml)	0.904	0.001*	0.845-0.963

Key: Value is significant when p≤0.05; *=significant TC = Total Cholesterol; TG = Triglyceride; LDL-C = Low-density Lipoprotein Cholesterol; HDL-C = High-density Lipoprotein Cholesterol; Lp-PLA$_2$ = Lipoprotein associated phospholipase A2; H-FABP = Heart-type fatty acid binding protein; sST2 = soluble suppression of tumorigenesis-2.

Table 4 shows the cut-off point, sensitivity and specificity of lipid profile, Lp-PLA2, H-FABP, sST2, and Troponin I. At >4.2 cut-off point, TC has a sensitivity and specificity of 74% and 68.75% respectively. At >1.00 cut-off point, TG has a sensitivity and specificity of 72% and 58.75% respectively. At ≤0.95 cut-off point, HDL-C has a sensitivity and specificity of 68% and 82.5% respectively. At >2.03 cut-off point, LDL-C has a sensitivity and specificity of 86% and 57.5% respectively. At cut-off point of >7.40, Lp-PLA2 has a sensitivity and specificity of 99% and 95% respectively. At the cut-off point of >6.00, H-FABP has a sensitivity and specificity of 95% and 100% respectively. At ≥10.70 cut-off point, sST2 has a sensitivity and specificity of 80% and 92% respectively. At ≤0.55 cut-off point, troponin I has a sensitivity and specificity of 24% and 95% respectively.

Table 4: The cut off point, sensitivity, and specificity, of Lipid profile, H-FABP, Lp-PLA2, sST2, and Troponin I.

Parameters	Cut-off point	Sensitivity (%)	Specificity (%)
TC (mmol/L)	>4.2	74.00	68.75
TG (mmol/L)	>1.00	72.00	58.75
HDL (mmol/L)	≤0.95	58.00	82.50
LDL (mmol/L)	>2.03	86.00	57.50
H-FABP (ng/ml)	>6.00	95.00	100.00
LP-PLA2 (ng/ml)	>7.40	99.00	95.00
sST2 (ng/ml)	>10.70	80.00	92.00
Troponin I (ng/ml)	≤0.55	24.00	95.00

Key: TC = Total Cholesterol; TG = Triglyceride; LDL-C = Low-density Lipoprotein Cholesterol; HDL-C = High-density Lipoprotein Cholesterol; Lp-PLA$_2$ = Lipoprotein associated phospholipase A2; H-FABP = Heart-type fatty acid binding protein; sST2 = soluble suppression of tumorigenesis-2.

Discussion

Though a lot of work has been done on the pathophysiology and epidemiology of heart failure in women, there is paucity of literature on the diagnostic utility of the studied biomarkers in female CHF. Therefore, this study aimed at determining the diagnostic usability of Lp-PLA2, H-FABP, and sST2 in female CHF.

This study pointed out that the test group had significantly higher systolic and diastolic blood pressure when compared with the control group. The findings are in agreement with the findings of the study conducted by Rapsomaniki *et al.* [28] and Mahmood *et al.* [29], where the authors stated that blood pressure has a strong association with occurrence of all cardiovascular diseases, and that also accounts for about one quarter of cardiovascular diseases. The higher blood pressure in the test group may also be associated with disturbances in sexual hormone production as they occur in the polycystic ovarian syndrome or during postmenopausal decline in estrogen levels [30] and this high blood pressure may have contributed to the development of chronic heart failure in these subjects.

Furthermore, this study found that the serum mean levels of TC, TG, and LDL-C were significantly higher (p =0.001, p = 0.007 and p = 0.001 respectively) in female CHF when compared with the control. This pattern of lipid profile from this study is consistent with series of epidemiological studies which have shown that high TC, LDL-C, TG, and low HDL-C are associated with increased risk of CVD such as chronic heart failure [31]. Patients with hyperlipidemia are about twice as likely to develop chronic heart failure, as some studies have shown that hyperlipidemia, in addition to well-known role in promoting atherosclerosis in the blood vessels, may directly affect the heart, leading to increased ischemia/reperfusion injury and weakened response to cardiac protective interventions such as ischemic preconditioning and post conditioning [32].

In the presented study, we observed that level of Lp-PLA2 was significantly higher in test group when compared with the control group. Different studies have proven that the level of LpPLA2 is found to be increased among the population with cardiovascular risk [33]. The recognition that atherosclerosis represents in part, an inflammatory process has created considerable interest in measurement of Lp-PLA2 as part of cardiovascular disease risk assessment. Elevated levels of serum Lp-PLA2 could be indicative of rupture prone plaque and a strong independent predictor of cardiovascular risk, including chronic heart failure [34]. The higher level of Lp-PLA2 along with the other markers like sST2, and H-FABP may indicate that Lp-PLA2 could be a reliable marker in diagnosing chronic heart failure events in the given population.

This present study also showed a significantly higher level of heart type fatty acid binding protein in CHF group. This result is in line with the study carried out by Lichtenauer *et al.* [35] where the authors found that H-FABP levels were significantly higher in patients with CHF compared to controls. The elevated level of H-FABP might indicate an ongoing myocardial damage in the test population, implying that this biomarker could be associated with higher disease stages of CHF [36]. The high levels of H-FABP obtained in this study is also in line with that obtained in a study by Niizeki *et al.* [37] who investigated serial measurements of hFABP levels in 113 chronic heart failure patients at the time of hospital admission and at the time of hospital discharge. The authors concluded that such serial measurement of hFABP can be informative for guiding therapy and management of chronic heart failure patients

This presented study revealed that sST2 was significantly elevated in CHF subjects when compared with the control group. This result is consistent with that from the study carried out by Dimitropoulos *et al.* [38], where patients with functional impairment were identified with higher values of sST2 when compared with the control groups. Report from Januzzi *et al*. [39] also revealed that the concentrations of sST2 in a group of patients with CHF were generally higher than in a healthy population.

Our study shows that H-FABp, Lp-PLA2, and sST2 possessed higher sensitivity and specificity than that of lipid profile and troponin I. A higher sensitivity and specificity of H-FABp, Lp-PLA2, and sST2 as observed in this present study could imply that they have a better diagnostic usability when compared with lipid profile and troponin I. This could also suggest a high chance that the novel biomarkers (LpPLA2, H-FABP, and sST2) have excellent discriminating ability and will correctly distinguish female CHF patients from the apparently healthy female subjects when compared with lipid profile and troponin I.

Conclusions

Our findings showed that the mean value of Lp-PLA2, H-FABP, and sST2 were higher in female with CHF when compared with the control group. Their sensitivity, specificity, and predictive values were also higher when compared with that of lipid profile and troponin I. This implies that these ll biomarkers could have significant diagnostic usability and could offer a precise diagnosis biomarkers could have significant diagnostic usability and could offer a precise diagnosis in chronic heart failure. Additional follow-up prospective studies with a larger sample size is needed to further evaluate the potential clinical benefits of these biomarkers in routine treatment of chronic heart failure.

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