MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 12(5) pp. 059-064, May, 2024
Available online http://www.meritresearchjournals.org/mms/index.htm

DOI: 10.5281/zenodo.11389999

Original Research Article

Validation of Soluble Suppression of Tumorigenesis-2, Heart-Type Fatty Acid-Binding Protein and Lipoprotein-Associated Phospholipase A2 as Tools in the Assessment of Chronic Heart Failure

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Abstract

Chronic heart failure (CHF) is a major public health and growing problem, which imposes a relevant burden, with high prevalence and mortality rates. Therefore, reliable cardiac biomarkers are needed to identify individuals with CHF. The goal of this study was to validate the diagnostic utility of some cardiac biomarkers as tools in the assessment of CHF. This was a hospital-based case-control study where a total of 180 participants (aged 30-85 years) consisting of 100 participants with CHF and 80 apparently healthy controls were recruited for the study. Serum troponin I, sST2, H-FABP, and LP-PLA$_2$, were measured using ELISA technique. The value of P≤ 0.05 was regarded as statistically significant. Serum levels of sST2, H-FABP, and Lp-PLA$_2$, were significantly higher in CHF participants when compared with control (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 (20% and 95% respectively).In conclusion, Lp-PLA2, H-FABP, and sST2 were higher in CHF individuals, indicating the possible presence of inflammation and myocardial injury. The markers were more sensitive and specific than troponin I, suggesting their potential use as reliable biomarkers for the assessment of chronic heart failure.

Keywords: Chronic heart failure, sST-2, H-FABP and Lp-PLA2

INTRODUCTION

Growing number of people is currently suffering from a range of major clinical heart and circulatory disease conditions, including coronary artery disease (CAD), peripheral arterial disease and cerebrovascular disease (Benjamin *et al.,* 2019). One of the major cardio-vascular diseases (CVDs), which have been found to be on the increase over the past 20 years in Nigeria, is chronic heart failure (CHF). CHF is a complex disease state characterized by impaired ventricular function and insufficient peripheral blood supply (Abassi *et al.,* 2022). It is a final common pathway to various heart diseases, a clinical syndrome characterized by dyspnea, fatigue and clinical signs of congestion, leading to frequent hospitalization, poor quality of life and shortened life expectancy (Adebayo *et al.,* 2017). In the face of such a huge disease burden and cost, getting a reliable biomarker that could help in the prevention, diagnosis and treatment of heart and vascular related diseases is of key importance. New parameters and markers are constantly being sought to help better assess patients with CHF, which could translate into better care and an improved prognosis. The presented study was therefore undertaken to measure diagnostic utility of the serum levels of soluble suppression of tumorigenesis-2 (sST2),

heart-type fatty acid-binding protein (H-FABP), lipoprotein-associated phospholipase A2 (LP-PLA_2) and compare them with troponin I in the assessment of chronic heart failure (CHF).

MATERIALS AND METHODS

A hospital-based case-control study was carried out among male and female subjects seeking care for chronic heart failure (CHF) at University of Nigeria Teaching Hospital (UNTH), Enugu State, Southeast Nigeria. A total of 180 participants (aged 30-85 years) consisting of 100 participants with CHF and 80 apparently healthy controls were recruited for the study. A total of 6mls of whole blood specimen was collected from the antecubital fossa vein of each participant, applying proper aseptic technique. The specimen was dispensed into plain tubes, and was spun at 3000 revolution per minute for 10min. The serum was separated and stored at -20^{o}C prior to analysis. The subjects' biochemical parameters like Troponin I, sST2, H-FABP, and LP-$PLA_{2,}$ were assayed by enzyme linked immunosorbent assay (Elabscience Biotechnology ELISA kit method) using a microplate reader, model MR96A (Mindray, China).Data were entered in Microsoft Excel 2016 and statistical processing was conducted through Statistical package for the Social Sciences (SPSS) statistical software, version 26.0. Continuous variables were expressed as mean ± SEM. To compare the biochemical levels of the parameters between chronic heart failure and controls groups, Independent-Samples Mann-Whitney U-Test was used. The relationship between the parameters were assessed by Spearman's rank correlation coefficient. The receiver operating characteristics (ROC) curve was used to assess the overall diagnostic performance of the novel biomarkers and to compare their performance with cardiac troponin I. The value of p≤ 0.05 was regarded as statistically significant.

RESULTS

Table 1 shows the demographic and anthropometric data for both CHF (Test) and apparently healthy (Control) participants. The mean levels of age for both CHF and control subjects were 59.53 ± 13.81 and 50.56 ± 13.52 respectively, showing that age was significantly higher in the CHF participants when compared with the control (p = 0.001). Systolic blood pressure (SBP) mean level for CHF and apparently healthy subjects were 145.83 ± 18.11, and 123.53 ± 18.56 respectively, indicating that SBP was significantly higher in CHF group when compared with the control group (0.001). Diastolic blood pressure (DBP) had a mean level of 101.02 ± 20.61 in CHF group and a mean level of 78.83 ± 16.14 in the control group. This shows a statistically significant higher level of DBP in CHF group when compared with the control (p = 0.001). The mean level of body mass index. (BMI) for both test and control groups were 44.47 ± 22.10 and 28.37 ± 8.22 respectively. BMI was significantly higher in CHF individuals when compared with the control (p = 0.001).

Table 2 shows the biochemical parameters of CHF and apparently healthy participants. The mean level of troponin I of both test and control subjects were 1.60 ± 1.49 and 1.72 ± 1.49 respectively. There was no significant difference in test group when compared with the control (P = 0.444). The mean level of lipoprotein associated phospholipase A2 (Lp-PLA2) of both test and control subjects were 15.58 ± 5.92 and 3.74 ± 2.84 respectively, showing that Lp-PLA2 was significantly higher in CHF subjects when compared with the control (P = 0.001). The mean level of heart-type fatty acid binding protein (H-FABP) of both test and control subjects were 9.22 ± 2.42 and 1.81 ± 1.51 respectively. There was a significant difference between the two groups as H-FABP was significantly higher in CHF subjects when compared with the control (P = 0.001). The mean level of soluble suppression of tomurigenesis 2 (sST2) of both test and control participants were 16.62 ± 6.98 and 5.94 ± 4.00 respectively. A significantly higher mean level of sST2 was observed in test group when compared with the control (P = 0.001).

Table 3 is the area under the receiver operating characteristic curve (ROC-curve) for the studied novel cardiac biomarkers and troponin I, with their sensitivity and specificity. The area under the ROC-curve for LP-PLA2, H-FABP, sST2, and troponin-I, and their asymptotic significance (p-value) at 95% Confidence interval were Area=0.961, p = 0.001 and 95% CI = 0.922-0.984; Area = 0.964, p = 0.001, and 95% CI = 0.926-986; Area = 0.900, p = 0.001, and 95% CI = 0.851-0.943; and Area = 0.467, p = 0.444. and 95%CI = 0.458-0.608, 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 20% and 95% respectively. The results show that LP-PLA2, H-FABP, and sST2 have higher sensitivity and specificity when compared with that of Troponin-I.

Table 4 This shows the correlation of novel cardiac biomarkers with troponin I in both CHF and control participants. In CHF group, sST2 and H-FABP positively correlated with troponin I ((r= 0.374, p = 0.001 and r = 0.287, p = 0.004 respectively). In control participants, there was no observed statistically significant difference when the novel cardiac biomarkers were correlated with troponin I (p>0.05).

Table 1. Demographic and anthropometric data for both CHF (Test) and apparently healthy (Control) participants

Parameter	CHF(TEST) n=100	Control n=80	U-test	P-value
AGE (year)	59.53 ± 13.81	50.56 ± 13.52	2487.50	0.001*
SBP (mmHg)	145.83 ±18.11	123.53 ± 18.56	1375.50	0.001*
DBP (mmHg)	101.02 ±20.61	78.83 ± 16.14	1599.00	0.001*
BMI (kg/m^2)	44.47 ± 22.10	28.37 ± 8.22	1696.00	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. Biochemical parameters of CHF and apparently healthy participants.

Parameters	CHF (Test) n=100	Control n=80	U-test	P-value
Troponin (ng/ml)	1.60 ± 1.49	1.72 ± 1.49	4265.50	0.444
Lp-PLA$_2$ (ng/ml)	15.58 ± 5.92	3.74 ± 2.84	311.00	0.001*
H-FABP (ng/ml)	9.22 ± 2.42	1.81 ± 1.51	286.00	0.001*
sST2 (ng/ml)	16.62 ± 6.98	5.94 ± 4.00	768.00	0.001*

Key Value is significant when p≤0.05; *=significant; CHF = Chronic heart failure; n = number of subjects in the group; Lp-PLA$_2$ = Lipoprotein associated phospholipase A2; H-FABP = Heart-type fatty acid binding protein; sST2 = soluble suppression of tumorigenesis-2.

Table 3. Area under the ROC-curve for the studied novel cardiac biomarkers and troponin I

Test Result Variable(s)	AUC	P-value	95%CI	Cut-off point	Sensitivity (%)	Specificity (%)
H-FABP (ng/ml)	0.964	0.001*	0.926-0.986	>6.00	95.00	100.00
LP-PLA2 (ng/ml)	0.961	0.001*	0.922-0.984	>7.40	99.00	95.00
sST2 (ng/ml)	0.900	0.001*	0.851-0.943	>10.70	80.00	92.00
Troponin I (ng/ml)	0.467	0.444	0.458-0.608	≤0.55	20.00	95.00

Key: Value is significant when p≤0.05; *=significant; H-FABP = Heart-type fatty acid binding protein; Lp-PLA$_2$ = Lipoprotein associated phospholipase A2; sST2 = soluble suppression of tumorigenesis 2.

Table 4. Correlation between the novel cardiac biomarkers with troponin I in both CHF and control subjects

CHF			**Control**	
Parameters	r	p-value	r	p-value
sST2 *vs* Troponin I	0.374	0.001*	0.168	0.137
H-FABP *vs* Troponin I	0.287	0.004*	-0.026	0.819
Lp-PLA2 *vs* Troponin I	-0.036	-0.724	-0.028	0.804

Key Value is significant when p≤0.05; r = correlation coefficient; sST2 = soluble suppression of tumorigenesis 2; Lp-PLA$_2$ = Lipoprotein associated phospholipase A2; H-FABP = Heart-type fatty acid binding protein.

DISCUSSION

Chronic heart failure (CHF) is a clinical syndrome which has been traditionally defined as a condition characterized by the reduced capability of the heart to pump and/or fill with blood, or alternatively as an abnormality of cardiac structure/function leading to an inadequate cardiac output or to an adequate cardiac output secondary to compensatory neurohormonal activation and increased left ventricular filling pressure (Gianluigi, 2022). In the presented study, we focused on validating the use of sST2, Lp-PLA2 and H-FABP as tools in the assessment of CHF.

The results of this study show that age, systolic blood pressure (SBP), diastolic blood pressure (DBP) and body mass index (BMI) were significantly higher in CHF individuals when compared with the control (p =0.001, p = 0.001, p = 0.001, and p = 0.001 respectively). These parameters have been established as risk factors for cardiovascular diseases,the terminal stage of which is the heart failure. The obesity is regarded as a significant risk factor for cardiovascular disease and has also been

linked to the development of CHF. The high levels of SBP and DBP in the test group may have caused numerous neurohormonal factors including the activation of the sympathetic nervous system and elevated levels of renin and aldosterone which could lead to the development of chronic heart failure. Hypertension causes left ventricular hypertrophy (LVH) and fibrotic changes, ultimately leading to diastolic dysfunction and when the pressure and volume overload is sustained, cardiac systolic dysfunction might ensue (Slivnick *et al*., 2019).

Also, the data of the current study revealed that lipoprotein-associated phospholipase A2 (Lp-PLA2) was significantly higher in CHF individuals when compared with the controls (p = 0.001). LP-PLA2 enhances the metabolism of oxidized lipoproteins, the local oxidative pressure increases, which causes damage of vascular endothelial cells and dysfunction, and eventually destroys its protective mechanism. Therefore, the vascular endothelial injury is related to the occurrence and prognosis of CHF (Zhu *et al.,* 2021).The presented work is in agreement with the results obtained by Takeki *et al.,* 2009, and van Vark *et al.,* 2006, where high plasma levels of Lp-PLA2 were observed in CHF subjects when compared with the non-CHF control group. Lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor acetylhydrolase, is an inflammation marker that could be used for CHF risk assessment (Libby *et al.,* 2019; Niccoli *et al.,* 2018). The release of these pro-inflammatory factors triggered by impaired endothelial cells in the arterial vessel wall aggravates ventricular remodeling, indicating that immune inflammatory activation is important in chronic heart failure progression and this enzyme is strongly expressed in advanced coronary plaques suggesting a potential role in promoting plaque instability (Batista *et al.,* 2021; Cao *et al.,* 2021).

Moreover, the presented study shows a significantly higher serum mean level of soluble suppression of tumorigenesis 2 (sST2) in CHF individuals when compared with the control group (p = 0.001). Our result is consistent with the study by Hicham*et al.,* (2020) and Stathis *et al.,* (2020), where a higher level of sST2 was observed in chronic heart failure subjects when compared with the healthy controls. sST2 binds to its specific ligand IL-33 to activate the IL-33/ST2L signaling pathway, which is considered as a mechanical activation system. When myocardial cells are subjected on mechanical stretch stimulation, they will release IL-33 molecules and form receptor complexes with ST2L on the myocardial membrane through paracrine action to activate downstream signaling pathways, thus playing a protective role in the heart by inhibiting inflammatory response and myocardial hypertrophy (Aimo *et al.,* 2019; Barutaut *et al*., 2020). sST2, as a competitive receptor of ST2L, can bind to IL-33 released from myocardial tissue cells, then block the cardiac protective effect of the IL-33/ST2L signaling pathway, participate in pathophysiological processes such as inflammatory response and myocardial injury, and regulate the remodeling of the extracellular matrix, leading to a series of cardiovascular negative events (Emdin *et al.,* 2018; Firouzabadi *et al.,* 2020). Therefore, when cardiomyocytes are damaged by mechanical stress, the myocardial tissue will lack sufficient IL-33 protection, resulting in myocardial remodeling and cardiac dysfunction, leading to promoting the occurrence and development of chronic heart failure (Kim *et al.,* 2021). Therefore, this biomarker, which is not easily affected by age, body mass index and kidney function damage, could be an important indicator for assessing the prognosis of chronic heart failure patients.

Furthermore, the study revealed that the mean serum levels of heart-type fatty acid binding protein (H-FABP) were also significantly higher in the CHF group when compared with the controls (p = 0.001). Our results are in agreement with the study by Niizeki *et al*., (2008), who investigated serial measurements of H-FABP levels in 113 chronic heart failure patients at the time of hospital admission and at the time of hospital discharge. The patients with consistently high levels of H-FABP had subsequent higher cardiac events in the follow-up period when compared to patients with normal H-FABP levels. The authors concluded that such serial measurement on the H-FABP can be informative for guiding therapy and management of chronic heart failure patients. Our results are also in confirmation of other studies, made by Dinh *et al.* (2011), Kazimierczyk *et al.,* (2018), Gruson *et al.,* (2021) and Ahmad *et al.* (2023), where a higher levels of H-FABP were observed in chronic heart failure subjects when compared with the controls. Heart type fatty acid binding protein (H-FABP), encoded by the fatty acid-binding proteins 3 (FABP3) gene, is a member of cytoplasmic protein group with a molecular weight of 15kDa, it is expressed abundantly in the cytoplasm of striated muscle cells which could likely be released immediately upon cardiomyocyte injury, and may be detectable earlier than cTn, therefore the protein is classified as a cardiac cell death marker (Piek*et al.,* 2018). H-FABP levels in serum rise immediately after cardiomyocyte injury, making it a promising molecule to investigate with respect to cardiac function and heart failure (Shrivastava *et al.,* 2020).

Comparing the sensitivity and specificity of the results onLp-PLA2, H-FABP, sST2 and troponin I from the ROC-AUC-curve, the sensitivity and specificity of Lp-PLA2, H-FABP and sST2 were significantly higher than that of troponin I. This finding could indicate that these biomarkers have excellent discriminating ability, suggesting a high chance that they might correctly distinguish CHF patients from the apparently healthy individuals when compared with troponin I. Therefore, the detection of serum LP-PLA2, H-FABP and sST2 in patients with CHF can promptly assess the condition and treatment, which will help to improved the prognosis and the quality of life of the patients.

Again, once there is strain or damage to the myocardium, there will be release of some cardiac biomarkers such as sST2, H-FABP and troponin I. The positive correlation of these biomarkers as we observed in the current study could indicate a continuous myocardial damage which resulted to simultaneous higher levels of these parameters in the blood.

CONCLUSION

The presented study concludes that individuals with CHF have higher levels of sST2, H-FABP and LP-PLA_2 compared to the apparently healthy controls, indicating the possible presence of inflammation and myocardial injury.sST2, H-FABP and LP-PLA_2 were more sensitive and specific with high discriminating ability than troponin I, suggesting their potential usability as reliable biomarkers for the assessment of chronic heart failure.

ACKNOWLEDGEMENT

The authors are appreciative to all the participants who volunteered themselves for this work. We also acknowledge the hospital management of University of Nigeria Teaching Hospital, Enugu and all the Clinicians in the cardiothoracic department for allowing us to use their patients, and all those who contributed in the execution of this work.

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