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RESEARCH ARTICLE

Role of Heart Type Fatty Acid Binding Protein(H-FABP) and NT-pro natriuretic peptide in Heart Failure Patients with Reduced and Preserved Ejection Fraction"

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Abstract: ***Background***: Heart failure (HF) is a prevalent condition with complex pathophysiology and with a significant morbidity and mortality rate, necessitating accurate biomarkers for timely diagnosis and differentiation between its subtypes: reduced ejection fraction (HFrEF) and preserved ejection fraction (HFpEF). ***Objective:*** The current study aimed to evaluate and compare the diagnostic value of heart-type fatty acid-binding protein (H-FABP) and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) in HF patients with HFrEF and HFpEF. ***Patients and Methods***: A case-control study was conducted involving 85 participants divided into three groups: 30 HFrEF patients (15 male, 15 female), 30 HFpEF patients (15 male, 15 female), and 25 controls. Serum levels of NT-proBNP and H-FABP were measured using ELISA kits. ***Results:*** H-FABP and NT-pro-BNP levels were statistically significantly elevated in HF patients compared to controls ($P < 0.001$). H-FABP was markedly elevated in HFrEF, while NT-pro-BNP levels were highest in HFrEF patients. H-FABP showed an AUC of 0.882 (sensitivity 91.7%, specificity 88%) and had a cut-off of 2.195 ng/mL, while NT-pro-BNP demonstrated an AUC of 0.923 (sensitivity 96.7%, specificity 88%) and had a cut-off of 165.025 pg/mL. NT-Pro BNP showed an odds ratio [OR] of 5.021 (95% CI: 2.35-10.08), and H-FABP: OR=4.474 (95% CI: 2.10-9.50). A significant positive correlation was noted between H-FABP and NT-pro-BNP levels ($r = 0.550$, $P < 0.001$). ***Conclusion:*** H-FABP and NT-pro-BNP showed statistically significantly higher levels in patients with heart failure, indicating their utility as significant biomarkers for diagnosing and differentiating heart failure phenotypes. According to the findings of this study, the combined use of these biomarkers improves the accuracy of distinguishing between different patterns of the disease, supporting their role as valuable tools in clinical evaluation, and also, it was shown that, in the future, H-FABP can be applied in the early detection of heart failure.

Keywords: H-FABP, NT-proBNP, heart failure, diagnosis Reduced ejection fraction, preserved ejection fraction

INTRODUCTION

Heart Failure (HF) is the final stage of a wide range of cardiac impairments and stands out as a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood. that results in the heart's failure to pump sufficient blood and oxygen to meet the metabolic requirements of other organs [1].

Heart failure is more shared as people age, whose incidence and mortality rates are persistently rising in conjunction with the aging demographic and an increasing load of comorbidities [2].

Rising from about 1% in individuals under 55 to >10% in individuals 70 years of age or older [3].

N-terminal pro-brain natriuretic peptide (NT-proBNP) is a cleavage product of proBNP that is released in response to ventricular volume expansion and heightened wall stress and has become a fundamental indicator in the diagnosis and risk assessment of heart failure [4].

NT-proBNP levels correlate with disease severity and prognosis and are integrated into major clinical guidelines, including those from the European Society of Cardiology (ESC) for the diagnosis of heart failure with reduced ejection fraction (HFrEF) and preserved ejection fraction (HFpEF) [5]. Considering its clinical utility, NT-proBNP levels may be affected by many confounding variables, including renal dysfunction, age, and obesity, necessitating the investigation of complementary biomarkers [6].

H-FABP is a small 15 kDa soluble protein composed of 132 amino acids. It is among the most prevalent proteins in the heart, constituting 5–15% of the total cytosolic protein pool. It participates in the intracellular transport of fatty acids for mitochondrial oxidation. [7]. H-FABP is released within 30 min to 3 h after myocardial injury, rendering it one of the earliest indicators identifiable in the bloodstream [8].

In chronic heart failure, a complete myocardial infarction may not occur; rather, there can be recurrent minor injuries, partial necrosis, and programmed cell death (apoptosis) during the process of cardiac remodelling. All of this leads to continuous cell breakdown or increased membrane permeability, resulting in a persistent or recurrent elevation of H-FABP levels. This promotion is associated with disease severity, hospitalization rates, and mortality [9].

The current study was designed to evaluate the diagnostic value of heart-type fatty acid-binding protein (H-FABP) and NT-proBNP in patients with heart failure, stratified by preserved and reduced ejection fraction, compared to controls. In addition, the current study aimed to assess the relationship between these biomarkers with demographic data (sex, age, and BMI) and risk factors such as DM, as well as their diagnostic sensitivity and specificity. By comparing the levels and performance of H-FABP with NT-proBNP, efforts were made to determine whether H-FABP could serve as a new complementary biomarker alongside NT-proBNP in the clinical evaluation of HF.

MATERIAL AND METHODS

A case-control study was carried out in the Karbala center for Cardiac Diseases and Al-Zahraa Center at Imam Hussain Medical City in Karbala, Iraq. And in the Department of Chemistry and Biochemistry, University of Karbala, College of Medicine. The participants were gathered over the duration spanning From December 2024 to March 2025 The study protocol was approved by the Ethical Committee of Karbala College of Medicine and the Karbala Health Directorate. Ninety participants were enrolled and divided into three groups: Group A: 30 patients diagnosed with HFrEF (EF <40%); Group B: 30 patients with HFpEF (EF ≥50%); Group C: 25 age- and sex-matched controls.

Inclusion Criteria:
Patients with heart failure were carried out in the Karbala Center for Cardiac Diseases and Al-Zahraa Center at Imam Hussain Medical City, Karbala, Iraq, diagnosed by cardiologists according to the history, clinical examination, electrocardiogram (ECG), and echocardiogram (Echo).

Exclusion Criteria:
Patients with chronic kidney disease, chronic liver disease, coronary heart disease, cancer, severe pulmonary conditions, patients with COVID-19, and pregnancy.

Sample Collection and Analysis: Venous blood samples of 5 ml were collected and centrifuged. All participants' serum H-FABP and NT-pro-BNP concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions. The concentrations of lipid profile analyses and fasting blood sugar (FBS) were analyzed using the Cobas C311 fully automated chemistry autoanalyzer device (Roche Inc., CH). while HbA1c was determined using a separate EDTA tube and analysed on the Lifotronic H8 device.
Statistical Analysis: Data were analysed using SPSS version 26. Continuous variables were expressed as mean ± SD. Intergroup comparisons were performed using one-way ANOVA and two independent samples T-tests. Diagnostic performance was assessed by ROC curve analysis. Correlation between H-FABP and NT-pro-BNP was determined using Pearson correlation coefficient. A P-value < 0.05 was considered statistically significant.

Ethical Approval: The study protocol was approved by the Ethical Committee of the University of Kerbala, College of Medicine, and Karbala Health Directorate. Informed consent was obtained in accordance with ethical standards, either directly from the patients or, when necessary, from their relatives.

RESULTS

The distribution of sex, age, and BMI across three groups:
The distribution of sex, age, and BMI across three groups Control, Preserved Ejection Fraction (EF), and Reduced Ejection Fraction (EF). Sex distribution was nearly equal among males and females across patient groups, with no significant difference (p=0.702). Age showed no significant difference (p=0.334). Regarding BMI, there was no significant difference (p=0.218), as shown in Table 1.

Comparison of study biomarker levels between two study subgroups
The heart failure groups exhibited statistically significantly higher levels of H-FABP; the HFrEF subgroup exhibited statistically significantly higher levels of H-FABP (4.271±1.166 ng/ml) compared to HFpEF (2.928±0.767 ng/ml), with a p-value (< 0.001)**,** compared to controls (1.811 ± 0.368 ng/ml), with a p-value < 0.001.
NT-proBNP showing statistically significant differences across HFrEF (415.988±56.462) and HFpEF (306.35±24.950), with a p-value (< 0.001), compared to controls (104.756±15.122 pg/ml), with a p-value < 0.001,
as shown in Table 2 and Figure 1.

Table 1: Socio-Demographic Characteristics

Characteristics		Groups			P-value
		Control (n=25)	Preserve EF (n=30)	Reduced EF (n=30)	
Sex (%)	Male	10 (40.0%)	15 (50.0%)	15 (50.0%)	0.702
	Female	15 (60.0%)	15 (50.0%)	15 (50.0%)	
Age n (%)	< 50 years	9(36.0%)	6 (20.0%)	5 (16.7%)	0.334
	50-60 years	10 (40.0%)	10 (33.3%)	8 (26.7%)	
	61-70 years	5 (20.0%)	10 (33.3%)	12 (40.0%)	
	> 70 years	1 (4.0%)	4 (13.33%)	5 (16.7%)	
BMI n (%)	Normal weight	13 (52.0%)	10 (33.3%)	13 (43.3%)	0.218
	Overweight	9 (36.0%)	13(43.3%)	12 (40.0%)	
	Obese	3 (12.0%)	7 (23.3%)	5 (16.7%)	

n: Number of Patients, %: Percent, Chi-Square test, Differences are significant at P-value < 0.05

Table 2. Comparison of Study Biomarkers According to Study Subgroups

Study Subgroups				
Biomarkers	**Control Mean ± SD (n=25)**	**Preserve EF Mean ± SD (n=30)**	**Reduced EF Mean ± SD (n=30)**	**P-value**
H-FABP (ng/ml)	1.811±0.368	2.928±0.767	4.271±1.166	<0.001
NT-PrBNP(pg/ml)	104.756±15.122	306.35±24.950	415.988±56.462	<0.001

n: Number of Patients, SD: Standard Deviation, two independent samples T-test, Differences are significant at P-value < 0.05

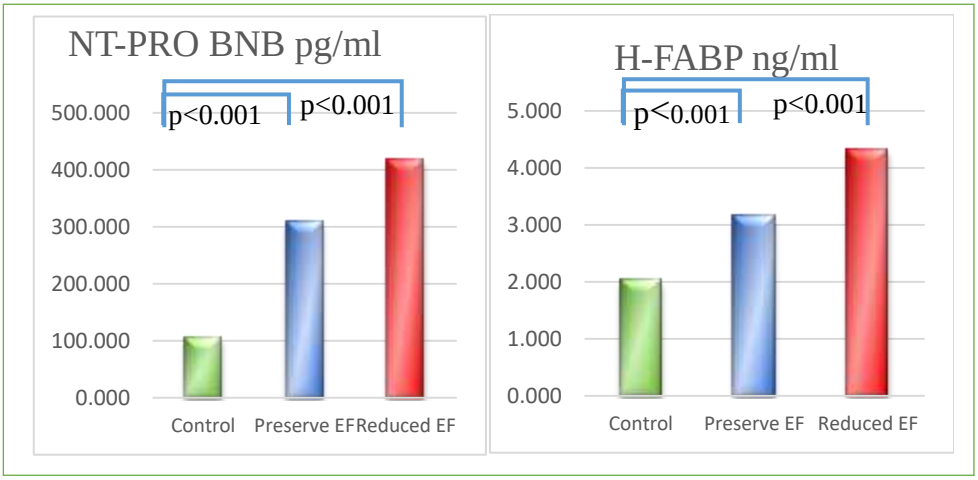

Fig. 1 Comparison of Study Biomarkers Based on Study Subgroups

H-FABP levels were higher in diabetic patients with heart failure (3.81±0.92 ng/ml) (n=41) compared to non-diabetic patients with heart failure (3.47±1.07 ng/ml) (n=19), but this difference was not statistically significant (p=0.240).
NT-proBNP levels were higher in diabetic patients (363.87±64.76 pg/ml) compared to non-diabetic patients (340.05±58.2 pg/ml), but this difference also was not statistically significant (p = 0.161), as shown in Table 3.

Table 3: Comparison of Study Biomarker Levels according to Diabetic Status in Patients with Heart Failure

Diabetic Mellitus			
biomarkers	**No Mean ± SD (n=19)**	**Yes Mean ± SD (n=41)**	**P-value**
H-FABP (ng/ml)	3.47 ±1.07	3.81±0.92	0.240
NT-Pro BNP (pg/ml)	340.05±58.2	363.87 ±64.76	0.161

n: Number of Patients, SD: Standard Deviation, Test: Two independent samples T-test, Differences are significant at a p-value < 0.05.

Comparison of Study Biomarker Levels with Body Mass Index (BMI) among Patients with Heart Failure.

The levels of H-FABP showed slight variation, being lowest in normal-weight patients (3.55 ± 0.99 ng/ml), higher in the obese group (3.83 ± 1.07 ng/ml), and higher in the overweight group (3.91 ± 0.99 ng/ml), though the differences were not statistically significant (p = 0.475).

However, NT-Pro BNP levels showed a statistically non-significant difference (p = 0.116). being high in the normal weight (366.162 ± 67.30 pg/ml), high in overweight (359.66 ± 71.11 pg/ml), and lowest in the obese group (319.51 ± 68.87 pg/ml), as shown in Table 4.

Table 4: Comparison of Study Biomarker Levels with Body Mass Index (BMI) among Patients with Heart Failure.

	BMI			
Biomarkers	**Normal weight (n=21) Mean ± SD**	**Overweight (n=24) Mean ± SD**	**Obese (n=15) Mean ± SD**	**P-value**
H-FABP (ng/ml)	3.55±0.99	3.91±0.99	3.83±1.07	0.475
NT-Pro BNP (pg/ml)	366.162±67.30	358.66±71.11	319.51±68.87	0.116

n: Number of Patients, SD: Standard Deviation, Test: One-way ANOVA test, Differences are significant at P-value < 0.05

Correlation Coefficients Between Study Biomarkers Among Patients with Heart Failure

The results demonstrate a moderate positive correlation between H-FABP and NT-Pro BNP (r=0.550, p<0.001) in heart failure patients. As shown in Table 5.

Table 5: Correlation Coefficients Between Study Biomarkers Among Patients with Heart Failure

biomarkers		**H-FABP (ng/ml)**	**NT-Pro BNP (pg/ml)**
H-FABP (ng/ml)	Pearson Correlation	1.000	0.550
	P-value	-	<0.001

Test: Pearson Correlation Coefficient. Correlations are significant at a p-value < 0.05.

Estimation of the association of study biomarkers in heart failure patients.

Table 6 evaluates the association of study biomarkers (H-FABP and NT-proBNP) with heart failure risk. Demonstrating highly significant predictive value for two study biomarkers (p<0.001). NT-Pro BNP showed an odds ratio [OR] = 5.021, 95% CI: 2.35-10.08).

H-FABP: OR=4.474, 95% CI: 2.10-9.50). As shown in Table 6.

Table 6: Estimation of the Association of Study Biomarkers in Heart Failure Patients.

biomarkers	**Odds Ratio**	**CL 95%**		**P-value**
		Lower	Upper	
H-FABP (ng/ml)	4.474	2.105	9.501	<0.001
NT-Pro BNP (pg/ml)	5.021	2.354	10.08	<0.001

CL: Confidence level, Associations are significant at P-value < 0.05

AUC, P-value, Cut-off Value, Sensitivity, and Specificity of H-FABP and NT-pro-BNP Levels in Heart Failure Patients Compared to Control Group.

H-FABP with an area under the ROC curve (AUC) of 0.882 (p<0.001). At a cut-off value of 2.195 ng/ml, H-FABP sensitivity (91.7%) and specificity (88%) for distinguishing heart failure patients from controls, these results suggest that H-FABP is a highly reliable biomarker for detecting heart failure, with both high true positive and true negative rates when using the identified cut-off level.

NT-pro-BNP with an area under the ROC curve (AUC) was 0.923, indicating excellent diagnostic performance. The optimal cut-off value for NT-Pro BNP was determined to be 165.025 pg/ml, at which point the test achieved a sensitivity of 96.7% and a specificity of 88%, with a highly significant p-value < 0.001.

as shown in Table 7 and Figure 2.

Table 7: AUC, P-value, Cut-off Value, Sensitivity, and Specificity of H-FABP and NT-pro-BNP Levels in Heart Failure Patients Compared to Control Group.

Biomarkers	AUC	P-value	Cutoff value	Sensitivity	Specificity
H-FABP (ng/ml)	0.882	<0.001	2.195 ng/ml	91.7%	88%
NT-proBNP (pg/ml)	0.923	<0.001	165.025 pg/ml	96.7%	88%

AUC: Area under Curve

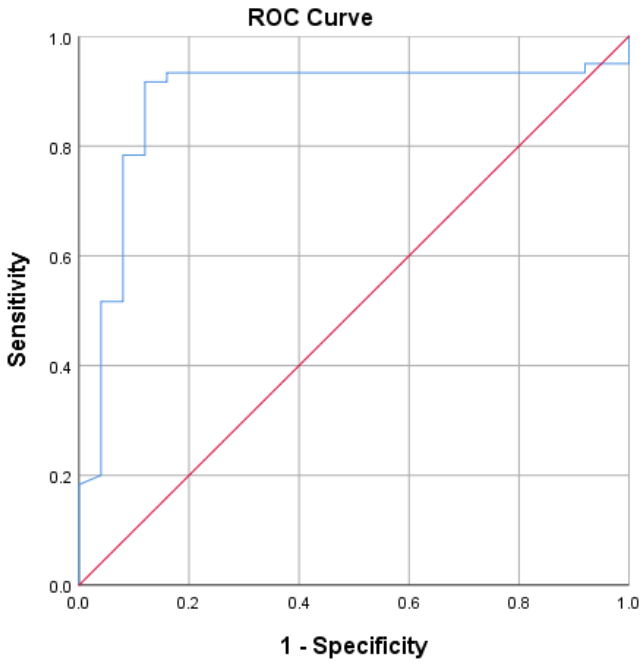

Fig. 2 Receiver Operation Curve (ROC) of H-FABP in Heart Failure Patients Compared to Control Group

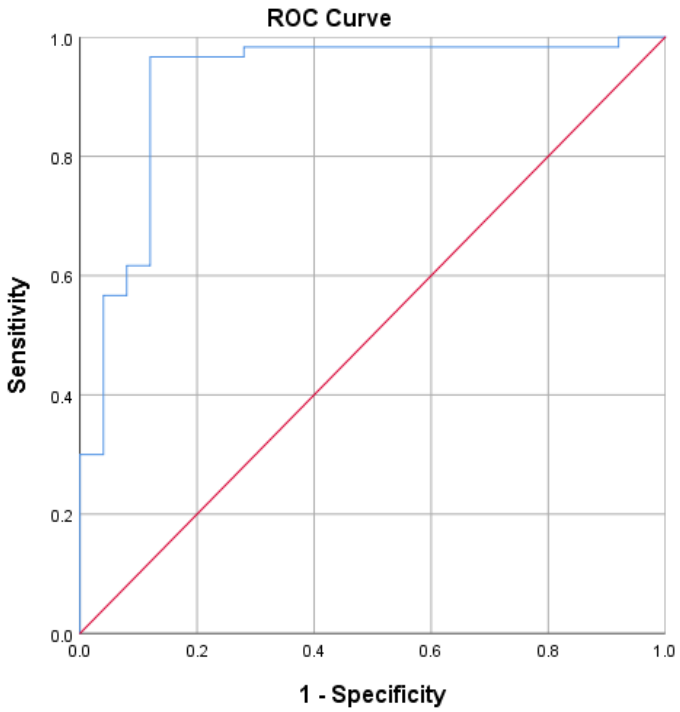

Fig. 3 Receiver Operation Curve (ROC) of NT-proBNP in Heart Failure Patients Compared to Control Group.

DISCUSSION

In the current study, the percentage of males is equal to that of females, with no statistically significant difference. Gender distribution was equal among males and females across heart failure patient groups, as shown in the table (1).

Research by Cediel et al. in 2021 showed numerous distinctions between women and men have been noted in heart failure, including the epidemiology, aetiology, pathogenesis, risk factors, and diagnosis. The prevalence of HF also differs between men and women, depending on the analysed study population. For example, women exhibited a reduced risk of incident heart failure compared to males among middle-aged to older persons; however, in the oldest age categories, women demonstrated a heightened risk of heart failure relative to men.

Men tended to be at higher risk of developing HF with reduced ejection fraction (HFrEF), and conversely, women exhibited a higher propensity to develop HFpEF [10].

Sex is a potential risk factor in ageing adults; older women possess an elevated risk of cardiovascular disease (CVD) relative to men of equivalent age. Nevertheless, in both males and females, the likelihood of developing CVD rises as they become older, which is linked to a general decrease in sex hormones, particularly oestrogen and testosterone [11].

In the current study, the age groups showed no statistically significant variation across three groups: Control, Preserved Ejection Fraction (EF), and Reduced Ejection Fraction (EF).

Indicating that they were age-matched and age had no effect on the results.

Research by Fang et al. in 2025 showed Many physical systems are impacted by the ageing process and lead to both structural and functional deterioration in heart tissue. These changes include an increased prevalence of left ventricular hypertrophy, a deterioration in left ventricular diastolic function, left atrial dilation, atrial fibrillation, myocardial fibrosis, and cardiac amyloidosis as a result, increasing the risk of CHF in the elderly. At the same time, endothelial dysfunction causes the circulatory system to undergo structural and functional changes that accompany ageing.

Arterial stiffness, impaired angiogenesis, oxidative stress, and inflammation impose additional strain on the heart [12].

In the current study, as shown in table 2, the levels of two study biomarkers
(H-FABP and NT-pro-BNP) significantly higher among patients of heart failure disease when compared to controls. The current study investigated the diagnostic utility of H-FABP and NT-pro-BNP levels among patients with heart failure, stratified by preserved and reduced ejection fraction (EF), in comparison to a control group.

H-FABP levels were elevated in patients, especially in reduced EF%. These results are in line with a study by Zhang et al. in 2025, which found that HFrEF patients had higher-than-average levels of H-FABP, indicating ongoing or progressive myocardial injury. Also, it showed that H-FABP is elevated in patients with chronic heart failure and HFrEF, signalling the progression of the disease and the worsening of the heart [13].

Also, these findings are consistent with research by Wang et al. in 2023 showing that combining H-FABP with the conventional biomarker NT-proBNP improves the ability to evaluate disease severity and predict clinical outcomes, verifying that H-FABP is an indicator of persistent heart damage and complements NT-proBNP in the diagnosis and monitoring of patients with HFrEF and HFpEF [14].

In the current study, patients, particularly those with decreased EF%, had higher NT-pro-BNP levels. And as shown in table 2 and figure (1).

Research by Cao et al. in 2019 showed that elevated levels of NT-pro-BNP serve as a recognised indicator of cardiac stress and dysfunction [15]. However, Rudolf et al. (2020) define that elevated NT-pro-BNP levels are related to poorer prognoses, encompassing a sensitive risk of all-cause mortality, cardiovascular mortality, and hospitalisation due to heart failure [16].

The results showed no statistically significant differences between level of H-FABP and NT-proBNP with diabetes mellitus (DM) and body mass index (BMI) in heart failure patients, as shown in tables (3) and (4).

In the current study, 68.33% of the heart failure patients were diagnosed with diabetes, as shown in Table 3, which indicates that diabetes is a major contributing factor in the development and progression of heart failure and causes metabolic changes, ultimately resulting in impaired cardiac function.

Also, H-FABP concentrations did not statistically significantly differ between HF with diabetes and HF without diabetes. These findings have been consistent with research by Rodríguez-Calvo et al. in 2023 showing H-FABP level was independently associated with all-cause death, and H-FABP concentrations were associated with HF mortality [17].
Also, consistent with other research, there was no statistically significant difference between H-FABP level and BMI categories [18].

This indicates that H-FABP is an independent biomarker in heart failure, reflecting myocardial injury rather than being influenced by metabolic or demographic variables. Also, the results showed here were no statistically significant correlations between NT-proBNP levels and any of the major cardiovascular risk factors, including diabetes mellitus (DM), BMI, demographic variables, or other risk factors. independent prognostic marker

These findings have been confirmed with a study by Siddiqui et al. in 2024 that showed no statistically significant difference was observed between NT-proBNP mean values in patients with or without diabetes in heart failure patients and revealed that NT-proBNP is an independent prognostic marker of mortality and morbidity in heart failure patients [19].

Also, consistent with other research by Suthahar et al. in 2021, the relationship between NT-proBNP levels and BMI categories was not found to be statistically significant. Also, elevated NT-proBNP levels were associated with incident HF, and BMI did not modify these associations [20].

In the current study, as shown in tables (5). The results showed a positive correlation between study biomarkers, H-FABP and NT-ProBNP, in heart failure patients. These results are consistent with Gruson et al. in 2021, showing the NT-proBNP biomarker is integral to the standard of treatment for diagnosing and managing heart failure patients.
and H-FABP had a strong correlation with NT-proBNP. H-FABP concentrations were also prognostic of cardiovascular (CV) death, and when combined with NT-proBNP, they may be synergistic for risk assessment.

[21].
In the current study, as shown in tables (6) and (7), the results showed statistically significant predictive value for study biomarkers (H-FABP and NT-proBNP) ($p<0.001$) in heart failure patients.

Also, the ROC curve analysis showed that both biomarkers, H-FABP and NT-proBNP, possess high diagnostic ability in differentiating heart failure patients from controls. H-FABP demonstrated a high area under the curve (AUC) with high sensitivity and strong specificity, indicating excellent accuracy in detecting diseased cases and distinguishing them from the control group.

Also, it is consistent with a study by Weng et al. in 2020. The study demonstrated that heart-type fatty acid-binding protein (H-FABP) surpasses traditional cardiac markers for the early detection of acute myocardial injury in heart failure patients.

due to higher sensitivity and specificity. Furthermore, H-FABP has a close correlation with the severity of heart failure [22].

In the current study NT-pro-BNP demonstrated strong diagnostic performance with an area under the ROC curve (AUC) of 0.923, sensitivity of 96.7%, and specificity of 88%.

NT-pro-BNP levels were elevated in patients, especially in reduced EF%. Elevated levels of NT-pro-BNP serve as a recognised indicator of cardiac stress and dysfunction and are linked to both the incidence and severity of heart failure [23].

CONCLUSION

H-FABP and NT-pro-BNP showed statistically significantly higher levels in patients with heart failure, indicating their utility as significant biomarkers for diagnosing and differentiating heart failure phenotypes. According to the findings of this study, the combined use of these biomarkers improves the accuracy of distinguishing between different patterns of the disease, supporting their role as valuable tools in clinical evaluation. And also, it was shown that, in the future, H-FABP can be applied in the early detection of heart failure.

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