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Evaluation of the diagnostic performances of tissue inhibitors of metalloproteinase-1 and fibronectin for heart failure

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Abstract

Previous research has linked an imbalance of the tissue inhibitors of metalloproteinase-1 (TIMP-1) and fibronectin (FN) to heart failure as a part of the extracellular matrix network (ECM) biochemistry profile, which is vital for cardiac homeostasis. This study aimed to assess the diagnostic performance of FN, TIMP-1, and CK-MB in heart failure (HF). Sixty patients (45 with acute and 15 with chronic HF) were recruited. Thirty individuals (20 with ischemic heart diseases, as other cardiac diseases, and 10 healthy individuals) were recruited as a control group. The biotin double antibody sandwich technology determined levels of human fibronectin and tissue inhibitors of metalloproteinase-1. FN was the most effective biomarker in differentiating HF patients from healthy individuals (AUC = 0.850) ($P < 0.001$), followed by TIMP (AUC = 0.74) and CK MB (AUC = 0.660). The sensitivity and specificity of FN were 82% and 70%, respectively, at a cutoff of 80 ng/ml. In addition, FN and TIMP had the same AUC (0.71) and efficiency (65%) in distinguishing HF patients from controls, followed by CK-MB (AUC = 0.70). We developed a novel model for HF diagnosis named the HFD model based on three biomarkers (FN, TIMP, and CK MB). The HFD model had an AUC of 0.77 in distinguishing HF patients from healthy individuals, with a sensitivity, specificity, and accuracy reaching 80%. For differentiating HF patients from controls, the HFD model had 0.8 AUC, 76% sensitivity, 75% specificity, and 76% accuracy.

Keywords: Extracellular matrix, Biochemical markers, Heart failure, Fibronectin, Tissue inhibitors of metalloproteinase-1

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1. Introduction

The main cause of death worldwide is cardiovascular disease (CVD). They are classified into five groups: atherosclerosis, acute myocardial infarction (AMI), heart failure (HF), stroke, and hypertension, conditions that can lead to death (Shi et al., 2020). Heart failure affects over 64.3 million people worldwide. In developed nations, the prevalence of diagnosed heart failure in the general adult population ranges between 1 and 2%. (Groenewegen et al., 2020; Hassanin et al., 2020).

According to the most recent guidelines issued by the European Society of Cardiology, the initial assessment of suspected heart failure patients should consist of clinical history, physical examination, laboratory profile, chest radiography, and electrocardiography. An echocardiogram can be used for confirmation. In addition to myocardial anomalies, other impairments such as abnormalities of the heart rhythm, pericardium, endocardium, valves, and conduction may be detected. To diagnose and manage heart failure, determining the underlying cause is critical (Ponikowski et al., 2016). To determine the severity and cause of heart failure, many investigations may be conducted, such as chest X-ray, electrocardiogram (EKG or ECG) (Vaidya, 2017), echocardiogram, magnetic resonance imaging (MRI) (Peterzan et al., 2016), NT-pro B-type natriuretic Peptide (BNP) (Tsai et al., 2010), cardiac catheterization, multi gated acquisition scan (MUGA scan) (Odak and Kayani, 2021), and stress test.

CKMB is a cardiac enzyme and is the most sensitive and specific myocardial cell necrosis indicator (Chang et al., 2015). Troponin is another cardiac enzyme with higher sensitivity and specificity than CKMB. As a result, cardiac enzymes are the preferred biochemical indicator of cardiac injury, detecting even tiny myocardial necrosis (Mair et al., 2018). Fibronectin and TIMP-1 are two of the most frequently used matrix indicators in the genesis and prognosis of cardiovascular diseases. In large community-based samples, circulating levels of FN and TIMP-1 have been linked to most cardiovascular disease risk factors. Furthermore, they have been linked to mortality in individuals with known cardiovascular diseases (Frangogiannis, 2019).

The matrix metalloproteinases (MMPs) are a class of enzymes capable of cleaving extracellular matrix components. Various MMPs are high in non-ischemic cardiomyopathy, and MMP-1 levels are related to prognosis in heart failure patients. Tissue inhibitors of metalloproteinases inhibit MMPs. TIMPs suppresses angiogenesis and apoptosis while preventing uncontrolled collagen breakdown. TIMP-1 deletion harms cardiac remodeling after myocardial infarction (Spinale, 2007).

Even though collagen is the most prevalent ECM protein in the heart, cellular fibronectin is critical in cardiac fibrosis. Patients with ischemia and dilated cardiomyopathy and HF animal models have higher FN levels (Valiente-Alandi et al., 2018). The current study aimed to evaluate the diagnostic performance of ECM components, including fibronectin, tissue inhibitors of metalloproteinase-1, and CK MB.

2. Material and Method

2.1. Patients

The study included sixty heart failure patients (45 acute and 15 chronic) and thirty individuals as a control group (20 with ischemic heart diseases and 10 healthy individuals). Heart failure diagnosis was based on the European Society of Cardiology (ESC) (Greer et al., 2018). Patients, less than 40 years or who had chronic kidney failure were excluded. All patients signed informed consent following the regulations of the National Heart Institute Research Board and the Helsinki Declaration.

2.2. Biochemical tests

Blood samples were taken from all patients after 12 hours of fasting. Routine laboratory investigations, including lipid profile, kidney function test, liver function test, and creatine kinase MB were done using an automated biochemistry analyzer (Cobas C 111, automated biochemistry analyzer, Japan). Complete blood count was measured using an automatic hematology analyzer (BC-2800, Mindray instruments, China). The serum was then separated to perform the troponin test using a rapid test (one-step troponin I test device (ABON card)). Levels of human fibronectin (Bioassay technology laboratory; Shanghai Korain Biotech Co., Ltd, Shanghai, China) and tissue inhibitors of metalloproteinase-1 (Bioassay technology laboratory; Shanghai Korain Biotech Co., Ltd, Shanghai, China) were determined. These assays were based on the biotin double antibody sandwich technology.

2.2. Statistical Analysis

Continuous normally distributed data were expressed as mean ±SD. The chi-square test (x^2), the Anova test, and Student's t-test assessed statistically significant differences between the studied groups. Statistical significance was defined as a *P*-value less than 0.05. Correlation analyses were done using Pearson's test for parametric data. A multiple logistic regression model was done to detect heart failure predictors. The diagnostic power of the studied markers was assessed using the ROC curve.

3. Results

3.1. Levels of routine markers in different studied groups

As shown in table1, no significant differences were detected between the studied groups regarding age, hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), cholesterol, triglyceride (TG), very low density lipoprotein (VLDL), and platelet count. In contrast, there were significant differences in alanine aminotransferase (ALT) (P = 0.014), aspartate aminotransferase (AST) (P = 0.009), creatinine (P = 0.001), urea (P < 0.001), high-density lipoprotein (HDL) (P = 0.024), and low-density lipoproteins (LDL) (P = 0.004), CK-MB (P = 0.002), FN (P = 0.009), and TIMP (P < 0.0001) (Figure 1). WBCs (P = 0.029), ALT (P = 0.029, and CK-MB (P = 0.01) significantly differed (P = 0.029) between acute and chronic heart failure, while FN and TIMP showed no significant differences (P = 0.435, 0.156, respectively) (Figure 2).

3.2. Correlation

FN was significantly positively correlated with TIMP (r = 0.679, P < 0.001) In contrast, FN showed a significant negative correlation with platelet count (r = -0.224, P = 0.038). In addition, TIMP and LDL were significantly negatively correlated (r = -0.222, P = 0.042).

3.3. Diagnostic performance

The diagnostic accuracy of potential markers to detect HF was assessed using ROC curves. FN was the most effective biomarker in distinguishing HF from healthy individuals (AUC = 0.85, sensitivity = 82%, specificity = 72%), followed by TIMP (AUC= 0.74) and CK-MB (AUC=0.66) (Table 3). FN and TIMP had the same AUC (0.71) and efficiency (65%) in distinguishing HF from controls, followed by CK-MB (AUC= 0.70) (Table 3). A regression model incorporating the three significant variables was employed to construct the optimal model to diagnose HF, namely the HFD model. It was represented as: (0.496+ CK-MB X 0.005 + TIMP-1 X 0.001 + FN X 0.001). The HFD model had an AUC of 0.77 with a sensitivity, specificity, and accuracy reaching 80% for distinguishing HF patients from healthy individuals. For differentiating HF patients from controls, it had an AUC of 0.80 with 76% sensitivity, 75% specificity, and 76% accuracy

Table 1: The levels of routine markers of different studied groups

Variables	Healthy	Other cardiac diseases	Control	HFD	P-value
Age (years)	54.7±5.7	54.1±8.2	54.3±7.4	57.7±11.1	0.129
Hb (gm/dl)	13.8±1.8	12.8±1.4	12.5±1.6	12.5±1.9	0.86
RBC ($x10^6$/uL)	5.0±0.54	4.7±0.39	4.8±0.47	4.6±0.71	0.31
WBC ($x10^3$/uL)	6.5±1.9	8.3±2.8	7.7±2.6	7.5±2.7	0.798
Platelets ($x10^9$/L)	237±56	241±65	239±61	216±63	0.105
ALT (IU/l)	13.8±3.1	18.9±7.6	17.2±6.8	50.8±73.2	**0.014**
AST (IU/l)	18.4±4.2	21.7±4.89	20.6±4.8	42.7±45.3	**0.009**
Creatinine (mg/dl)	0.82±0.17	0.94±0.21	0.9±0.2	1.3±0.68	**0.001**
Urea (mg/dl)	35.7±8.5	33.3±6.75	34.1±7.3	73.8±45.6	**< 0.0001**
Cholesterol (mg/dl)	140.2±22.3	157.7±43.9	151.8±38.6	139.2±43.1	0.18
Triglyceride (mg/dl)	113.9±43.9	128.4±55.1	123.6±51.3	134.1±97.1	0.58
HDL (mg/dl)	39.1±6.6	38.5±7.7	38.7±7.3	34.2±9.2	**0.024**
LDL (mg/dl)	123.2±20.5	92.0±36.2	102.4±34.8	79.8±33.6	**0.004**
VLDL (mg/dl)	27.3±5.7	26.5±10.3	26.7±8.9	26.0±16.4	0.825
CK MB (U/L)	15.1±5.7	14.6±4.8	14.8±5.0	24.9±16.8	**0.002**
FN (ng/ml)	65.7±24.1	101.4±38.7	89.5±38.2	128.6±74.4	**0.009**
TIMP (ng/ml)	75.1±23.0	82.1±39.8	79.6±34.6	150.8±88.3	**< 0.0001**

P-value tested by Anova test

Table 2: The levels of routine markers between chronic and acute heart failure groups

Variables	Chronic	Acute	P^2
Age (years)	56.5±8.6	58.1±11.9	0.26
Hb (gm/dl)	12.8±2.2	12.5±1.8	0.568
RBC ($x10^6$/uL)	4.8±0.58	4.6±0.75	0.262
WBC ($x10^3$/uL)	6.99±1.7	7.76±3.06	**0.029**
Platelets ($x10^9$/L)	224.2±48.2	214.4±68.2	0.18
ALT (IU/l)	68.6±112.7	44.9±54.87	**0.029**
AST (IU/l)	37.6±27.0	44.4±50.1	0.19
Creatinine (mg/dl)	1.2±1.19	1.37±0.40	0.104
Urea (mg/dl)	46.8±32.4	82.88±46.1	0.232
Cholesterol (mg/dl)	145.4±47.4	137.2±41.9	0.515
Tri-glyceride (mg/dl)	152.8±140.8	127.9±78.6	0.62
HDL (mg/dl)	35.6±7.6	33.8±9.7	0.183
LDL (mg/dl)	82.2±36.4	79.0±33.0	0.788
VLDL (mg/dl)	27.6±19.0	25.5±15.7	0.267
CK MB (U/L)	19.2±6.3	26.8±18.7	**0.01**
FN (ng/ml)	170.8±71.4	113.2±70.1	0.435
TIMP (ng/ml)	208.3±66.8	129.3±86.3	0.156

P value tested by T-Test test

Table 3: Diagnostic performance of CK, FN, TIMP, and HFD model to differentiate among studied groups

Marker	ROC	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Healthy VS HFD							
CK MB	0.66	17	60	60	90	20	60
FN	0.85	80	82	70	94	41	80
TIMP	0.74	84	76	60	91	32	74
*****HFD model	0.86	0.76	80	80	95	44	80
Control VS HFD							
CK MB	0.70	17	60	63	77	44	61
FN	0.71	80	82	33	70	50	65
TIMP	0.71	84	76	45	72	50	65
*****HFD model	0.80	0.76	76	75	84	64	76

*****HFD model= combined three markers (Ck, FN, TIMP)**.** It can be represented as: (0.496+ CK MB X 0.005 + TIMP-1 X 0.001 + FN X 0.001)

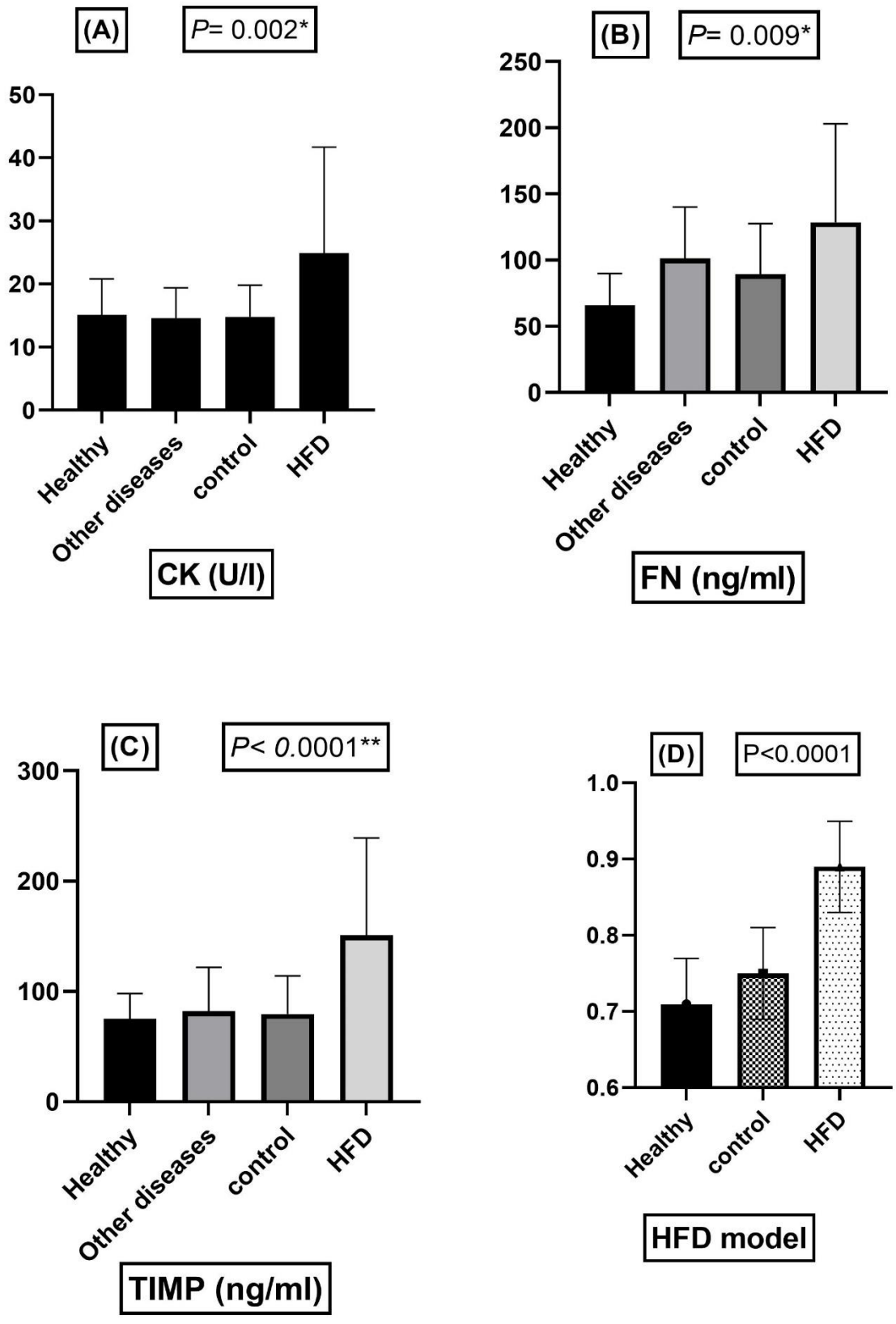

Figure 1. levels of single candidate markers and combined markers in studied groups.

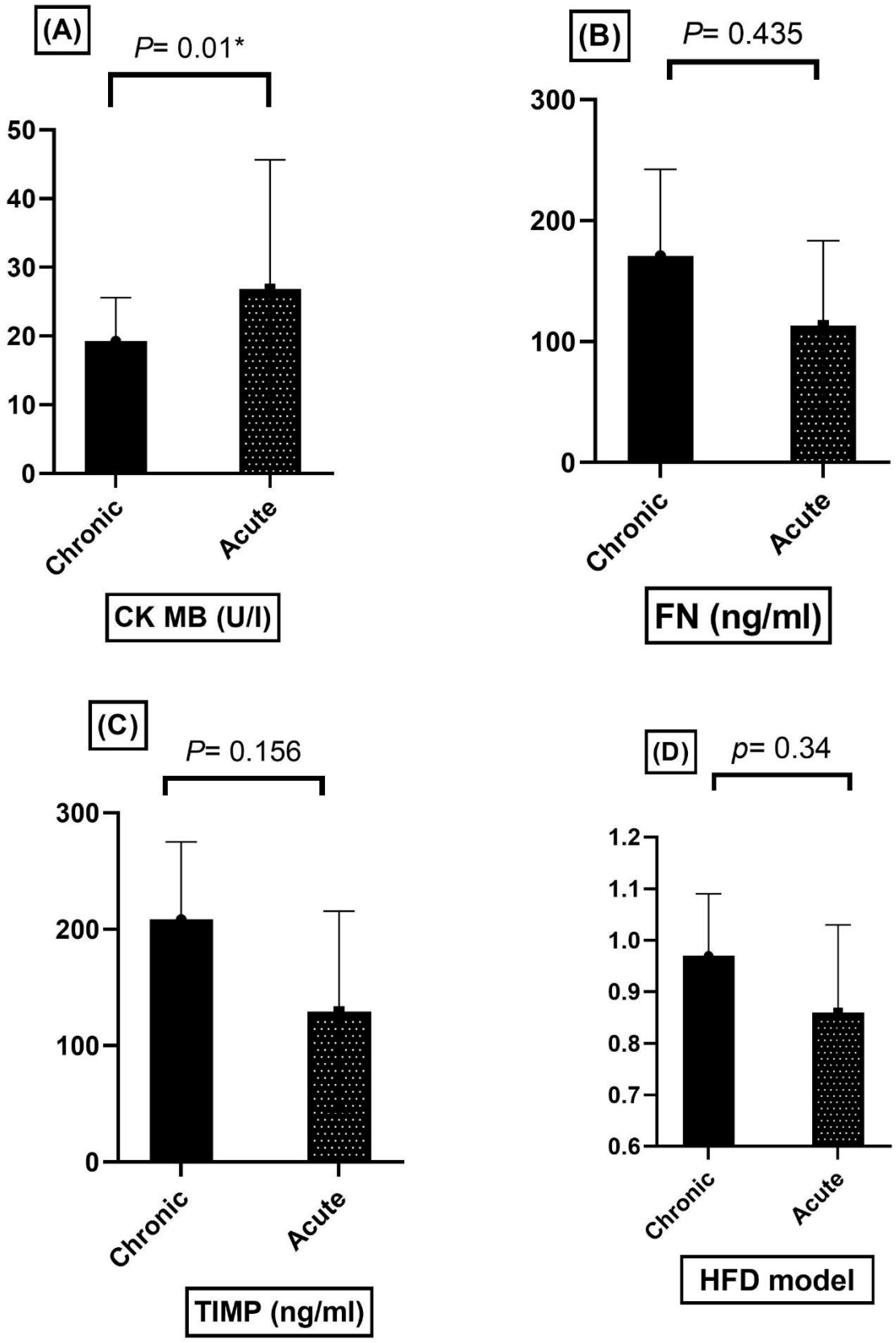

Figure 2. levels of single candidate markers and combined markers in acute and chronic HFD

4. Discussion

Ideal heart failure biomarkers should be highly expressed and detectable within cardiac tissue early after symptoms such as chest pain, with high clinical sensitivity and specificity (Wang et al., 2020). CK-MB can be released due to myocardial damage. Its levels are elevated in disorders unrelated to the heart or skeletal muscle, such as myeloma, head injuries, and brain diseases (Chang et al., 2015). According to Valiente-Alandi et al., FN is quickly expressed, creating a scar and remodeling of the myocardial wall. A substantial increase in FN expression defines the ultimate heart damage in hypertension (Bowers et al., 2019). Previous studies found that patients with heart failure have significantly higher FN levels ($P < 0.001$) than healthy participants (Ziffels et al., 2016). In the current study, the TIMP-1 had an AUC, sensitivity, and specificity of 0.85, 82%, and 70%, respectively, for distinguishing heart failure patients from healthy individuals. According to Akahane et al., TIMP-1 is responsible for aortic smooth muscle cell proliferation, and its levels are significantly lower in CHF patients ($P = 0.001$). In the present study, TIMP-1 had an AUC of 0.74 with 76% sensitivity and 60% specificity for distinguishing heart failure patients from healthy individuals. The AUC of the serum heart-type fatty acid-binding protein in diagnosing acute heart failure is 0.84, with a sensitivity and specificity of 90% and 71%, respectively (Shirakabe et al., 2016). According to Fan et al., fibroblast growth factor 21 (FGF21) has an AUC of 0.96 in predicting prognosis in heart failure patients. Fibrinogen (FIB) is the hallmark of inflammation and thrombosis and is related to the prognosis of many disorders. The AUC of fibrinogen for chronic heart failure is 0.65 (Meng et al., 2021).

In the current study, no single biomarker had an ideal AUC. However, FN was the most efficient one. Consequently, our model was based on three biochemical markers (FN, TIMP-1, CK MB) that increased the area under the curve to 0.86.

Chen et al. investigated the role of N-terminal pro-B-type natriuretic peptide (NT-pro BNP) combined with echocardiography in evaluating diastolic heart failure (DHF). They reported a higher AUC for the combination than for the single application. Heart failure leads to a hypercatabolic state, enhancing the catabolic activity of branched-chain amino acids (isoleucine, leucine, and valine) in the heart and skeletal muscles and reducing protein synthesis in the liver. Consequently, free plasma aromatic amino acids (phenylalanine and tyrosine) increase. The leucine/phenylalanine ratio accurately predicts heart failure with an AUC of 0.76, 78% sensitivity, and 67% specificity (Hiraiwa et al., 2021). Insulin-like growth factor binding protein-4 (IGFBP-4) could predict acute heart failure with an AUC of 0.73, which is significantly higher than that of NT-proBNP (0.68) and CRP (0.67). The combination of these three biomarkers leads to a higher AUC (0.79) than any of them alone (Konev et al., 2020). Claus et al. reported that a multimarker model, including NT-proBNP, growth differentiation factor-15, and circulating neprilysin, best differentiates HF from controls (AUC = 0.9).

Conclusion: Combining CKMB, FN, and TIMP-1 provide a good marker for diagnosing heart failure with high sensitivity and specificity.

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