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Diagnostic value of mirna-208a and pregnancy-associated plasma protein-A (papp-a) in relation to n-terminal pro-b-type natriuretic peptide (nt-probnp) for early detection of heart failure with preserved ejection fraction

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Abstract
Objective: Heart failure with preserved ejection fraction (HFpEF) remains diagnostically challenging owing to non-specific symptoms and limitations of conventional biomarkers. This study evaluated the diagnostic value of pregnancy-associated plasma protein-A (PAPP-A) and microRNA-208a (miRNA-208a), alone and in combination with N-terminal pro-B-type natriuretic peptide (NT-proBNP), for early detection of HFpEF.
Methods: A case-control study enrolled 119 patients with confirmed HFpEF (left ventricular ejection fraction ≥50%) and 120 age- and sex-matched healthy controls. Serum PAPP-A (ELISA), plasma miRNA-208a (real-time PCR), and NT-proBNP (chemiluminescence immunoassay) were measured. Clinical and metabolic parameters were also recorded. Group comparisons, correlation analyses, receiver operating characteristic (ROC) curves, and multiple linear regression were performed.
Results: All three biomarkers were significantly elevated in HFpEF patients compared with controls ($p<0.001$). miRNA-208a showed an 11-fold increase (61.26±11.06 vs 5.53±2.62), PAPP-A a 7-fold increase (48.63±12.32 vs 7.12±5.71 ng/mL), and NT-proBNP a 12-fold increase (618.70±247.93 vs 50.85±21.19 pg/mL). Strong positive correlations with NT-proBNP were observed for miRNA-208a ($\rho=0.775$ overall; $\rho=0.708$ within HFpEF) and PAPP-A ($\rho=0.809$ overall; $\rho=0.445$ within HFpEF). ROC analysis demonstrated excellent diagnostic accuracy (area under the curve >0.95 for all), with optimal cut-off values: PAPP-A ≥31.0 ng/mL, miRNA-208a ≥40.0 (relative expression), and NT-proBNP ≥239.0 pg/mL. In regression analysis, miRNA-208a and body mass index were the strongest independent predictors of NT-proBNP.
Conclusions: Circulating miRNA-208a and PAPP-A are significantly elevated in HFpEF and correlate strongly with NT-proBNP. A multi-biomarker panel including these markers may improve early identification of HFpEF, particularly in resource-limited settings where advanced imaging is unavailable.

Keywords: Heart failure with preserved ejection fraction, MicroRNA-208a, Pregnancy-associated plasma protein-A, NT-proBNP, Early diagnosis

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Plain English Summary
Heart failure with preserved ejection fraction (HFpEF) is a condition where the heart's pumping ability appears normal, but the heart muscle is stiff, making it hard to fill with blood. Diagnosing HFpEF is difficult because its symptoms – such as breathlessness and fatigue – are similar to those of other diseases. This study examined whether two newer blood markers (PAPP-A and miRNA-208a) could help detect HFpEF earlier and more accurately than the standard marker (NT-proBNP). We measured these markers in 119 patients with HFpEF and 120 healthy people. All three markers were much higher in patients with HFpEF, and the two new markers were as good as, or even better than, the standard test at distinguishing people with and without the disease. Using all three markers together could provide a simple blood test to help doctors diagnose HFpEF earlier, especially in places where heart ultrasound is not easily available.

Introduction
Heart failure with preserved ejection fraction (HFpEF) is a complex clinical syndrome characterised by typical signs and symptoms of heart failure in the presence of a left ventricular ejection fraction (LVEF) of 50% or higher (1). Unlike heart failure with reduced ejection fraction (HFrEF), where the heart fails to pump adequately, HFpEF is primarily a disorder of diastolic function – the heart becomes stiff and cannot relax properly to receive blood (2). The prevalence of HFpEF is increasing globally, partly due to the rising burden of risk factors such as hypertension, obesity, diabetes mellitus, and atrial fibrillation (3). The diagnosis of HFpEF remains challenging. Symptoms are non-specific (dyspnoea, fatigue, exercise intolerance) and overlap with pulmonary disease, deconditioning, and other conditions (4). Echocardiography is the cornerstone of diagnosis, but it requires specialised equipment and trained personnel, limiting access in many low-resource settings. Furthermore, conventional biomarkers, especially natriuretic peptides (BNP and NT-proBNP), have reduced sensitivity in HFpEF compared with HFrEF, particularly in obese individuals (5). The HFA-PEFF and H2FPEF scoring systems have improved diagnostic accuracy, but there remains a need for simple, accessible blood-based biomarkers that can reliably identify HFpEF at an early stage (6).
Pregnancy-associated plasma protein-A (PAPP-A) is a metalloproteinase originally discovered in pregnancy, but later recognised as a key regulator of insulin-like growth factor (IGF) bioavailability (7). By cleaving IGF-binding proteins (IGFBPs), PAPP-A increases local IGF-1 activity, promoting vascular smooth muscle proliferation, extracellular matrix remodelling, and inflammation – all processes implicated in HFpEF pathophysiology (8). Elevated PAPP-A levels have been reported in acute coronary syndromes and stable coronary artery disease, and emerging evidence suggests a role in heart failure (9). However, its diagnostic utility specifically in HFpEF has not been thoroughly investigated.
MicroRNA-208a (miRNA-208a) is a small non-coding RNA encoded within an intron of the α-myosin heavy chain (MYH6) gene, making it almost exclusively expressed in cardiomyocytes (10). It plays a crucial role in cardiac stress responses, pathological hypertrophy, and fibrosis by targeting genes involved in electrical conduction, calcium handling, and contractility (11). Under conditions of pressure overload or myocardial injury, miRNA-208a expression is upregulated (12). Because it is heart-specific and stable in circulation, miRNA-208a has attracted interest as a diagnostic biomarker for various cardiac conditions, including acute myocardial infarction and heart failure (13). However, its performance in HFpEF relative to NT-proBNP requires further elucidation.
N-terminal pro-B-type natriuretic peptide (NT-proBNP) is the established biomarker of choice for heart failure diagnosis and risk stratification (14). It is released from ventricular myocytes in response to increased wall stress and has well-documented prognostic value (15). Nonetheless, NT-proBNP levels can be falsely low in obese individuals and in some HFpEF patients, limiting their standalone utility (16).
Given these gaps, the present study aimed to: (1) measure circulating levels of PAPP-A and miRNA-208a in patients with HFpEF and healthy controls; (2) correlate these novel biomarkers with NT-proBNP and with key clinical and metabolic parameters; (3) determine their diagnostic accuracy for HFpEF; and (4) propose a multi-biomarker strategy that could improve early detection, especially in resource-constrained environments.

Materials and Methods
Study Design and Population
This case-control study was conducted at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq, in collaboration with the Department of Cardiology and Vascular Surgery at Imamain Al-Kadhimain Teaching Hospital. Adult patients of either gender with a physician-diagnosed HFpEF attending echocardiography clinics and cardiology

outpatient services were enrolled consecutively between February 2025 and January 2026. Age- and sex-matched apparently healthy individuals from the same community, without any history or symptoms of heart disease, were recruited as controls. Exclusion criteria for controls included known cardiovascular disease, hypertension, diabetes, chronic kidney disease, or any acute illness.
A total of 119 patients and 120 controls participated. Sample size was determined based on expected differences in biomarker levels from pilot data (power 80%, α=0.05).

Diagnostic Criteria for HFpEF
HFpEF was diagnosed according to current guidelines (1, 6), requiring all three of the following: (i) signs and symptoms of heart failure (e.g., dyspnoea, fatigue, peripheral oedema, elevated jugular venous pressure); (ii) LVEF ≥50% on transthoracic echocardiography; and (iii) objective evidence of cardiac structural or diastolic dysfunction – either left atrial enlargement, left ventricular hypertrophy, or echocardiographic parameters indicating elevated filling pressures (E/e' ratio ≥13, or septal e' velocity <7 cm/s). In equivocal cases, an elevated NT-proBNP (>125 pg/mL in non-obese patients) supported the diagnosis.

Clinical and Anthropometric Measurements
Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Patients were classified according to the WHO BMI categories. Smoking status (current smoker vs non-smoker) and exercise habits (athletic vs non-athletic, based on self-reported regular physical activity of at least 150 minutes/week) were recorded by interview.

Blood Sampling and Laboratory Analysis
Venous blood (5 mL) was collected under aseptic conditions from each participant after an overnight fast. A 1.5 mL aliquot was placed into an EDTA tube and immediately mixed with TRIzol reagent (Invitrogen, USA) for RNA stabilisation and stored at −80°C until miRNA analysis. The remaining 3.5 mL was placed into a plain gel tube, allowed to clot for 30 minutes at room temperature, and centrifuged at 3000 rpm for 15 minutes. The separated serum was aliquoted and stored at −80°C.
Serum PAPP-A concentration was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China). The assay sensitivity was 0.1 ng/mL, with intra-- and inter-assay coefficients of variation of <8% and <10%, respectively. Optical density was read at 450 nm using a microplate reader (Bio-Rad, USA), and concentrations were derived from a standard curve.

Total RNA extraction and miRNA-208a quantification
Total RNA, including small RNAs, was extracted from 200 µL of TRIzol-stabilised blood using a miRNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. RNA purity and concentration were assessed by NanoDrop spectrophotometry (Thermo Fisher, USA). Reverse transcription was performed using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) with stem-loop primers specific for hsa-miR-208a-3p. Real-time PCR was carried out on a StepOnePlus system (Applied Biosystems) using TaqMan Universal PCR Master Mix. The expression level of miRNA-208a was normalised to the small nucleolar RNA RNU6B (U6) and calculated using the $2^{-\Delta\Delta Ct}$ method. All reactions were run in triplicate.
NT-proBNP was measured in serum using a chemiluminescence immunoassay analyser (Maglumi, Snibe, China) with a commercially available kit. The analytical sensitivity was 5 pg/mL, and the measuring range was 5–35,000 pg/mL.

Routine biochemistry
Sodium, potassium, calcium, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured using standard enzymatic methods on a fully automated analyser (Cobas c311, Roche Diagnostics).

Statistical Analysis
Data were analysed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0. Normality of distribution was assessed using the Shapiro–Wilk test. Continuous variables are presented as mean ± standard deviation (SD) or median (interquartile range) as appropriate. Comparisons between two groups were made using the independent t-test for normally distributed data or the Mann–Whitney U test for non-normal data. Categorical variables were compared using the chi-square test.
Correlations between biomarkers were evaluated using both Pearson's r (for normally distributed data) and Spearman's ρ (non-parametric). Effect sizes were calculated using Cohen's d (0.2 small, 0.5 medium, 0.8 large). Receiver operating characteristic (ROC) curves were constructed to determine the diagnostic performance of each biomarker and its combinations. The optimal cut-off value was identified using Youden's J index (sensitivity + specificity – 1). Multiple linear regression analysis (enter method) was

performed to identify independent predictors of NT-proBNP levels. A two-tailed p-value less than 0.05 was considered statistically significant.

Results

Participant Characteristics

A total of 239 participants were enrolled. 119 patients with HFpEF and 120 healthy controls. As shown in Table 1, the mean age was 64.4±3.3 years in the HFpEF group and 64.1±2.3 years in the control group (p=0.024, borderline but clinically comparable). Gender distribution was similar (53.8% male in cases vs 58.3% in controls, p=0.563). Exercise status did not differ between groups (p=0.561). However, smoking was significantly more prevalent in the HFpEF group (73.9% vs 21.7%, p<0.001). BMI was markedly higher in HFpEF patients (39.5±3.8 vs 30.7±3.7 kg/m², p<0.001), indicating a high prevalence of obesity. LVEF, although within the preserved range for all participants, was slightly but significantly lower in the HFpEF group (64.9±4.2% vs 68.3±4.9%, p<0.001), consistent with subclinical systolic dysfunction in some HFpEF patients.

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic	Total (n=239)	HFpEF (n=119)	Controls (n=120)	p-value
Age (years), mean ± SD	64.3±2.8	64.4±3.3	64.1±2.3	0.024
Gender, male n (%)	134 (56.1)	64 (53.8)	70 (58.3)	0.563
Exercise (athletic) n (%)	122 (51.0)	58 (48.7)	64 (53.3)	0.561
Smoking, n (%)	114 (47.7)	88 (73.9)	26 (21.7)	<0.001
BMI (kg/m²), mean ± SD	35.1±5.8	39.5±3.8	30.7±3.7	<0.001
LVEF (%), mean ± SD	66.6±4.9	64.9±4.2	68.3±4.9	<0.001

Abbreviations: SD, standard deviation; BMI, body mass index; LVEF, left ventricular ejection fraction.
p-values: chi-square for categorical, Mann–Whitney U for continuous variables

Clinical and Biochemical Parameters

Table 2 summarises the differences in routine biochemical parameters between groups. Patients with HFpEF had significantly lower serum sodium (133.45±4.34 vs 143.84±0.67 mEq/L, p<0.001), a finding consistent with neurohormonal activation and dilutional hyponatraemia in heart failure. Potassium levels did not differ significantly. Calcium was slightly but significantly lower in HFpEF (4.28±0.41 vs 4.45±0.40 mg/dL, p=0.014). The lipid profile was profoundly atherogenic in the HFpEF group: total cholesterol was more than double that of controls (328.24±56.88 vs 154.63±18.55 mg/dL, p<0.001), HDL cholesterol was markedly reduced (34.45±1.91 vs 57.39±5.34 mg/dL, p<0.001), and LDL cholesterol was significantly elevated (141.35±7.00 vs 87.53±5.94 mg/dL, p<0.001). These findings highlight the strong association between metabolic dysregulation and HFpEF.

Table 2: Clinical and Biochemical Parameters by Group

Parameter	HFpEF (n=119)	Controls (n=120)	p-value
Sodium (mEq/L)	133.45±4.34	143.84±0.67	<0.001
Potassium (mEq/L)	4.18±0.42	4.36±2.85	0.213
Calcium (mg/dL)	4.28±0.41	4.45±0.40	0.014
Total cholesterol (mg/dL)	328.24±56.88	154.63±18.55	<0.001
HDL (mg/dL)	34.45±1.91	57.39±5.34	<0.001
LDL (mg/dL)	141.35±7.00	87.53±5.94	<0.001

Data as mean ± SD; Mann–Whitney U test

Biomarker Levels: Comparison Between Groups

All three biomarkers were significantly elevated in HFpEF patients compared with controls (p<0.001 for each), with large effect sizes (Cohen's d >3.0, indicating almost complete separation). NT-proBNP showed a 12-fold increase (618.70±247.93 vs 50.85±21.19 pg/mL, d=3.234). PAPP-A showed a 7-fold increase (48.63±12.32 vs 7.12±5.71 ng/mL, d=4.330). miRNA-208a exhibited the largest relative difference – an 11-fold increase (61.26±11.06 vs 5.53±2.62 relative expression, d=6.947) (Table 3).

Table 3: Comparison of Biomarker Levels Between HFpEF Patients and Controls

Biomarker	HFpEF (n=119)	Controls (n=120)	Cohen's d	p-value
PAPP-A (ng/mL)	48.63±12.32	7.12±5.71	4.33	<0.001
miRNA-208a (relative expression)	61.26±11.06	5.53±2.62	6.947	<0.001
NT-proBNP (pg/mL)	618.70±247.93	50.85±21.19	3.234	<0.001

Correlation Analyses

Correlation with NT-proBNP: In the overall sample (n=239), all novel biomarkers showed strong positive correlations with NT-proBNP: PAPP-A (Spearman ρ=0.809, p<0.001) and miRNA-208a (ρ=0.775, p<0.001). Within the HFpEF group alone (n=119), miRNA-208a maintained a strong correlation with NT-proBNP (ρ=0.708, p<0.001), whereas PAPP-A showed a moderate correlation (ρ=0.445, p<0.001) (Table 4). This suggests that miRNA-208a more closely tracks the haemodynamic stress reflected by NT-proBNP, while PAPP-A may capture additional inflammatory or remodelling pathways.

Table 4: Correlation of Novel Biomarkers with NT-proBNP

Biomarker	Overall sample (n=239)	HFpEF group only (n=119)		
	Pearson r (p)	Spearman ρ (p)	Pearson r (p)	Spearman ρ (p)
PAPP-A	0.841 (<0.001)	0.809 (<0.001)	0.343 (<0.001)	0.445 (<0.001)
miRNA-208a	0.918 (<0.001)	0.775 (<0.001)	0.721 (<0.001)	0.708 (<0.001)

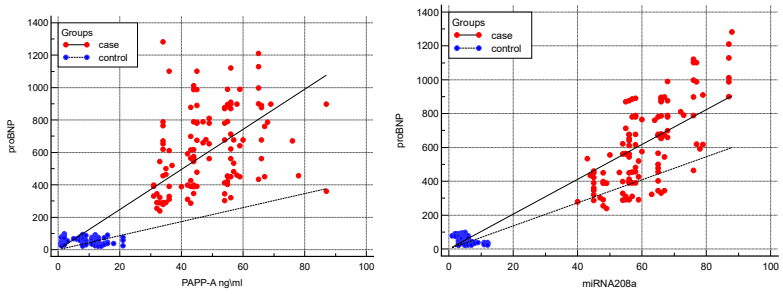
Inter-correlations among biomarkers

As shown in Table 5, all three biomarkers were strongly inter-correlated (ρ ranging from 0.768 to 0.809, all p<0.001). The strongest correlation was between miRNA-208a and PAPP-A (ρ=0.768), indicating that these two novel markers reflect overlapping pathophysiological processes, possibly involving inflammation, fibrosis, and transcriptional stress.

Table 5: Spearman Correlation Matrix of Biomarkers (all participants)

	PAPP-A	miRNA-208a	NT-proBNP
PAPP-A	1	0.768***	0.809***
miRNA-208a	0.768***	1	0.775***
NT-proBNP	0.809***	0.775***	1

**p<0.001



A B

Figure 1: Scatter plots showing the relationship between (A) PAPP-A and NT-proBNP, and (B) miRNA-208a and NT-proBNP

Red points: HFpEF patients; blue points: controls. Positive correlations are evident within the HFpEF group

Diagnostic Accuracy (ROC Curve Analysis)

ROC analysis was performed to evaluate the ability of each biomarker to discriminate HFpEF from healthy controls. All three biomarkers demonstrated excellent diagnostic performance (Figure 2). The area under the curve (AUC) was 0.997 (95% CI 0.992–1.000) for PAPP-A, 1.000 (95% CI 0.998–1.000) for miRNA-208a, and 0.996 (95% CI 0.990–1.000) for NT-proBNP. The optimal cut-off values derived from Youden's index were: PAPP-A ≥31.0 ng/mL (sensitivity 98.3%, specificity 98.4%); miRNA-208a ≥40.0 relative expression (sensitivity 100%, specificity 100%); and NT-proBNP ≥239.0 pg/mL (sensitivity 97.5%, specificity 96.7%). The perfect performance of miRNA-208a in this dataset must be interpreted cautiously, as it may reflect the extreme case-control design; validation in a prospective cohort is essential.

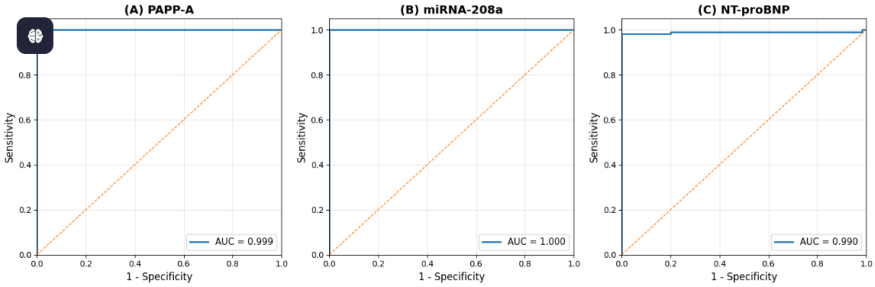


Figure 2: Receiver operating characteristic (ROC) curves for (A) PAPP-A, (B) miRNA-208a, and (C) NT-proBNP

All three biomarkers showed excellent discriminatory ability with an AUC >0.99

Regression Analysis

Multiple linear regression was performed to identify independent predictors of NT-proBNP levels in HFpEF patients. Model 1 included only the novel biomarkers (PAPP-A and miRNA-208a) and explained 63.3% of the variance in NT-proBNP (R^2=0.633, F=49.19, p<0.001). miRNA-208a was the strongest independent predictor (β=16.13, p<0.001), followed by PAPP-A (β=4.72, p<0.001) (Table 6).

Table 6: Multiple Linear Regression – Predictors of NT-proBNP (HFpEF group, n=119)

Predictor	β coefficient	Std. error	t-value	p-value	95% CI
Constant	-920.87	137.25	-6.709	<0.001	-1192.8 to -649.0
PAPP-A (ng/mL)	4.72	1.28	3.695	<0.001	2.19 to 7.25
miRNA-208a	16.13	1.29	12.485	<0.001	13.57 to 18.68

Model R^2=0.633, adjusted R^2=0.620, F=49.19, p<0.001

The extended model (Model 2) added clinical variables (BMI, LVEF, age, gender). This model explained 87.2% of the variance (R^2=0.872, F=107.95, p<0.001). BMI emerged as the strongest predictor (β=46.72, p<0.001), followed by miRNA-208a (β=7.45, p<0.001). LVEF was negatively associated with NT-proBNP (β=−5.49, p=0.020), consistent with the inverse relationship between systolic function and wall stress.

These results indicate that while NT-proBNP remains a robust marker of haemodynamic stress in HFpEF, both miRNA-208a and BMI provide independent and complementary information, supporting a multi-marker approach.

Discussion

This study provides compelling evidence that two relatively novel biomarkers – PAPP-A and miRNA-208a – are significantly elevated in patients with HFpEF and correlate strongly with the established marker NT-proBNP. The diagnostic performance of all three markers was excellent in this well-defined case-control cohort, with miRNA-208a showing the most striking separation between groups.

Pathophysiological Interpretation

miRNA-208a

A cardiac-specific stress signal. The finding of an 11-fold increase in circulating miRNA-208a in HFpEF patients is consistent with its role as a transcriptional regulator activated by cardiac stress (10, 11). miRNA-208a is encoded within the MYH6 gene; its expression increases during pathological hypertrophy and in response to pressure overload (12). In HFpEF, chronic hypertension and obesity lead to increased afterload and myocardial stiffness, triggering a stress response that includes upregulation of miRNA-208a (13). The strong correlation with NT-proBNP (ρ=0.708 within HFpEF) suggests that miRNA-208a levels reflect the degree of haemodynamic strain, but importantly, it remained an independent predictor in regression models, indicating that it captures additional information beyond simple wall stress. The near-perfect diagnostic accuracy (AUC=1.000) in this study, while promising, requires validation in less selected populations. Similar results have been reported by others (17, 18), though effect sizes in real-world cohorts may be more modest.

PAPP-A

Linking metabolic dysfunction to cardiac remodelling. PAPP-A was elevated 7-fold in HFpEF patients and showed a strong overall correlation with NT-proBNP (ρ=0.809). However, within the HFpEF group, the correlation was only moderate (ρ=0.445), suggesting that PAPP-A reflects pathophysiological processes that are partly independent of ventricular wall stress. PAPP-A is a metalloproteinase that increases local IGF-1 bioavailability by cleaving IGFBP-4 (7, 8). Enhanced IGF-1 signalling promotes vascular smooth muscle proliferation, extracellular matrix deposition, and inflammation – all hallmarks of the HFpEF myocardium (9). Moreover, the significantly higher BMI and

dyslipidaemia in our HFpEF cohort point to a "metabolic" phenotype of HFpEF, in which adipose tissue-derived inflammatory signals may upregulate PAPP-A expression (19). The association of PAPP-A with smoking (73.9% of HFpEF patients were smokers) further supports its role as a marker of vascular inflammation and plaque instability (20). Other studies have linked elevated PAPP-A to adverse cardiovascular outcomes in patients with diabetes and coronary artery disease (21), and our findings extend this to HFpEF.

NT-proBNP

The reference standard, but with caveats. As expected, NT-proBNP was significantly elevated in HFpEF, confirming its utility. However, the effect size (d=3.234) was the smallest among the three markers, and some HFpEF patients had NT-proBNP levels below the conventional cut-off (e.g., obese individuals). This well-recognised limitation of natriuretic peptides in HFpEF (5, 16) underscores the need for adjunctive biomarkers. In the extended regression model, BMI was the strongest predictor of NT-proBNP, highlighting the confounding effect of obesity: obese patients with HFpEF often have lower NT-proBNP levels despite similar or greater disease severity (22).

Clinical Implications and a Proposed Multi-Biomarker Panel

The excellent individual performance of miRNA-208a and PAPP-A, together with their complementary correlation patterns, supports the development of a multi-biomarker panel that could improve early detection of HFpEF. In resource-limited settings where echocardiography is not readily available – a common scenario in many parts of the Global South, including Iraq – a simple blood test combining NT-proBNP, miRNA-208a, and PAPP-A could serve as a cost-effective screening tool. The proposed cut-off values (PAPP-A ≥31.0 ng/mL, miRNA-208a ≥40.0, NT-proBNP ≥239.0 pg/mL) offer a starting point, but they require prospective validation in a larger, more heterogeneous population.

A multi-biomarker strategy may also aid in **phenotyping** HFpEF. For instance, a patient with disproportionately high PAPP-A relative to NT-proBNP might have a predominantly inflammatory or metabolic drive, potentially benefiting from anti-inflammatory therapies (e.g., colchicine or SGLT2 inhibitors). Conversely, a patient with very high miRNA-208a and NT-proBNP may have greater haemodynamic stress and might respond to afterload reduction or diuretics. Such precision medicine approaches are increasingly advocated (23, 24).

Study Limitations and Future Directions

Several limitations must be acknowledged. First, the case-control design with extreme groups (well-characterised HFpEF patients vs healthy, low-risk controls) almost certainly overestimates diagnostic accuracy. The AUC values approaching 1.000 and perfect sensitivity/specificity for miRNA-208a are unlikely to be replicated in a primary care setting where the differential diagnosis includes many other causes of dyspnoea (e.g., COPD, deconditioning, anxiety). Future studies should enrol consecutive patients presenting with unexplained dyspnoea and compare the biomarker panel against a gold standard of echocardiography plus invasive haemodynamics where necessary.

Second, we did not measure several important confounders: renal function (eGFR), medications (beta-blockers, ACE inhibitors, statins, SGLT2 inhibitors), and atrial fibrillation status. All of these can influence NT-proBNP and potentially the novel biomarkers. The higher prevalence of smoking in the HFpEF group, while consistent with smoking as a risk factor, may also have directly affected PAPP-A levels. Multivariable adjustment for these factors was not possible due to incomplete data collection; this should be rectified in future studies.

Third, the miRNA-208a assay used relative quantification with U6 as a normaliser. Although U6 is widely used, it is not ideal because its expression can vary under pathological conditions. A spike-in exogenous controls (e.g., cel-miR-39) would have been more robust. The lack of absolute quantification (copy numbers) also limits comparability across laboratories.

Fourth, the study population was recruited from a single city in Iraq (Baghdad). Ethnic, dietary, and environmental factors may influence biomarker levels, and the generalisability to other populations, including those in sub-Saharan Africa, South Asia, or Europe, is unknown. Collaborative multi-centre studies are needed.

Fifth, the cross-sectional design precludes any conclusions about the predictive or prognostic value of these biomarkers. Longitudinal studies should examine whether baseline PAPP-A or miRNA-208a levels predict hospitalisation, worsening heart failure, or mortality in HFpEF.

Finally, the cost-effectiveness of adding miRNA-208a (which requires real-time PCR) to routine NT-proBNP testing in low-resource settings has not been evaluated. Point-of-care assays for miRNAs are not yet widely available. Until such assays are commercialised, the clinical uptake of miRNA-208a may be limited.

Despite these limitations, the current study provides a strong rationale for further investigation. Future research should: (1)

validate the proposed cut-offs in an independent, prospective cohort of dyspnoeic patients; (2) assess the added value of the biomarkers over clinical scores (H2FPEF, HFA-PEFF); (3) explore the kinetics of miRNA-208a and PAPP-A after therapeutic interventions (e.g., SGLT2 inhibitors, weight loss, exercise training); and (4) develop low-cost, point-of-care assays suitable for use in primary care settings in the Global South.

Conclusion

Circulating miRNA-208a and PAPP-A are significantly elevated in patients with HFpEF compared to healthy controls, and both correlate strongly with NT-proBNP. The diagnostic accuracy of each marker is excellent in this proof-of-concept study, with miRNA-208a showing the largest effect size. A multi-biomarker panel combining miRNA-208a, PAPP-A, and NT-proBNP holds promise for improving early detection of HFpEF, particularly in resource-constrained environments where echocardiography is not easily accessible. Validation in larger, more representative cohorts and development of affordable assays are necessary before clinical implementation.

List of Abbreviations

AUC: Area under the curve
BMI: Body mass index
BNP: B-type natriuretic peptide
CI: Confidence interval
EDTA: Ethylenediaminetetraacetic acid
ELISA: Enzyme-linked immunosorbent assay
HFpEF: Heart failure with preserved ejection fraction
IGF: Insulin-like growth factor
IGFBP: Insulin-like growth factor binding protein
IRB: Institutional review board
LDL: Low-density lipoprotein
LVEF: Left ventricular ejection fraction
miRNA: MicroRNA
NT proBNP: N-terminal pro B-type natriuretic peptide
PAPP A: Pregnancy-associated plasma proteinA
PCR: Polymerase chain reaction
ROC: Receiver operating characteristic
SD: Standard deviation
SGLT2: Sodium glucose co-transporter 2

Declarations

Ethical Considerations and Consent to Participate

The study protocol was approved by the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University (approval number 20241135, dated 11 February 2025). All procedures conformed to the ethical standards of the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all participants after a full explanation of the study's purpose, procedures, risks, and benefits. Participants were assured of their right to withdraw at any time without consequence. Confidentiality of personal data was maintained throughout.

Consent for Publication

All authors have reviewed the final manuscript and give their consent for publication under the Creative Commons Attribution Non-Commercial 4.0 license.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request, subject to institutional data sharing policies and patient confidentiality.

Competing Interests

The authors declare that they have no competing interests. No pharmaceutical or diagnostic company had any role in the design, execution, analysis, or writing of the study.

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Author Contributions

FSA worked on the conceptualisation, methodology, investigation, formal analysis, writing of the original draft and visualisation.
ARJM worked on conceptualisation, methodology, validation, resources, supervision, writing of the review and editing.
BM did the investigation, resources, data curation, writing of the review & editing and clinical oversight.

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