

**THE ROLE OF MATRIX METALLOPROTEINASES
2 AND 9 IN VASCULAR AND VENTRICULAR
REMODELLING IN PATIENTS WITH HEART FAILURE
WITH PRESERVED EJECTION FRACTION**

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Abstract

Imbalance of activation and inhibition of matrix metalloproteinases (MMPs) lead to an increase in their activity and the occurrence of pathological changes in the vascular wall. The purpose of this paper is to determine the role of MMP-2 and MMP-9 in vascular and ventricular remodelling in patients with heart failure with preserved ejection fraction. The patients were divided into three groups: 15 patients with heart failure with preserved ejection fraction (HFpEF), 72.73 ± 10.44 years old; 15 patients with arterial hypertension (AH), 63.73 ± 7.06 years old; 10 healthy controls, 58.7 ± 5.87 years old. Arterial stiffness was assessed by pulse wave velocity (PWV). Diastolic dysfunction was assessed by the ratio of early diastolic mitral flow velocity and early diastolic myocardial velocity (E/Em ratio). Left ventricular mass was calculated by area-length method and indexed to body surface area (LVMI). MMP-2, MMP-9 and Brain Natriuretic Peptide (BNP) were measured by ELISA technique.

MMP-2 was higher in patients with HFpEF and AH versus controls (13987 ± 4464 ng/ml and 13040 ± 5060 ng/ml vs 9260 ± 4135 ng/ml, $p = 0.047$). MMP-9 was similar across the groups. BNP was higher in HFpEF versus AH and controls (775.33 ± 443.59 pg/ml vs 370.00 ± 158.29 pg/ml and 345.00 ± 94.39 pg/ml, $p = 0.002$). In HFpEF patients, PWV (12.04 ± 2.46 m/s vs 10.06 ± 1.94 m/s vs 7.22 ± 1.19 m/s, $p < 0.0001$), LVMI (134.11 ± 29.40 g/m² vs 122.45 ± 23.73

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g/m² vs 101.66 ± 11.92 g/m², $p < 0.0001$), E/Em (16.30 ± 6.80 vs 9.57 ± 2.70 and 8.15 ± 1.63, $p < 0.0001$) were significantly higher, compared to hypertensive patients and controls. Higher MMP-2 was associated with higher PWV ($r = 0.43$, $p = 0.007$), E/Em ($r = 0.40$, $p = 0.011$) and LVMI ($r = 0.46$, $p = 0.003$). The measuring of MMP-2 could be useful for early detection of high risk patients and initiation of therapy before the development of organ damage.

Key words: arterial stiffness, heart failure with preserved ejection fraction, matrix metalloproteinases

Introduction. Except impaired diastolic function, there are different pathophysiological mechanisms in the development of heart failure with preserved ejection fraction. One of them is the increased arterial stiffness [1].

The increased arterial stiffness is a result of changes of extracellular matrix and cells in the arterial wall. Mechanical factors and circulating biochemical substances alter the structure and function of the vessel wall [2].

Matrix metalloproteinases (MMPs) are zinc-dependent proteolytic enzymes. They are responsible for vascular remodelling, cell migration and extracellular matrix degradation [3-5]. Imbalance of their activation and inhibition leads to an excessive increase of their activity and the occurrence of pathological changes of the vessel wall [3-5].

MMPs are discussed in the pathogenesis of cardiovascular disease. Several studies show that changes in serum levels of MMP-2 and MMP-9 are associated with aging [6], arterial and ventricular stiffening [7] in patients with arterial hypertension [8-14] and patients with heart failure with preserved ejection fraction [15-17]. Their role in the evaluation of vascular and ventricular remodelling and determining the prognosis of the patient is still controversial.

The aim of our study is to determine the role of MMP-2 and MMP-9 in vascular and ventricular remodelling in patients with heart failure with preserved ejection fraction.

Methods. This is a cross sectional study. 15 patients with heart failure with preserved ejection fraction (HFpEF), NYHA III-IV functional class, 72.73 ± 10.44 years old, 53% female; 15 patients with arterial hypertension (AH), 63.73 ± 7.06 years old, 53% female; 10 healthy controls, 58.7 ± 5.87 years old, 50% female were examined. The inclusion and the exclusion criteria are listed in Table 1.

All patients signed an informed consent. Medical history was obtained. A brief physical examination was done. A blood sample was taken. All study procedures were performed on the same day. The patients avoided food, alcohol, caffeine, nicotine consumption before the study procedures.

Blood pressure was measured in sitting position after 10 min resting. It was measured every two minutes three consecutive times of hand with reported higher values. The mean value was calculated.

T a b l e 1

The inclusion and the exclusion criteria

Inclusion criteria	Exclusion criteria
Patients with heart failure with preserved ejection fraction	
Symptoms of heart failure EF > 50% Diastolic dysfunction -E/E' > 15 -8 < E/E' < 15 + BNP > 200 pg/ml Age above 50 years old	EF < 50% Severe valvular disease Coronary artery disease Atrial fibrillation Pericardial disease Congenital heart disease Inflammatory disease Neoplastic disease Alcohol and drug abuse Unsigned informed consent
Patients with arterial hypertension	
Arterial hypertension Age above 50 years old	Heart failure EF < 50% Severe valvular disease Coronary artery disease Atrial fibrillation Pericardial disease Congenital heart disease Inflammatory disease Neoplastic disease Alcohol and drug abuse Unsigned informed consent
Healthy controls	
No history of disease Age above 50 years old	Unsigned informed consent

Arterial stiffness was assessed by pulse wave velocity (PWV) using applanation tonometry and validated transfer function (SphygmoCor, AtCor Medical) [18]. The procedure was performed in a quiet room with a temperature of 22 °C in supine position. A consecutive record synchronized with ECG of pulse waves of carotid and femoral artery was done. The time difference between the arrival of pulse wave on carotid and femoral artery was determined. The distance travelled by two waves was measured as the difference in distance between the sternum and the place of records on the carotid and femoral arteries on the body surface. The pulse wave velocity was calculated in m/s.

Diastolic function was assessed by the E/E' ratio – the ratio of early diastolic mitral flow velocity (measured by PW-Doppler with sample volume of 2.5 mm placed between the mitral leaflet tips) and early diastolic myocardial velocity (measured by tissue Doppler imaging with sample volume of 10 mm placed in basal segments of medial mitral annulus) [19].

Left ventricular mass was calculated by area-length method and indexed to body surface area (LVMI) [20]. The echocardiography was performed on Philips iE33 with transducer with 3.5 MHz.

Blood sample was taken and the serum was separated and stored at -20°C . Total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were determined using standard methodology. MMP-2, MMP-9 and BNP were measured by ELISA technique.

T a b l e 2
Patients' characteristics

Parameter	HFpEF ($N = 15$)	Hypertension ($N = 15$)	Controls ($N = 10$)	p -value
Demographic characteristic				
Age, years	$72.73 \pm 10.44^{**}$	$63.73 \pm 7.06^{\wedge}$	58.7 ± 5.87	<0.0001
Sex, female %	53	53	50	NS
BMI, kg/m^2	$28.50 \pm 6.08^{\wedge}$	$28.36 \pm 3.15^{\wedge}$	23.53 ± 1.94	0.014
Hypertension, %	93 [^]	100 [^]	0	<0.0001
Diabetes mellitus, %	33	27	0	NS
Smokers, %	20	27	10	NS
Clinical characteristic				
SBP, mm Hg	135.60 ± 18.63	130.87 ± 10.85	121.70 ± 9.78	NS
DBP, mm Hg	$76.07 \pm 11.01^*$	83.87 ± 7.12	77.50 ± 5.40	0.041
PWV, m/s	$12.04 \pm 2.46^{**}$	$10.06 \pm 1.94^{\wedge}$	7.22 ± 1.19	<0.0001
E/Em	$16.30 \pm 6.80^{**}$	9.57 ± 2.70	8.15 ± 1.63	<0.0001
LVMI, g/m^2	$134.11 \pm 29.40^{**}$	$122.45 \pm 23.73^{\wedge}$	101.66 ± 11.92	0.008
Laboratory measurements				
Cholesterol, mmol/l	5.75 ± 1.55	5.43 ± 1.00	5.83 ± 1.48	NS
HDL, mmol/l	$0.95 \pm 0.29^{\wedge}$	1.16 ± 0.35	1.43 ± 0.25	0.017
LDL, mmol/l	3.65 ± 1.23	2.87 ± 0.99	3.39 ± 1.41	NS
Triglycerides, mmol/l	2.07 ± 1.15	2.24 ± 1.43	1.27 ± 0.70	NS
MMP-2, ng/ml	$13987 \pm 4464^{\wedge}$	$13040 \pm 5060^{\wedge}$	9260 ± 4135	0.047
MMP-9, ng/ml	144.91 ± 29.94	152.06 ± 26.81	143.34 ± 45.95	NS
BNP, pg/ml	$775.33 \pm 443.59^{**}$	370.00 ± 158.29	345.00 ± 94.39	0.002

Values are mean \pm SD or %. Final column reflects overall group analysis of variance (ANOVA) or chi square. For between group comparison: $*p < 0.05$ vs Hypertension; $^{\wedge}p < 0.05$ vs Controls (ANOVA after Bonferroni). BMI – body mass index; SBP and DBP – systolic and diastolic blood pressure; PWV – pulse wave velocity; LVMI – left ventricle mass index

Data were reported as mean \pm SD with p -value < 0.05 considered significant. Chi-square test for comparison between categorical variables, one-way analysis of variance (ANOVA), using post-hoc Bonferroni correction and Pearson's correlation test were performed. Data were analyzed using SPSS version 19.0.

Results. Demographic, clinical and laboratory characteristics are presented on Table 2. The patients with heart failure with preserved ejection fraction were older (72.73 ± 10.44 years old) than the hypertensive patients and healthy individuals (63.73 ± 7.06 years old and 58.7 ± 5.87 years old, $p < 0.0001$). The heart failure and hypertensive patients had higher body mass index (28.50 ± 6.08 kg/m² and 28.36 ± 3.15 kg/m²) compared to the control group (23.53 ± 1.94 kg/m², $p = 0.014$). The HDL-cholesterol was lower in the patients with heart failure (0.95 ± 0.29 mmol/l) compared to the hypertensive and healthy subjects (1.16 ± 0.35 mmol/l and 1.43 ± 0.25 mmol/l, $p = 0.017$). There were no statistically significant differences between the groups for sex, diabetes mellitus, smoking, systolic and diastolic blood pressure, total cholesterol, LDL-cholesterol, triglycerides (Table 2).

The pulse wave velocity was significantly higher in the patients with heart failure with preserved ejection fraction (12.04 ± 2.46 m/s) compared to the hypertensive patients (10.06 ± 1.94 m/s), higher in the hypertensive patients than in the healthy subjects (7.22 ± 1.19 m/s, $p < 0.0001$) (Table 2).

The heart failure patients had higher E/Em ratio (16.30 ± 6.80) than the hypertensive and control groups (9.57 ± 2.70 and 8.15 ± 1.63 , $p < 0.0001$) (Table 2).

The left ventricle mass index was significantly higher in the heart failure group (134.11 ± 29.40 g/m²) compared to the hypertensive group (122.45 ± 23.73 g/m²), and higher in the hypertensive patients than in the healthy subjects (101.66 ± 11.92 g/m², $p < 0.0001$) (Table 2).

The heart failure and hypertensive patients had significantly higher levels of MMP-2 (13987 ± 4464 ng/ml and 13040 ± 5060 ng/ml) than the healthy subjects (9260 ± 4135 ng/ml, $p = 0.047$) (Table 2). MMP-2 was similar between the heart failure and hypertensive group. BNP was significantly higher in patients with heart failure with preserved ejection fraction (775.33 ± 443.59 pg/ml) compared to the hypertensive and healthy subjects (370.00 ± 158.29 pg/ml and 45.00 ± 94.39 pg/ml, $p = 0.002$) (Table 2). MMP-9 was similar across the groups (Table 2).

Higher MMP-2 (Fig. 1A) was associated with higher PWV (Fig. 1B), E/Em (Fig. 1C) and LVMI (Fig. 1D). The results were similar after adjusting for age. The serum levels of MMP-2 did not correlate with the BNP in serum.

Discussion. The current study assessed the role of MMP-2 and MMP-9 in vascular and ventricular remodelling in patients with heart failure with preserved ejection fraction. The main findings were that higher values of matrix metalloproteinase 2 are associated with higher arterial stiffness and higher left ventricle mass index even in healthy and hypertensive subjects.

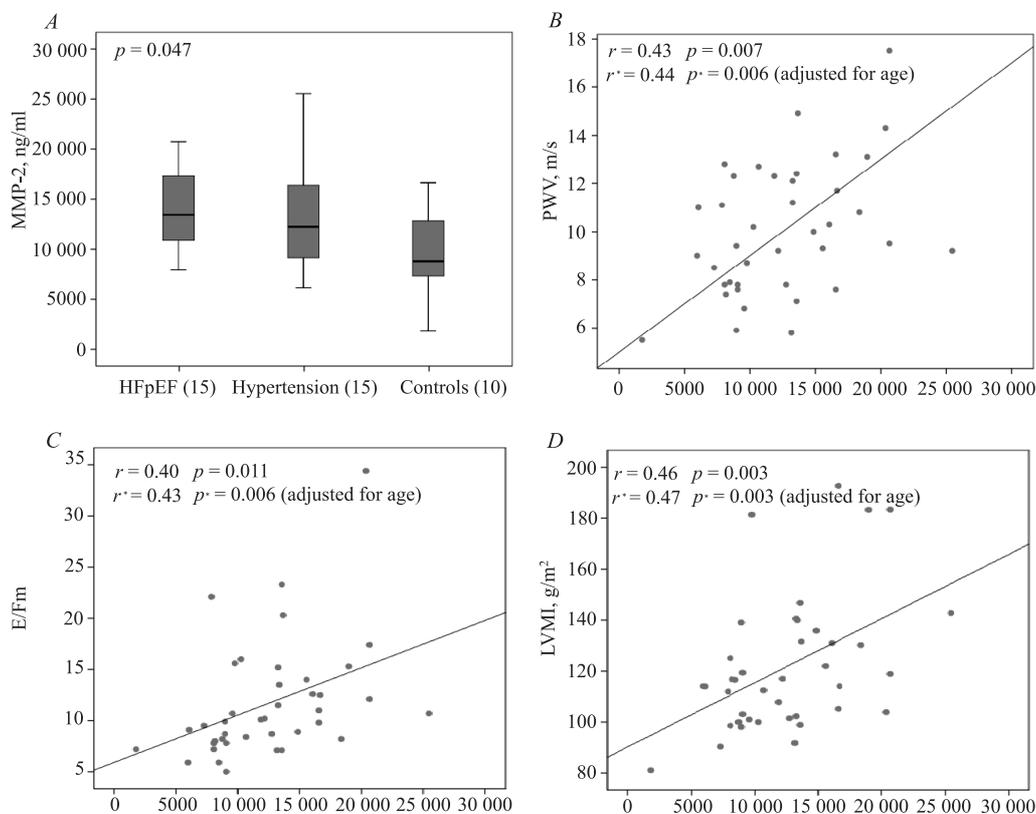


Fig. 1. A – Comparison of MMP-2 between groups; B – Association between MMP-2 and PWV; C – Association between MMP-2 and E/Fm; D – Association between MMP-2 and LVMI

Two control groups were included in the study design: healthy controls and hypertensive patients. The patients with arterial hypertension without symptoms of heart failure had similar characteristics to the patients with heart failure with preserved ejection fraction. In this way, signs of early vascular and ventricular damage could be detected.

MMP-2 was significantly higher in patients with arterial hypertension compared to healthy subjects. MMP-2 did not differ significantly between hypertensive and heart failure patients. We suppose that the levels of MMP-2 in serum increase at the early stage of the vascular and ventricular alteration before the development of symptoms of heart failure.

The serum levels of MMP-2 were higher in hypertensive patients, similar to the results of previous studies of YASMIN et al. [10], DEROSA et al. [12] and FRIESE et al. [14]. MMP-2 was increased in heart failure patients, similar to the results of MATOS et al. [15] and COLLIER et al. [16]. In contrast to our study, AHMED et al [11] showed that MMP-2 was decreased in patients with arterial hypertension and left ventricle hypertrophy.

TAYEBJEE et al. [9], Yasmin et al. [10], Ahmed et al. [11], Derosa et al [12], TAN et al. [13], Friese et al. [14] showed that MMP-9 was increased in patients with arterial hypertension. The serum levels of MMP-9 in heart failure patients were also increased in the study results of Matos et al. [15], Collier et al. [16] and VITLYANOVA [17]. LI-SAW-HEE et al. [8] found that MMP-9 was decreased in hypertensive patients. In contrast to the previous studies, MMP-9 did not differ significantly across the groups in our analysis.

In our study, the higher serum levels of MMP-2 were associated with increased arterial stiffness, similar to the results of Yasmin et al. [10], and with left ventricle hypertrophy and severe diastolic dysfunction, similar to the results of Matos et al. [15].

The present study indicates that MMP-2 is increased in patients with AH and HFpEF, and the serum levels of MMP-2 correlate independently and significantly with PWV. This suggests that MMP-2 may be involved in the process of arterial stiffening and the development of AH and HFpEF.

Limitations of the study. 1) The age of the heart failure patients was significantly higher compared to the hypertensive and healthy subjects. The age is associated with arterial stiffening. Our results did not change significantly after adjusting for age. 2) The patients with atrial fibrillation were excluded from the study because one of the conditions for the measurement of pulse wave velocity with SphygmoCor System is sinus rhythm. 3) The cross-sectional nature of the present study limits our ability to determine the causality. 4) Small sample size.

Conclusions. Higher values of matrix metalloproteinase 2 are associated with higher arterial stiffness and higher left ventricle mass index before occurrence of symptoms of heart failure. Further studies are needed to determine the role of MMP-2 as a potential biomarker for early detection of high risk patients and initiation of therapy before the development of organ damage.

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