

ASSOCIATION BETWEEN SERUM MATRIX METALLOPROTEINASE-12/ANGIOTENSIN II PROFILE AND LEFT VENTRICULAR HYPERTROPHY IN PATIENTS WITH HEART FAILURE

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ABSTRACT

Introduction. Matrix metalloproteinases (MMPs) are important target genes for angiotensin II (AII), which subsequently alters MMP expression in extracellular matrix (ECM) and lead to remodeling process.

Objective. To investigate whether an association between serum levels of MMP-12, AII and left ventricular hypertrophy exists in patients with heart failure with mid-range ejection fraction (HFmrEF) and arterial hypertension (AH).

Material and methods. 56 patients with HFmrEF and AH were examined, mean age 65.62±9.69 years, and 22 age and sex-matched healthy subjects, mean age 56.4±5.53 years. 41 patients had hypertension-mediated organ damage and 15 did not. Patients were divided in two subgroups: subjects with left ventricular hypertrophy (n=32), (HFmrEF+LVH), and subjects without left ventricular hypertrophy (n=24), (HFmrEF-LVH). ELISA was used for measuring AII and MMP-12.

RÉSUMÉ

Association entre le profil de la métalloprotéinase matricielle dans le sérum-12/angiotensine II et l'hypertrophie ventriculaire gauche chez des patients atteints d'une insuffisance cardiaque

Introduction. Les métalloprotéinases matricielles (MMP) sont des gènes cibles importants pour l'angiotensine II (AII), qui modifie par la suite l'expression des MMP dans la matrice extracellulaire (ECM) et conduisent à un processus de remodelage.

L'objectif de l'étude a été de déterminer s'il existe une association entre les taux sériques de MMP-12, AII et l'hypertrophie ventriculaire gauche chez les patients atteints d'insuffisance cardiaque avec une fraction d'éjection moyenne (HFmrEF) et d'hypertension artérielle (AH).

Matériel et méthodes. 56 patients avec HFmrEF et AH ont été examinés, âge moyen 65.62±9.69, et 22

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Results. Serum MMP-12 levels were statistically significant lower in patients, than in controls: 0.0033 ng/mL (0.0022 ÷ 0.0071) vs. 0.0075 ng/mL (0.0068 ÷ 0.016) (KW=7.37; p=0.006). MMP-12 showed correlation with grade of AH (r=0.28; p=0.03). Patients with HFmrEF+LVH showed statistically significant higher levels of AII: 8.533 pg/mL (1.477÷13.009) than HFmrEF-LVH 1.333 pg/mL (0.477÷6.932) and healthy controls 1.539 pg/mL (0.274÷5.218); (KW=3.48; p=0.04). AII correlated with stage of AH (r=0.47; p=0.001). MMP-12/AII ratio was significantly higher in patients with HFmrEF+LVH than in HFmrEF-LVH 3.867 vs. 0.0025 (p<0.001).

Conclusions. Our data suggest an association between serum MMP-12 levels, AII and grade and stage of AH. Determination of serum MMP-12/Angiotensin II profile may be a useful method for monitoring of development and progression of left ventricular hypertrophy.

Keywords: arterial hypertension, heart failure, left ventricular hypertrophy, extracellular matrix.

Abbreviations list:

- ECM- extracellular matrix
- CHF- chronic heart failure
- MMP- matrix metalloproteinase
- MME- macrophage metalloelastase
- ME- macrophage elastase
- AII- angiotensin II
- TIMP- tissue inhibitor of matrix metalloproteinase
- AH- arterial hypertension
- HfmrEF- heart failure with mid-range ejection fraction
- LVEF- left ventricular ejection fraction
- ESC- European Society of Cardiology
- HMOD- hypertension-mediated organ damage
- HFmrEF+LVH- subjects with mid-range ejection fraction and left ventricular hypertrophy
- HFmrEF-LVH- subjects with mid-range ejection fraction without left ventricular hypertrophy
- CVD- cardiovascular disease
- ECG- electrocardiography
- LVH- left ventricular hypertrophy
- HDL- high density lipoprotein cholesterol
- LDL- low density lipoprotein cholesterol
- CKD- chronic kidney disease
- GFR- glomerular filtration rate
- ELISA- enzyme-linked immunosorbent assay
- SNP- single nucleotide polymorphisms
- HETE- hydroxyeicosatetraenoic acid
- CYP450- cytochrome P450
- ACE- angiotensin-converting enzyme inhibitors
- ARB- angiotensin II receptor blockers
- BMI- body mass index
- SBP- systolic blood pressure
- DBP- diastolic blood pressure
- CK-MB- creatine kinase myocardial band
- SMCs- smooth muscle cells

sujets sains appariés selon l'âge et le sexe, âge moyen 56.4±5.53. 41 des patients étaient atteints de lésions organiques médiées par l'hypertension et 15 étaient sans. Les patients étaient divisés en deux sous-groupes: sujets avec hypertrophie ventriculaire gauche (n=32), (HFmrEF+LVH), et sujets sans hypertrophie ventriculaire gauche (n=24), (HFmrEF-LVH). ELISA a été utilisée pour mesurer AII et MMP-12.

Résultats. Les taux sériques de MMP-12 étaient statistiquement significativement plus faibles chez les patients que chez les témoins: 0.0033 ng/ml (0.0022 ÷ 0.0071) contre 0.0075 ng/ml (0.0068 ÷ 0.016) (KW= 7.37; p= 0.006). MMP-12 a montré une corrélation avec le grade d'AH (r= 0.28; p= 0.03). Les patients avec HFmrEF + LVH ont montré des niveaux statistiquement significativement plus élevés d'AII: 8.533 pg/ml (1.477 ÷ 13.009) que HFmrEF-LVH 1.333 pg/ml (0.477 ÷ 6.932) et des témoins sains 1.539 pg/ml (0.274 ÷ 5.218); (KW= 3.48; p= 0.04). L'AII était corrélé avec le stade de l'AH (r= 0.47; p= 0.001). Le rapport MMP-12/AII était significativement plus élevé chez les patients avec HFmrEF + LVH que chez HFmrEF-LVH 3.867 contre 0.0025 (p<0,001).

Conclusions. Nos données suggèrent une association entre les niveaux sériques de MMP-12, l'AII et le grade et le stade d'AH. La détermination du profil sérique MMP-12/angiotensine II peut être une méthode utile pour surveiller le développement et la progression de l'hypertrophie ventriculaire gauche.

Mots-clés: hypertension artérielle, arrêt cardiaque, hypertrophie ventriculaire gauche, matrice extracellulaire.

INTRODUCTION

Collagen fibrils, elastin fibers and fibroblasts are major structural components of extracellular matrix (ECM)¹. Macrophages, macromolecules, such as glycoproteins, glycosaminoglycans, together with other molecules such as growth factors, cytokines and extracellular proteases take part in this structure's props too. Myocardial collagen matrix may be conceptualized as scaffolding providing structural integrity². Traditionally, cardiac ECM is considered a relatively inactive structure, playing an important framework role for cardiac myocytes and vessels. Recent data provide evidence that cardiac ECM is a dynamic and metabolically active structure. Cardiac extracellular matrix changes are suspected of contributing to the genesis and progression of heart failure³.

Elastin is a connective tissue protein characterized by highly elasticity. It is a major ECM component and allows many tissues in the body to resume their shape after stretching or contracting⁴. Elastin is involved in cardiac ECM structure and function⁵. Elastin turnover is regularized mainly by MMP-12 and TIMP-3. Currently, there are insufficient data of elastin turnover in chronic heart failure (CHF). Investigation of elastin turnover should help to better understanding the role of ECM in the pathogenesis of CHF. Abnormal changes in cardiomyocytes structure might not be the only pathophysiological mechanism involved in development and progression of heart failure. That is why the focus of our study is myocardial ECM. This might help for a better understanding of CHF pathogenesis and possible future therapies.

Matrix metalloproteinase-12 (MMP-12), also known as macrophage metalloelastase (MME) or macrophage elastase (ME), is an enzyme encoded by the MMP12 gene. The major MMP-12 substrate is elastin. Moreover, MMP-12 is capable of degrading other ECM constituents (but not gelatin) and many nonmatrix proteins *in vitro*. Most MMP's are secreted as inactive proproteins. The enzyme degrades soluble and insoluble elastin⁶. MMP12 plays a role in aneurysm formation and Stanford-A acute aortic dissection and studies in mice and humans suggest a role in the development of emphysema. MMP-12 is also involved in tissue remodeling in Fas-Induced lung fibrosis, and in fibrotic diseases, including systemic sclerosis (SSc), and correlates with vasculopathy and fibrosis, potentiating skin and heart fibrosis in these patients⁷.

There is evidence that plasma level of MMP-7 and-12 are elevated in type 2 diabetes mellitus, associated with more severe atherosclerosis and an increased incidence of coronary events. These observations provide clinical support to previous experimental studies, demonstrating MMPs' role in plaque

development. Data suggest that MMP-7 and-12 are potential biomarkers of atherosclerosis' burden and cardiovascular disease risk⁸.

A study showed that „early matrix metalloproteinase-12 inhibition worsens post-myocardial infarction cardiac dysfunction by delaying inflammation resolution in mice. Their results reveal a novel protective mechanism for MMP-12 in neutrophil biology. Post-myocardial infarction, MMP-12 impaired CD44-HA interactions to suppress neutrophil apoptosis and prolong inflammation, which worsened left ventricular function“⁹.

Angiotensin II (AII) is the main component of the renin-angiotensin system. AII is associated with cardiovascular diseases such as hypertension, vascular remodeling and inflammation. Dysregulation of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) results in a remodeling process. Moreover, MMPs are considered as important target genes for angiotensin II.

There is growing evidence which supports the opinion that AII may directly cause cardiovascular and renal diseases, independent of its hypertensive effect. Recent scientific data have provided new insights into the molecular and cellular mechanisms of AII-mediated cardiovascular and renal diseases. AII exerts direct effects on vascular remodeling and function¹⁰. Arterial hypertension (AH) is a leading cause for a large number of heart failure (HF) cases. Heart failure with mid-range ejection fraction (HFmrEF) is a syndrome defined by: (1) left ventricular ejection fraction (LVEF) 40-49%; (2) symptoms and/or signs of heart failure; (3a) elevated levels of natriuretic peptides; (3b) at least one additional criterion: relevant structural heart disease (left ventricular hypertrophy/left atrial enlargement) or diastolic dysfunction, according to the 2016 European Society of Cardiology (ESC) Guidelines for the diagnosis and treatment of acute and chronic heart failure¹¹.

In 2005, researchers showed that AII stimulates matrix metalloproteinase secretion in human vascular smooth muscle cells via nuclear factor-kappa B and activator protein 1 in a redox-sensitive manner. „The renin-angiotensin system contributes to atherogenesis. Matrix metalloproteinases (MMP) are thought to participate in plaque destabilization through degradation of extracellular matrix“¹².

In 2010, investigators revealed that AII differentially induces matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 production and disturbs MMP/TIMP balance¹³. The aim of the study was to determine the effects of angiotensin II on MMP-9 and TIMP-1 production and MMP/TIMP balance in a monocytic cell type. It is induced more efficiently than TIMP-1 by AII that leads to MMP/

TIMP imbalance. Authors' data also showed the pivotal participation of these cells in pathological cardiovascular remodeling mediated by AII¹³.

THE OBJECTIVE OF THE STUDY was to investigate whether an association between serum levels of MMP-12, AII and left ventricular hypertrophy exists in patients with heart failure with mid-range ejection fraction (HFmrEF) and arterial hypertension (AH).

MATERIAL AND METHODS

Patient population

All patients were residing in the vicinity of the Pleven University Hospital, Pleven, Bulgaria. Subjects' serum was taken by experienced staff. All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000. The approval of local Ethics Committee was obtained and informed consent from adult research participants was obtained, too.

The study group consisted of 56 patients with AH and HFmrEF, mean age 65.62±9.69 years.

Forty-one of patients had hypertension-mediated organ damage (HMOD) and 15 did not. Patients were divided in two subgroups: subjects with mid-range ejection fraction and left ventricular hypertrophy (n=32), mean age 62.5±12.58 years, (HFmrEF+LVH); subjects with mid-range ejection fraction without left ventricular hypertrophy (n=24), mean age 60.4±8.4 years, (HFmrEF-LVH) (Table 1). These values were compared to serum MMP-12/AII profile in 22 age and sex-matched controls, with no family history of diabetes, atherosclerosis or hypertension, mean age 56.4±5.53 years.

Hypertension-mediated organ damage assessment

All patients were examined for HMOD via history (for established cardiovascular or premature CVD, renal, cerebrovascular or ophthalmological damage), physical examination and pulse pressure assessment (in older people) ≥60 mmHg.

Blood pressure

Arterial blood pressure was measured using a standard aneroid sphygmomanometer, to the nearest 2 mmHg, in the dominant arm, after at least 10-min

Table 1. Clinical data of patients and healthy controls

Clinical data	HFmrEF+LVH	HFmrEF-LVH	Controls
Age	62.5±12.58	60.4±8.4	56.4±5.53
Gender (M/F)	18/14	14/10	12/10
Mean duration AH	9.57±6.63	7.63±5.26	N/A
Mean duration HF	5.6±2.9	4.89±1.60	N/A
SBP (mmHg)	151.35±16.05	139.11±18.42	118.26±13.74
DBP (mmHg)	89.05±10.32	84.41±11.73	76.4±8.4
BMI	28.52±5.99	28.36±4.96	22.11±3.27
Total cholesterol (mmol/L)	4.99±1.30	4.79±0.83	3.99±0.65
HDL-cholesterol (mmol/L)	0.87±0.29	1.16±0.20	0.96±0.20
LDL-cholesterol (mmol/L)	3.27±1.17	3.01±1.07	2.43±0.64
Triglycerides (mmol/L)	1.49±1.66	1.50±1.39	1.31±0.61
Blood glucose	6.00±0.52	6.11±0.83	N/A
Creatinine	101.21±2.87	99.58±3.69	N/A
Hypertensive heart damage	(n=21)	-	
Hypertensive brain damage	(n=4)	(n=2)	
Hypertensive kidney damage	(n=6)	(n=4)	
Hypertensive eye damage	(n=2)	(n=2)	
Smoking	21	16	8
Count	32	24	22

Data are presented as mean±SD

Legend: BMI- body mass index; DBP- diastolic blood pressure; HDL- high density lipoprotein cholesterol; HFmrEF+LVH- heart failure with mid-range ejection fraction and left ventricular hypertrophy; HFmrEF-LVH- heart failure with mid-range ejection fraction without left ventricular hypertrophy; LDL- low density lipoprotein cholesterol; SBP- systolic blood pressure

rest in supine position. Blood pressure measuring was performed by „Riester“ blood pressure measuring tool - Type- Precisa® N; Ø 64 mm aluminum, Single-tube, Cotton hook cuff, adult, No. 1362-104.

Electrocardiography

Electrocardiography (ECG) was performed for LVH assessment (Sokolow-Lyon index >35 mm, or R in aVL ≥11 mm; Cornell voltage duration product >2440 mm.ms, or Cornell voltage >28mm in men or >20mm in women)¹⁴. ECG was performed by 12-lead ECG machine „Fukuda“- Type FX 8322.

Echocardiography

Echocardiography was performed with General Electric (Vivid S5) with 4-MHz transducer. All measurements were obtained according to the European Association of Cardiovascular Imaging (EACVI) and The American Society of Echocardiography (ASE) criteria for Cardiac Chamber Quantification

by Echocardiography¹⁵: echocardiographic LVH [LV mass index: men >50 g/m²; women >47 g/m². (height in m); indexation for BSA may be used in normal weight patients; LV mass/BSA g/m² >115 (men) and >95 (women)] (Table 2).

Laboratory tests

1. Serum uric acid, glucose were determined. Total cholesterol, triglycerides concentrations, HDL were measured by enzyme assay (Boehringer Mannheim, Mannheim, Germany). LDL was calculated via Friedewald's formula.

2. Serum creatinine levels were measured and moderate CKD with eGFR>30-59 mL/min/1.73 m² (BSA) or severe CKD with GFR<30 mL/min/1.73 m² were assessed. If any HMOD was found, then patient was further consulted by a referring specialist (cardiologist, nephrologist, neurologist, ophthalmologist).

ELISA

Enzyme-linked immunosorbent assay (ELISA) was used for measuring AII and MMP-12 levels.

MMP-12 levels were measured in serum samples using (NV-DDXKE-MMP12-1) Human MMP-12 ELISA kit, 96-well plate- Novus Biologicals according to the manufacturer's instructions.

AII levels were measured in serum samples using ELISA kit by ALPCO Diagnostics (Salem, MA, USA, 74-ANGHU-E01) according to the manufacturer's instructions.

Statistical analyses

The research data were processed with the computer programs EXCEL (Microsoft Corporation, Redmond, WA) and STATGRAPHICS plus

Table 2. Echocardiographic data.

	Patients
LVEDD	48.57
LVESD	36.49
LVEDV	111.31
LVESV	58.22
IVS	12.20
LVPWD	12.22
EF%	47.5
LA	35.5
Count	56

Data are presented as mean value

Table 3. Serum levels of MMP-12 in patients with essential hypertension and HFmrEF and healthy controls.

	MMP-12 (ng/mL) M±(Q1toQ3)	Comparison with		MMP-12 (ng/mL) M±(Q1toQ3)	Comparison with
Patients	0.0033 (0.0022,0.0071)	Controls p=0.006	HFmrEF+LVH	0.0033 (0.0019,0.0059)	HFmrEF-LVH (p>0.05)
Controls	0.0075 (0.0068,0.016)	Patients p=0.006	HFmrEF-LVH	0.0034 (0.002,0.0068)	HFmrEF+LVH (p>0.05)

Table 4. Serum levels of angiotensin II in patients with essential hypertension and HFmrEF and healthy controls according to the presence of LVH.

	Angiotensin II (ng/mL) M±(Q1toQ3)	Comparison with		Angiotensin II (ng/mL) M±(Q1toQ3)	Comparison with
Patients	8.952 (1.680,13.923)	Controls p=0.03	HFmrEF+LVH	8.533 (1.477,13.009)	HFmrEF-LVH p=0.04
Controls	1.539 (0.585,7.141)	Patients p=0.03	HFmrEF-LVH	1.333 (0.477,6.932)	HFmrEF+LVH p=0.04

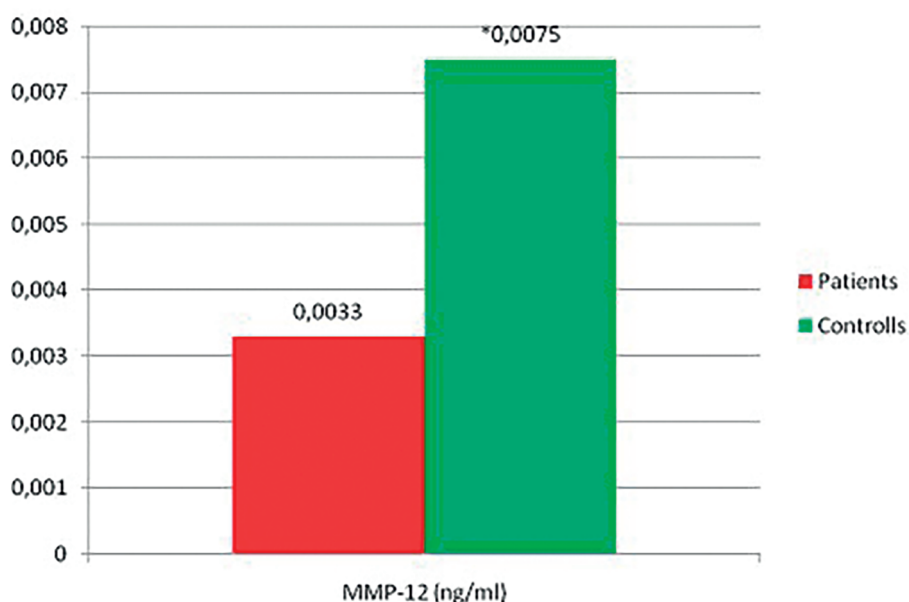


Figure 1. Serum levels of MMP-12 in patients with essential AH and HFmrEF and healthy controls. Serum MMP-12 levels were statistically significantly lower in patients, than in controls: 0.0033 ng/mL (0.0022 ÷ 0.0071) vs. 0.0075 ng/mL (0.0068 ÷ 0.016) (KW=7.37; p=0.006)

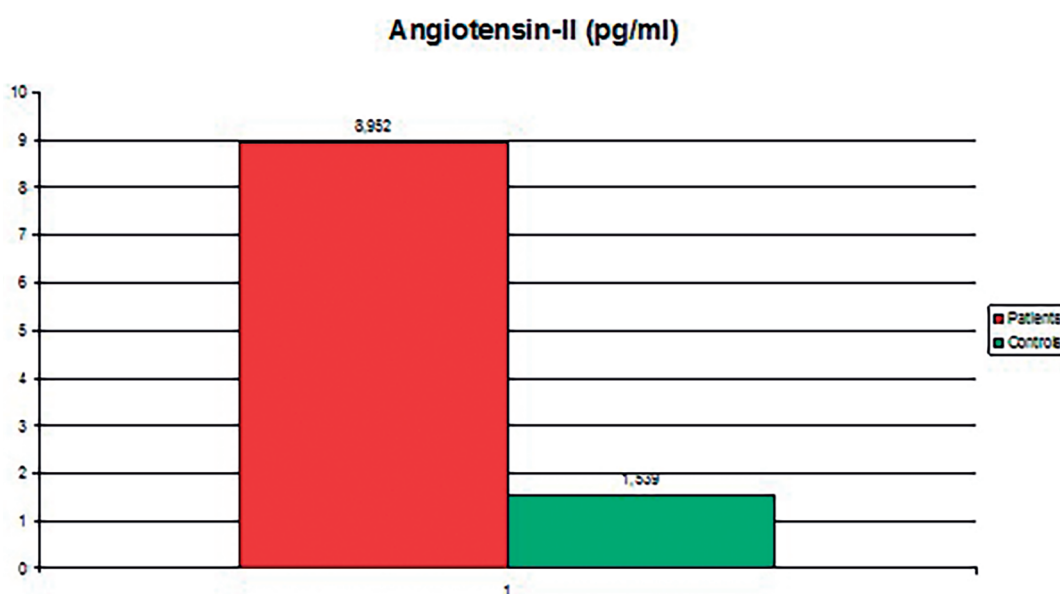


Figure 2. Serum levels of AII in patients with essential AH and HFmrEF and healthy controls. AII serum levels were significantly higher in patients compared to controls: 8.952 pg/mL (1.680÷13.923) vs. 1.539 pg/mL (0.585÷7.141) (p=0.03).

(Manugistics, Rockville, MD) for WINDOWS. All results were described in tables, graphs, numerical values (mean ± SD, share indicators and correlations). The level of significance was determined as p<0.05. K-W (Kruskal-Wallis) test was performed in cases with different from normal distribution. Median (M) was used in K-W test, together with first and third quartile Q1 and Q3; (twenty-fifth and seventy-fifth percentile P25 and 75P). The Pearson's test was used

for assessing of correlations. Multivariate regression analysis was performed too.

RESULTS

Serum MMP-12 levels were statistically significant lower in patients, than in controls: 0.0033 ng/mL (0.0022÷0.0071) vs. 0.0075 ng/mL (0.0068÷0.016) (KW=7.37; p=0.006) (Table 3), (Fig. 1). There was not

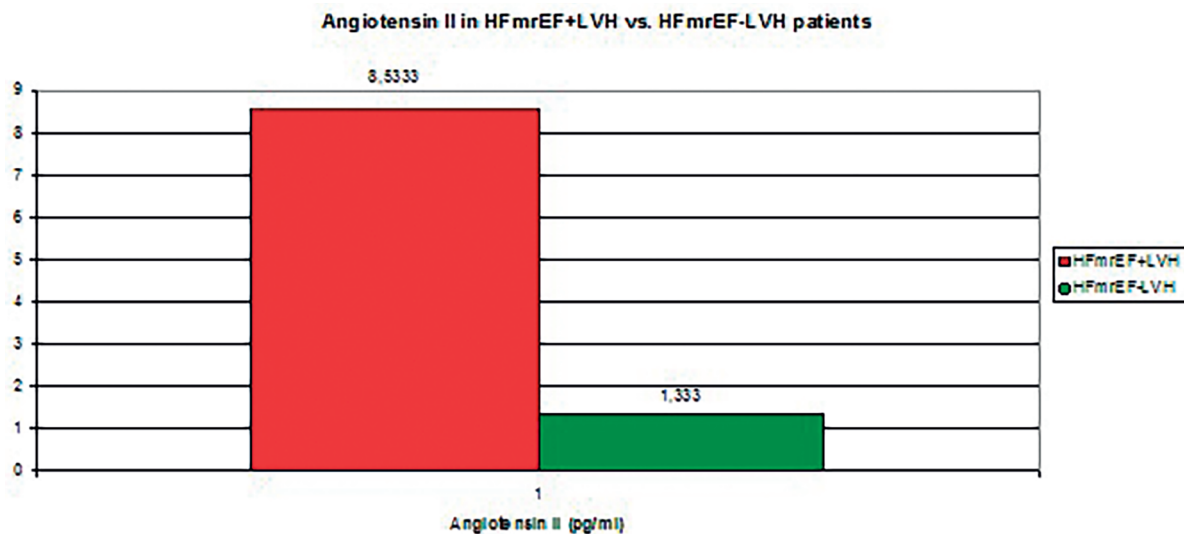


Figure 3. Serum levels of AII in patients with essential AH and HFmrEF and healthy controls according to presence of LVH. Patients with HFmrEF+LVH showed statistically significant higher levels of AII: 8.533 pg/mL (1.477÷13.009) than HFmrEF-LVH 1.333 pg/mL (0.477÷6.932) and healthy controls 1.539 pg/mL (0.274÷5.218); (KW=3.48; p=0.04).

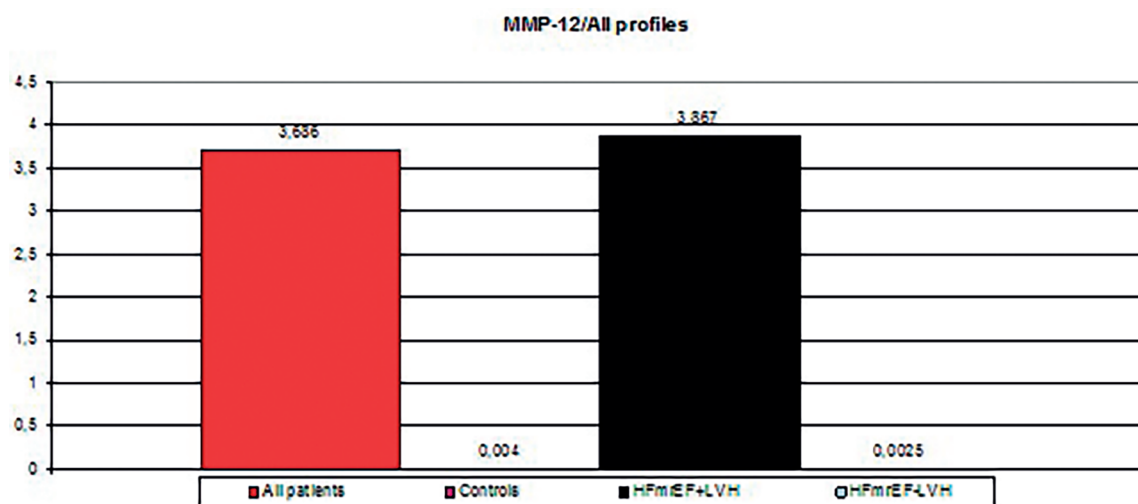


Figure 4. MMP-12/AII serum profiles of all subjects taking part in the current study. MMP-12/AII ratio was significantly higher in all patients than in controls 3.686 vs. 0.004 ($p<0.001$). MMP-12/AII ratio was significantly higher in patients with HFmrEF+LVH than in HFmrEF-LVH 3.867 vs. 0.0025 ($p<0.0001$).

statistically significant difference between MMP-12 levels in patients with HFmrEF+LVH compared to HFmrEF-LVH ($p>0.05$). MMP-12 showed correlation with the grade of AH ($r=0.28$; $p=0.03$). AII serum levels were significantly higher in patients compared to controls: 8.952 pg/mL (1.680÷13.923) vs. 1.539 pg/mL (0.585÷7.141) ($p=0.03$). Patients with HFmrEF+LVH showed statistically significant higher levels of AII: 8.533 pg/mL (1.477÷13.009) than HFmrEF-LVH 1.333 pg/mL (0.477÷6.932) and healthy controls 1.539 pg/mL (0.274÷5.218); (KW=3.48; $p=0.04$) (Table 4), (Fig. 2, 3). AII correlated with the stage of AH ($r=0.47$; $p=0.001$). The MMP-12/AII ratio was significantly higher in all patients than controls

3.686 vs. 0.004 ($p<0.001$). MMP-12/AII ratio was significantly higher in patients with HFmrEF+LVH than in HFmrEF-LVH 3.867 vs. 0.0025 ($p<0.001$) (Fig. 4).

DISCUSSION

The conceptual role of AII as a local mediator of fibrosis is suggested by several studies. Data show that circulating AII is chronically increased from either endogenous or exogenous sources¹⁶. Mounting evidence points towards an authentic signalling capacity for TIMPs distinct from their MMP-inhibitory activity. It also seems to play an important role in the regulation of apoptosis, cell survival, growth, migration,

differentiation, angiogenesis, inflammation and overall ECM remodelling. Due to these mechanisms, TIMPs could play a vital role in the process of cardiac remodelling^{17,18}. A suggestion about a link between TIMPs and the renin-angiotensin-system is reported¹⁹. The authors identified human angiotensin-II-type-2-receptor as a novel TIMP-3 interacting partner, linking TIMP3 with the renin-angiotensin system¹⁹. Although the patho-physiological roles and signaling mechanisms of angiotensin-II-type-2-receptor are still largely unknown, they were shown to be increased during hypertrophy and ischemic heart disease.

Our results show decreased MMP-12 levels and increased AII in all patients. There was not a statistically significant difference between MMP-12 levels in patients with HFmrEF+LVH compared to HFmrEF-LVH. Patients with HFmrEF+LVH showed statistically significantly higher levels of AII than HFmrEF-LVH and healthy controls. Interestingly, the MMP-12/AII profile is different according to presence of LVH. These results can summarize with three major findings:

MMP-12/AII ratio is significantly higher in all patients than in controls.

Patients with HFmrEF-LVH show decreased MMP-12/AII ratio.

The profile of patients with HFmrEF+LVH shows increased MMP-12/AII ratio.

Data from the literature about the interaction between MMP-12 and AII and their complex turnover in patients with essential AH and HFmrEF are still insufficient. In 2017, a study showed that matrix metalloproteinase-12 is causally implicated in cardiovascular disease²⁰. Researchers evaluated and extended the reported association by examining the relationship between MMPs and vascular disease in both clinical and experimental samples. Plasma concentrations of MMP-1, MMP-3, MMP-7, MMP-10 and MMP-12 in ng/mL were measured in 3394 subjects with high-risk for cardiovascular disease, using the Olink ProSeek CVD array. The plasma MMP-12 concentration showed an association with incident cardiovascular events (199 events over 36 months) and intima-media thickness progression over time ($p=3.6 \times 10^{-5}$). The single nucleotide polymorphisms (SNP) variant rs1892971 was strongly associated with plasma MMP-12 concentration ($p=8 \times 10^{-29}$) and weakly with susceptibility to coronary heart disease in the CardiogramplusC4D consortium study ($p=8.8 \times 10^{-5}$). The same SNP was also significantly associated with MMP-12 gene expression in peripheral blood mononuclear cells using microarrays from patients with carotid atherosclerosis ($n=96$; $p=1.8 \times 10^{-4}$). The

expression of MMP-12 was strongly increased in carotid plaques ($n=127$) compared with undiseased arteries ($n=10$; $p<0.001$) and in plaques from symptomatic ($n=87$) compared to asymptomatic patients ($n=40$; $p=0.03$) and localised to CD68+ macrophages. Using proximity ligation assay, MMP-12 and elastin were demonstrated to co-interact in plaques in situ, particularly in regions with moderate to strong MMP-12 expression. In conclusion, this study suggests that MMP-12 is a causal factor in cardiovascular diseases, that is highly upregulated in human atherosclerotic plaques, where it interacts with elastin and appears to enhance macrophage invasion.

Arterial stiffening is a hallmark of aging and a risk factor for cardiovascular disease, yet its regulation is poorly understood²¹. Arterial stiffness plays a causal role in the development of systolic hypertension^{22,23}. A study in 2015 showed that matrix metalloproteinase-12 is an essential mediator of acute and chronic arterial stiffening. The authors used mouse modeling to show that matrix metalloproteinase-12, a potent elastase, is essential for acute and chronic arterial stiffening²⁴. MMP-12 was induced in arterial smooth muscle cells (SMCs) after acute vascular injury. In human aortic samples, MMP12 levels strongly correlate with markers of SMC stiffness. Authors concluded that MMP-12 causes arterial stiffening in mice and suggest that it functions similarly in humans.

In a study from 2018, the authors reported that elevated 20-hydroxyeicosatetraenoic acid (20-HETE) in metabolic syndrome regulates arterial stiffness and systolic hypertension via MMP-12 activation²⁵. 20-HETE, a cytochrome P450 (CYP450)-derived arachidonic acid metabolite, is known to be elevated in resistance arteries in hypertensive animal models and loosely associated with obesity in humans. However, the role of 20-HETE in the regulation of large artery remodeling in metabolic syndrome has not been investigated. Soler A et al showed that elevated 20-HETE increases matrix metalloproteinase 12 activation in metabolic syndrome²⁵. This process leads to increased degradation of elastin, increased large artery stiffness and increased systolic blood pressure. These findings may have implications in the management of systolic hypertension in patients with metabolic syndrome²⁶.

Data from 2018 reported the relationship between matrix metalloproteinase-12, low-grade inflammation and kidney function in patients with resistant hypertension²⁷. The authors found that matrix metalloproteinase-12 (MMP-12) modulates glomerular fibrogenesis and inflammation. MMP-12 plasma activity is elevated in resistant hypertension patients compared to patients with controlled hypertension. Moreover, MMP-12 plasma activity is

associated with the deterioration of kidney function and hs-CRP. These findings suggest the important roles of MMP-12 in both hypertensive kidney injury and resistant hypertension development.

Our findings showed significantly higher MMP-12/AII ratio in all patients than in controls. Patients with HFmrEF-LVH showed decreased MMP-12/AII ratio. Patients with HFmrEF+LVH showed a profile presented by increased MMP-12/AII ratio. This study reveals altered elastin metabolism, focusing on pathological elastin degradation. We supposed that patients with essential AH and HFmrEF have reduced elastin degradation and blocked arterial stiffening. These alterations could accelerate higher grade and stage of hypertension and favor development and progression of left ventricular hypertrophy.

In our study, we report an association between changes in MMP-12/AII profile and left ventricular hypertrophy. Determination of serum MMP-12/AII profile may be an useful method for monitoring the development and progression of LVH in patients with essential AH and HFmrEF. This is a pilot study. Although these findings should be confirmed in a larger study, our data suggest that alterations of MMP-12/AII balance may play an important role in the structural, functional, and clinical manifestations of cardiac remodeling. However, further examination and prospective studies are needed to clarify the MMP-12 independent biological functions and fully understand its relevance and effects on left ventricular hypertrophy.

CONCLUSIONS

Our data suggest an association between changes in the levels of serum MMP-12, AII and grade and stage of AH in patients with HFmrEF. Determination of serum MMP-12/AII profile may be a useful method for monitoring the development and progression of LVH.

Author Contributions:

Conceptualization, A.N.; methodology, A.B. software, A.N., A.B.; validation, M.T.; formal analysis, A.N.; investigation, A.N., M.T.; resources, A.N.; data curation, A.N. and K.K.; writing—original draft preparation, A.N.; writing—review and editing, M.T.; visualization, K.K.; supervision, M.T.; project administration, A.N. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the

Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law“

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