# Ischemic Heart Disease and Matrix Metalloproteinase-9 in Chronic Heart Failure.

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## ABSTRACT

**Background:** There are limited data on the complex relationship between metalloproteinases (MMPs) and ischemic heart disease (IHD) in patients with chronic heart failure (CHF). The aim of the study was to investigate the relationship between plasma MMP-9 and IHDif any, as well as the effect of certain clinical and laboratory determinants on that relationship. **Methods:** The study was conducted on 59 patients with CHF (mean age 69.3 ±7.9), women 42.4%. The total number of definite IHD assessed by angiography was 28. Assessment of the correlations between the investigated variables was done using logistic regression analysis (LRA). **Results:** MMPs are not significantly higher in ischemic CHF patients [2.4 ±1.7, vs. 1.8 ±0.8 (ng/ml), p>0.05]. The two groups differed significantly in mean values of the left ventricle ejection fraction (LVEF) (36.1±8.6, vs. 46.8±15.7 %), proportion of female gender (21.4 vs. 61.3%) and chronic atrial fibrillation (35.7, vs. 61.3%) found to be lower in IHD group. The number of diabetics is almost fourfold higher in ischemic patients (p<0.05). Significant and independent predictors of MMP-9 are LVEF and etiology (F=2.75, p<0.01). Additionally, in the IHD group the diabetic status independently correlated to the higher plasma MMP-9 (β=0.18, p<0.05).**Conclusion:** The relationship between MMP-9 and IHD is complex and significantly dependent on diabetes mellitus and the degree of the left ventricle dysfunction, measured by the values of LFEF. The observed relationships in case of DM and LVEF were not observed in the non-ischemic group.

**Keywords:**chronic heart failure, ischemic heart disease, metalloproteinases.

## INTRODUCTION

Chronic heart failure (CHF) is the final common pathophysiological pathway of the evolution of all heart diseases, a major cause of morbidity and mortality in developed countries. The metallo-proteinase MMP-9 is a representative of a family of zinc-dependent proteases regulating the synthesis and degradation of myocardial extracellular matrix proteins.[1] The processes of post-infarction remodeling are not completely understood, despite the availability of experimental data on the intensive destruction of the extracellular matrix and its components as a result of cardiac remodeling in patients with CHF. Data from *in-vitro* studies demonstrated that neurohormonal stimulation in CHF may enhance MMP expression and activity.[2] There is a limited amount of data on the complex relationship between neurohormonal activation, MMP and ischemic heart disease (IHD) in patients with CHF.

The AIM of the study was to examine the relationship between plasma MMP-9 and IHD as well as the influence of certain clinical and laboratory parameters on this dependence in patients with CHF.

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## MATERIALS AND METHODS

**Study population and design**

The study was conducted in full compliance with the Declaration of Helsinki. [3] All participants were tested after previously signing the informed consent to participate.

MMP-9 was determined in 59 CHF patients, mean age 69.3 ± 7.9, women 42.4% (25) admitted to the Cardiology clinic of the Department of Propedeutics of Internal Medicine at Aleksandrovska University Hospital. Systolic HF was defined in case of left ventricular ejection fraction (LVEF) ≤50%. Patients who experienced an acute myocardial infarction (AMI) within 3 months from the beginning of the study were excluded. Ischemic etiology of CHF was defined in the presence of angiographic evidence of significant coronary artery disease. The total number of patients with CHF and defined IHD was 28. Blood was taken from all patients for biochemical analysis, including: blood count, enzymes, troponin T. Blood samples for brain Natriuretic peptide (BNP) and MMP-9 were taken at the discharge of the patients from the clinic. Plasma MMP-9 protein and BNP concentrations were examined by the enzyme-linked immunosorbent assay ELISA (EIA- ELISA assays, ELISA reader Trinitron and ELISA reader from Biolab) at the Institute of Immunology and Biology of Reproduction – Bulgarian Academy of Sciences with ready tests (IBL Hamburg).

All patients included in the study were interviewed and examined by a standardized protocol, including

history data and objective status, as well as clarifying questions about: demographic, biological, cardiovascular risk factors, clinical characteristics (NYHA functional class, signs and symptoms of HF; comorbidities: arterial hypertension (AH), diabetes mellitus (DM); laboratory tests for which, according to data in literature, there is an established correlation with MMP-9 (cardiac specific creatine phosphokinase (CPK), Troponin T and leukocytes). LVEF was determined in % using Simpson's rule in a standard two chamber view. [4]

**Statistical analysis**

The quantitative indicators were presented with mean and standard deviation (SD). The categorical factors were described by relative shares by category. The comparative analysis of the groups included: non-parametric analysis using the chi-squared test and parametric analysis; Student-Fisher's t-test for the quantitative variables.

The evaluation of correlations between the studied parameters was carried out by linear regression analysis (ANOVA for multiple comparisons of means for a given factor using the categories of another factor). All indicators in the linear regression analysis, with the exception of diastolic blood pressure (DAP) and number of leukocytes (LEU), were analyzed as qualitative variables. The reference categories were: male gender, non-diabetics, non-ischemic etiology, sinus rhythm, LVEF≥50%.

Statistical analysis was performed using SPSS, version 13.0.

## RESULTS

The baseline clinical parameters in CHF patients tested for MMP-9are presented in [Table1 and 1a]**.**

**Comparative analysis of the groups depending on the presence of underlying IHD**

The values of MMP-9 did not differ statistically significantly between patients with ischemic and non-ischemic etiology of CHF [2.4 ± 1.7 vs 1.8 ± 0.8 ng/ml, p>0.05].

**Table 1:** Distribution of studied patients with CHF (n = 59) by indicator

| Characteristic | (%/n)/X±SD |
|---|---|
| AH | 83.1 (49) |
| DM | 25.4 (15) |
| **Ischemic *etiology*of HF** | 47.2 (28) |
| *Clinical data* | |
| CAF | 49.2 (29) |
| SAP* (mmHg) | 130.2±22.0 |
| DAP (mmHg) | 78.9±12.7 |
| NYHA functional class | |
| II | 25.4 (15) |
| III | 27.1 (16) |
| IV | 25.4 (15) |
| *Echocardiographic data* | |
| LVEF (%) | 41.9±13.8 |
| LVH* | 12.2±1.9 |

*systolic arterial pressure

** left ventricular hypertrophy = left ventricular hypertrophy score = (thickness of LV posterior wall + thickness of LV septum)/2

**Table 1a:** Laboratory parameters in patients with CHF*

| | **Patients withCHF (*n*=59)** |
|---|---|
| MMP-9 (ng/ml) | 2.1±1.4 |
| BNP (ng/ml) | 0.54±1.1 |
| troponin T (ng (ml) | 0.2±0.7 |
| leukocytes (*$10^9$/l) | 7.8±2.1 |
| CPK (U/l) | 93.9±65.2 |

*data are represented as mean (X) ± standard deviation(SD)

The mean value of LVEF, DAP, relative share of women patients and chronic atrial fibrillation (CAF) were significantly lower in the IHD group; the incidence of diabetes mellitus (DM) was almost four times higher in the IHD group compared to the patients without IHD [Table 2]. Based on laboratory indicators, statistically significant difference between the groups was observed only for the number of leukocytes, with higher values in the IHD group. The cardio-specific markers CPK and Troponin T had insignificantly higher values in IHD patients. The degree of hormonal activation measured through BNP valuesdid not differ significantly in both groups [Table 2a].

**Linear regression analysis (LRA)**

Simple LRA demonstrated a significant linear relationship between the dependent variable MMP-9 and presence of atrial fibrillation data. The correlation between MMP-9 and CAF is negative: its registering in the course of CHF correlated with lower MMP-9 levels. There were no significant correlations of MMP-9 with the other studied factors [Table 3].

The conducted multiple LRA, with control of the effect of all the factors on each other, established a significant and independent correlation between MMP-9 and the degree of left ventricular dysfunction (LVEF), as well as with IHD: higher MMP-9 correlated with lower values of LVEF and presence of underlying ischemic etiology [Table 3]. Stratification by etiology of CHF established an independent relationship between MMP-9 and diabetic status, and LVEF, but only in the IHD group. The observed correlation between MMP-9 and IHD is dependent on the effects of left ventricular dysfunction and diabetes mellitus (DM) comorbidity [Table 4].

**Table2:** Distribution of studied patients with CHF by indicator and etiology

| Characteristic (%) / X±SD | Ischemic CHF (n=28) | Non-ischemic CHF (n= 31) | *P* |
|---|---|---|---|
| age (years) | 70.8±7.2 | 67.9±8.3 | *NS** |
| women | 21.4 | 61.3 | *0.002* |
| AH | 94.9 | 74.2 | *NS* |
| DM | 39.3 | 12.9 | *0.020* |
| *Clinical data* | | | |
| CAF | 35.7 | 61.3 | *0.050* |
| SAP (mmHg) | 128±25.6 | 131.8±18.5 | *NS* |
| DAP (mmHg) | 74.8±13.1 | 82.7±11.2 | *0.015* |
| NYHA functional class | | | |
| II | 27.8 | 35.7 | |
| III | 44.4 | 28.6 | |
| IV | 27.8 | 35.7 | *NS* |
| *Echocardiographic data* | | | |
| LVEF (%) | 36.1±8.6 | 46.8±15.7 | *0.019* |
| LVH | 11.9±2.1 | 12.5±1.7 | *NS* |

*NS – statistically not significant

**Table 2a:** Distribution of studied patients with CHF by laboratory indicators and etiology

| Characteristic | Ischemic CHF (n=28) | Non-ischemic CHF (n= 31) | *P* |
|---|---|---|---|
| BNP(ng/ml) | 0.70±0.98 | 0.39±1.12 | *NS* |
| LEU (*$10^9$/l) | 8.4±2.1 | 7.2±1.7 | *0.021* |
| CPK (U/l) | 112±82.5 | 76.3±36.7 | *0.07* |
| Troponin T (ng/ml) | 0.34±0.94 | 0.02±0.01 | *NS* |

**Table 3:** Simple and multiple LRA of the studied indicators[1]

| Indicators | SimpleLRA | | | | MultipleLRA | | | |
|---|---|---|---|---|---|---|---|---|
| | $R^2$ | F | B | *P* | $R^2$ | F | B | *P* |
| Gender | 0.29 | 1.65 | 0.47 | *NS* | 0.30 | 2.75 | 0.53 | *NS* |
| DM | 0.55 | 3.21 | 0.72 | *NS* | | | 0.39 | *NS* |
| CAF | 0.08 | 4.62 | -0.26 | *0.036* | | | -0.44 | *NS* |
| DAP | 0.023 | 1.28 | 0.02 | *NS* | | | 0.02 | *NS* |
| LVEF | 0.035 | 2.01 | 0.02 | *NS* | | | 0.03 | *0.033* |
| LEU | 0.03 | 4.40 | 0.11 | *NS* | | | -0.002 | *NS* |
| IHD | 0.050 | 2.9 | 0.60 | *NS* | | | 1.04 | *0.027* |

[1]MMP-9 – dependent variable

**Table 4:** Multiple LRA: stratification by etiology*

| Indicators | Ischemic ethiology | | | | Non-ischemic ethiology | | | |
|---|---|---|---|---|---|---|---|---|
| | $R^2$ | F | B | P | $R^2$ | F | B | P |
| Gender | 0.55 | 3.9 | 0.27 | NS | 0.13 | 0.45 | 0.09 | NS |
| CAF | | | -0.23 | NS | | | 0.01 | NS |
| DM | | | 0.18 | 0.037 | | | 0.15 | NS |
| LVEF | | | 0.05 | 0.005 | | | 0.01 | NS |
| LEU | | | -0.04 | NS | | | -0.02 | NS |
| DAP | | | -0.01 | NS | | | 0.003 | NS |

* MMP-9 - dependent variable,reference category - women

## DISCUSSION

Our results support the involvement of MMP-9 in the long-lasting process of cardiac remodeling in patients with CHF. MMPs are secreted in latent pro-forms requiring activation to transform them into active for proteolysis forms. Their activity is lower in healthy tissues. Increased expression of MMP has been observed in various pathological processes, including inflammation and ventricular remodeling after MI, which corresponds to the established by us increased plasma levels of MMP-9 in the IHD group. The role of MMPs in IHD is explained by the evidence that MMPs are involved in the pathogenesis of atherosclerosis by facilitating the process of migration of vascular smooth muscle cells through the internal elastic layer to the intima of the vessel, where proliferation

and their participation in the formation of atherosclerotic plaques takes place.[5]

The existing in literature claims, that MMPs in the myocardium are the driving force responsible for the progressive deterioration of the pumping function, are in accordance with the established by us direction of the correlation between MMP-9 and LVEF.[6] It is believed that MMPs may be used to track and prognosticate the ventricular remodeling in a given patient. There are, however, a number of limiting factors in the described chain of interactions. In fact, LV remodeling is a long-lasting process, taking months and years after the onset of myocardial damage.[7]

The healing process involves hemodynamic forces, sympathetic activation, inflammation, formation of new vessels, rather than just changes in the extracellular matrix. The regulation of the extracellular collagen matrix is a slow process (with a half-life of about 25 days) with a huge impact on the processes responsible for maintaining the most effective form of the chambers. Recovery after MI is an extremely complex process involving overlapping phases of inflammation, formation of new tissues as well as remodeling.[8] Thus the examination of the role of MMP in LV remodeling should not be limited to a cause-and-effect type of relationship, but rather a complex interaction of different substances, their action and inhibition. Regardless of their possibly beneficial effect in the post-myocardial infarction period, the activation of MMP can also accelerate a number of adverse effects, resulting from the degradation of the components of the extracellular matrix and destruction of the myocyte-matrix network.

According to Kelly et al. except in the formation of adverse LV modeling, MMP-9 is probably also involved in the processes of healing and scarring of damaged areas, depending on the duration and phase of the ventricular remodeling after AMI, which could explain to some extent the lack of significant difference in plasma MMP-9 between the studied groups of patients.[9,10] The results reveal a statistically significant correlation between higher levels of MMP-9 and DM, but only in the IHD group. Data from published studies to date on the issue are controversial.[11-13] Uemara et al. Reported similar to our results in experimental animal models. According to Dobos et al. the difference in the obtained results regarding the relationship between MMP-9 and diabetic status can be explained by differences in the type of the performed studies and duration of diabetes. It is assumed that the changes in MMP-9 are time dependent with an initial peak in the early stages of diabetes resulting in enhanced migration of smooth muscle cells and subsequent intimal hyperplasia. The progression of diabetes leads to suppression of the MMP system with subsequent changes in the extracellular matrix and fibrosis.[14, 15]

## CONCLUSION

The relationship between MMP-9 and IHD is complex and significantly dependent on DM comorbidity and the degree of left ventricular dysfunction, measured through the values of LVEF. The observed correlations between MMP-9, DM and LVEF were not registered in the group without underlying IHD.

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