Original Article

Serum Levels of Matrix Metalloproteinases 2 and 9 in Patients with Acute Myocardial Infarction

(myocardial infarction / MMP-2 / MMP-9 / microarray)

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Abstract. The myocardial extracellular matrix plays an important role in maintaining the structural and functional integrity of the heart and is centrally involved in post-myocardial infarction repair processes. We analysed some genetic and proteomic aspects that could play an important role in the development of myocardial infarction. Matrix metalloproteinases are enzymes that contribute strongly to the degradation of extracellular matrix components. In this study the serological levels of MMP-2 and MMP-9 were investigated using immunological testing in 34 patients with myocardial infarction and 34 matched control subjects. The serum levels of MMPs were determined by ELISA. Changes in serum levels were characterized within 24 h and after 6 months post myocardial infarction. Significantly higher levels of MMP-2 (299.47 ± 117.61 ng/ml) and MMP-9 (93.56 ± 53.74 ng/ml) were determined in patients with myocardial infarction compared to the controls, in both cases P < 0.001. MMP-9 levels decreased significantly in the 6 months after cardiac event, whereas the levels of MMP-2 were almost equal to the post-infarction ones. While comparing the results from four patients that died of cardiovascular cause within 6 months we found significantly higher MMP-2 (435.00 ± 55.83 ng/ml, P = 0.003) and MMP-9 (166.25 ± 41.07 ng/ml, P = 0.018) values. Microarray analysis was used to determine the gene expression of selected genes for MMPs and their regulators from peripheral blood. The selected genes did not show satisfactory results that could have a potential implication for diagnostics of tissue degeneration.

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Abbreviations: BMI – body mass index, ECM – extracellular matrix, MI – myocardial infarction, MID6 – patients that died within 6 months from initial MI, MMP – matrix metalloproteinase, MIO6 – sample collection within 6 h from MI, MIO24 – sample collection within 24 h from MI, TIMP – tissue inhibitor of matrix metalloproteinase.

Introduction

Cardiovascular diseases are considered to be the most common cause of death worldwide. In the Czech Republic approximately 600 deaths in 100,000 inhabitants are reported per year. This comprises more than 50 % of all deaths. Ischemic heart disease, which can result in myocardial infarction (MI), is one of the leading causes of death in the developing world. Although great strides have been made to reduce its impact, the mechanisms responsible for initiating and propagating damage to the heart are still poorly understood. Post-myocardial infarction changes are accompanied by increased proteolytic activity. One group of enzymes that is important in mediating the destructive effects in extracellular matrix (ECM) is the family of matrix metalloproteinases (MMPs).

MMPs represent a large family of zinc-dependent proteases with a wide range of substrates, including extracellular matrix components, cytokines, receptors, and cell motility factors (Yong et al., 2007; Morrison et al., 2009). They are recognized as the main proteolytic enzyme group involved in remodelling of the ECM and modifying cell-cell and cell-matrix interactions (Murphy and Nagase, 2008). MMPs are not generally expressed in normal, healthy, resting tissue. Up-regulation of the activity of individual MMPs is seen in a wide range of diseased or inflamed tissues. Many different human MMPs have been identified (Yong et al., 2007). On the basis of substrate specificity and homology, MMPs can be divided into six groups: collagenases (MMP-1, -8, -13 and -18), gelatinases (MMP-2 and -9), stromelysins

(MMP-3, -10, and -11), matrilysins (MMP-7 and -26), membrane-type MMPs (MT-MMPs: MMP-14, -15, -16, -17, -24 and -25) and other MMPs (MMP-12, -19, -20, -21, -22, -23, -28 and -29) (Zhang et al., 2009; Sekhon, 2010). Most MMPs are initially synthesized as enzymatically inactive zymogens (pro-MMPs). Some MMPs are stored within the cell after synthesis, but most of them are either secreted freely into the extracellular space or anchored to the surface of cell membranes (Page-McCaw et al., 2007; Raffetto and Khalil, 2008). Rigorous regulation of MMP expression and activity is a crucial part of ECM homeostasis. This regulation occurs primarily at the level of gene expression, pro-MMP secretion, extracellular localization, zymogen activation, and enzyme inhibition by interaction with endogenous inhibitors (Zhang et al., 2009).

Acute MI is associated with enhanced inflammatory response, which includes the release of a variety of inflammatory mediators and infiltration of leukocytes into the infarcted myocardium. Inflammatory cells recruited into myocardium have been suggested to be an important source of regional MMPs, especially MMP-9 (Lindsey et al., 2001), which are elevated in infarcted hearts (Fang et al., 2007, 2008). MMPs have an important role in ECM degradation of the collagen network and contribute significantly to post-MI left ventricular remodelling (Ducharme et al., 2000; Hayashidani et al., 2003). MMP-2 and MMP-9 display diverse changes in their plasma levels after MI. Although plasma MMP-9 is consistently elevated (Kai et al., 1998; Kaden et al., 2003; Webb et al., 2006), the changes in plasma MMP-2 remain inconsistent (Kai et al., 1998; Hojo et al., 2001; Kaden et al., 2003; Webb et al., 2006). In addition, plasma MMP-9 increases early after MI (Kai et al., 1998; Kaden et al., 2003; Webb et al., 2006); however, a delayed increase in plasma MMP-2 has been observed (Kai et al., 1998; Hojo et al., 2001). The reasons for such diversities are not fully understood.

The aim of our research was to assess the concentration of selected metalloproteinases participating in degradative processes early after MI using peripheral blood samples. We evaluated the levels of serum MMP-2 and MMP-9 collected within 6 h and within 24 h from evidence of the first signs of MI and during a follow-up visit performed after six months from the initial event. Additionally, we aimed to determine whether the results from microarray analysis could provide us with the expression profile of the selected MMPs and their regulators.

Material and Methods

In the present study 34 patients with confirmed diagnosis of primary acute MI were enrolled between September 2006 and January 2011. The diagnosis had to satisfy the clinical criteria, ECG outcome and laboratory findings according to medical guidelines. Coronary angiography was performed in most patients. Inclusion criteria were less than 80 years of age and no other systemic disease (e.g. cancer, acute or chronic inflammatory disease). Cases were divided into two groups based on time of sample collection. Twenty-one samples were collected within 6 h from the first signs of MI (MIO6) and 13 samples were collected within 24 h (MIO24). Six months following the cardiac event the cardiovascular survival status and MMP levels were assessed. Four patients died of cardiovascular cause within six months from MI.

The control group consisted of 34 healthy individuals with stable and physiological biochemical laboratory results, without any manifestation of cardiovascular or chronic infections or autoimmune disease and with no acute or inflammatory disease or cancer. Paired controls were matched to cases based on their gender, age (the controls could be up to five years older than cases), diabetes status and smoking status.

Blood samples were collected from all patients with MI within 24 h from the occurrence of the first signs and from controls and at a follow-up visit after six months.

The study complied with the Declaration of Helsinki and was approved by the ethical committee. Written informed consent was obtained from all subjects.

Clinical data collection

The following biochemical markers were evaluated from blood according to standard methods (see internal laboratory normal values): total cholesterol in mmol/l (reference ranges: 2.90–5.00 (15–40 years), 2.90–5.20 (40–110 years)), LDL in mmol/l (reference ranges: 1.2–3.0), HDL in mmol/l (reference ranges: 1.0–2.1 for males, 1.2–2.7 for females), apolipoprotein A (ApoA) in g/l, apolipoprotein B (ApoB) in g/l, triglyceride (TAG) in mmol/l (reference ranges: 0.45–1.70), glycaemia in mmol/l (reference ranges: 3.9–5.6), uric acid in μmol/l (reference ranges: 220–420 for males, 140–340 for females) and creatinine in μmol/l (reference ranges: 44.0–110.0 for males, 44.0–104.0 for females). BMI is a measure of fat based on height and weight. Based on the BMI value the normal weight is considered 18.5–24.9, overweight is 25–29.9.

Immunoassay of MMP-2, MMP-9

MMP-2 and MMP-9 were measured from the blood serum in all individuals using the sandwich method of enzyme-linked immunosorbent assay kits (ELISA). All the sera were separated within an hour of blood collection and stored at -70 °C until assayed. We used a commercial kit USCN (Life Science Inc., Houston, TX). The serum samples were diluted 300-fold before determination of MMP-2 and 20-fold for MMP-9.

Microarray analysis

Upon collection samples of peripheral whole blood were mixed with RNAlater® (Ambion, Inc., Carlsbad, CA) and stored at -70 °C. The RNA was isolated using RiboPure™-Blood Kit (Ambion, Inc.). Samples were precipitated and purified with GLOBINclear™ Whole Blood Kit (Ambion Inc.). The quantification was made in Nanodrop ND 1000 Spectrophotometer (Thermo

Scientific Scientific Inc., Carlsbad, CA) (1 μl) and the integrity of the RNA was measured in Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) (1 μl).

Illumina direct hybridization whole-genome expression assays were used to analyse intact RNA samples. The required input of total RNA was 150 ng in 11 μl. RNA was reverse-transcribed into complementary DNA (cDNA), purified and used for *in vitro* synthesis of complementary RNA (cRNA). cRNA samples were hybridized on Human WG6-v2 (3,4) Expression BeadChips (Illumina Inc., San Diego, CA) according to manufacturer's instructions. After washing and staining steps, BeadChips were scanned in Illumina® BeadArray Reader (Illumina).

The study utilized Illumina microarray technology to analyse gene expression intensities across the full human genome. Statistical analysis used the R system for statistical computing, graphic and data analysis. The "beadarray" package was used for reading in the gene expression data from Illumina analyser scans. The normal exponential convolution method was adopted to separate the background noise from the signal. The $\log_2$-transformed quantile-normalized gene expression intensities were modelled using two explanatory variables that matched the pair indicator using the "limma" package accounting for correlated data due to several biologically replicated samples. Statistical significance was reached for transcripts with a q value below 0.05, and the clinical significance was reached when the $\log_2$-fold change was greater or equal to 2.0 in absolute value. Modelling results were validated by RT-qPCR analysis using RNA samples from four patients, their matched controls, and six other randomly selected controls. Gene expression levels were normalized against the housekeeping genes including *18S* and *HPRT* (Valenta et al., 2012).

The expression profile of selected genes was compared between patients with MI and controls and among patients within 24 h from MI. Microarray analysis was performed in 34 patients with MI and in 34 controls matched to these patients. Twenty-one genes were selected for investigation of the expression rate: genes for selected MMPs (*MMP1*, *MMP2*, *MMP3*, *MMP7*, *MMP8*, *MMP*9, *MMP13* and *MMP14),* genes for their tissue inhibitors – TIMPs (*TIMP1, TIMP2, TIMP3, TIMP4),* genes for matrix proteins (*FN1 – Homo sapiens* fibronectin 1, *SPARC* – osteonectin, *SPP1* – osteopontin), and genes for selected cytokines (*IL1A* – interleukin 1α, *IL1B* – interleukin 1β, *IL7* – interleukin 7, *IL18* – interleukin 18 and *TNF* – tumour necrosis factor).

Statistical analysis

Results are presented as mean value ± standard deviation (SD). Two types of statistical software, Statistica (version 10) and R software (version 2.15.1), were used to detect the difference between the groups. A difference at $P < 0.05$ was considered as statistically significant. The two-sample *t*-test was used to determine the significance of MMP concentrations between samples. The assumptions were normality of both samples and equality of variances. Normality of distribution was checked by histograms and Shapiro-Wilk's test; homogeneity of variances in the two groups was tested by F-test. If these assumptions were not met, we applied both logarithmic transformation of data and Mann-Whitney's test. Both of these methods helped to choose variables whose evidence against the hypothesis of equality of group averages was the greatest. The linear regression model was used to express the dependence of MMP levels on the time between the event (i.e. myocardial infarction) and sample collection. Gene expression values were compared using multivariate analysis of variance.

Results

Characteristics of the MI distribution, ST-segment elevation and significant clinical variables (including height, weight, waist circumference, BMI, blood pressure, glycaemia, lipids, creatinine and urea) were collected and body mass index (BMI) was calculated for each patient (Tables 1, 2). We did not find any signifi-

Table 1. Baseline characteristic

Characteristic	MI		MID6		Controls	
	N	"yes" answer (%)	N	"yes" answer (%)	N	"yes" answer (%)
Gender (male)	30	20 (67 %)	4	2 (50 %)	34	22 (65 %)
Smoking	30	6 (20 %)	4	0 (0 %)	34	6 (18 %)
DM type II	30	8 (27 %)	4	2 (50 %)	34	11 (32 %)
Dyslipidaemia	30	11 (37 %)	4	2 (50 %)	34	13 (38 %)
Hypertension	30	22 (73 %)	4	2 (50 %)	34	22 (65 %)
STEMI	30	17 (57 %)	4	3 (75 %)	-	-
ACEI	30	14 (47 %)	4	1 (25 %)	34	14 (41 %)
Sartan	30	1 (3 %)	4	1 (25 %)	34	4 (12 %)
Betablockers	30	14 (47 %)	4	0 (0 %)	34	12 (35 %)
Diuretics	30	7 (23 %)	4	1 (25 %)	34	11 (32 %)
Ca blockers	30	9 (30 %)	4	3 (75 %)	34	10 (29 %)
Statins	30	10 (33 %)	4	2 (50 %)	34	14 (41 %)
Fibrates	30	2 (7 %)	4	0 (0 %)	34	2 (6 %)

ACEI – angiotensin-converting-enzyme inhibitor, DM – diabetes mellitus, MI – patients with acute myocardial infarction that survived a 6-month follow-up period, MID6 – patient that died of cardiovascular cause within 6 months from myocardial infarction, STEMI – myocardial infarction with ST elevation on ECG.

Table 2. Descriptive characteristics

Characteristic	MI		MID6		Controls	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Age (years)	30	64.93 ± 9.14	4	72.03 ± 4.72	34	66.89 ± 9.07
Height (cm)	28	166.50 ± 11.47	3	163.33 ± 10.41	33	164.21 ± 10.43
Weight (kg)	27	84.26 ± 18.54	2	81.00 ± 16.97	33	79.47 ± 13.44
BMI	27	30.04 ± 5.15	2	29.54 ± 1.16	33	29.55 ± 4.75
Waist (cm)	20	95.80 ± 11.41	2	103.00 ± 2.83	30	99.47 ± 9.60
SBP (mmHg)	29	135.31 ± 24.42	4	139.00 ± 9.87	34	137.94 ± 10.65
DBP (mmHg)	29	79.21 ± 12.57	4	84.38 ± 16.36	34	82.43 ± 5.95
Glycaemia (mmol/l)	30	6.69 ± 2.12	2	10.45 ± 6.43	34	6.84 ± 2.31
Chol (mmol/l)	28	5.35 ± 1.31	1	4.70 ± 0.00	34	5.23 ± 1.48
LDL (mmol/l)	28	3.39 ± 1.05	1	2.59 ± 0.00	34	3.29 ± 1.21
HDL (mmol/l)	28	1.28 ± 0.32	1	1.91 ± 0.00	34	1.44 ± 0.44
TAG (mmol/l)	28	1.54 ± 0.97	1	0.80 ± 0.00	34	1.32 ± 0.56
ApoA (g/l)	9	1.30 ± 0.34	1	1.09 ± 0.00	31	1.40 ± 0.29
ApoB (g/l)	9	1.04 ± 0.33	1	0.69 ± 0.00	31	0.94 ± 0.27
Creatinine (μmol/l)	30	81.67 ± 93.26	3	184.33 ± 178.55	34	80.68 ± 14.48
Uric acid (μmol/l)	28	330.46 ± 93.26	3	339.33 ± 141.61	34	299.26 ± 99.08

Chol – total cholesterol, DBP – diastolic blood pressure, MI – patients with acute myocardial infarction that survived a 6-month follow-up period, MID6 – patients that died of cardiovascular cause within 6 months from myocardial infarction, SBP – systolic blood pressure, TAG – triglycerides

cant differences in these variables while comparing values among patients during hospitalization and during the follow-up visit. The mean value of BMI in patients with MI was 30.04 ± 5.15 and during the follow-up visit the mean value was 29.78 ± 4.58. The mean value recorded in controls was similar to those in patients (29.55 ± 4.75). Patients from all groups can be considered to be overweigt or obese. Four enrolled patients died of cardiovascular cause within six months from the initial cardiac event (MID6).

Proteomic analysis

The mean values of MMP-2 and MMP-9 were significantly higher in patients with MI as compared to controls, regardless of whether they were measured early after MI (MI vs. controls) or after six months (MI follow-up vs. controls). The MMP-9 levels decreased significantly in six months after MI, whereas the levels of MMP-2 were almost equal to the post-infarction ones (Table 3).

While comparing the results of four patients that died of cardiovascular cause within six months from the initial cardiac event to controls we found significantly higher levels of both MMPs in these patients. The mean MMP-2 value was 435.00 ± 55.83 ng/ml (P = 0.003) and the mean MMP-9 value was 166.25 ± 41.07 ng/ml (P = 0.018).

Blood samples were collected at different time points after the occurrence of first signs of acute MI; however, it was always collected within 24 hours. The dependence on the time point of collection was evaluated for increased serum levels of MMP-2 and MMP-9. We hypothesized that the level of MMP is changing with increased time between cardiac event and sample collection. To verify this hypothesis we used the model of linear regression. The resultant curve proved a decrease in MMP-2 levels within 24 hours, whereas MMP-9 levels were stable (Fig. 1). These results were confirmed when we compared the mean serum MMP-2 and MMP-9 levels in patients divided into groups based on the time of sample collection (MIO6 vs. MIO24) (Table 4).

Microarray analysis

All samples of total RNA met the following key criteria: the ratio of the absorbance at 260 and 280 nm (A260/280) was > 1.8, RNA Integrity Number (RIN) was > 7.0 and the ratio of ribosomal subunits (28S/18S) was > 0.7.

The majority of the genes selected for microarray analysis demonstrated very low intensity and low expression among other genes. As a result they were eliminated from further statistical analysis. Only two genes – *MMP8* (gene for MMP-8) and *SPP1* (gene for osteonectin) – showed adequate intensity to be further analysed (Table 5). The results of the statistical analysis did not prove significant statistical or clinical differences in the expression levels of these two genes. The set of se-

Table 3. Serum levels of MMP-2 [ng/ml] and MMP-9 [ng/ml]

Marker	MI		MI Follow-up		Controls		P value		
	Mean ± SD	(N)	Mean ± SD	(N)	Mean ± SD	(N)	MI vs. MI-FU	MI vs. controls	MI Follow-up vs. controls
MMP-2	299.47 ± 117.61	(34)	263.20 ± 167.08	(30)	70.50 ± 39.58	(34)	0.317	< 0.001	< 0.001
MMP-9	93.56 ± 53.74	(34)	49.87 ± 27.77	(30)	29.91 ± 20.45	(34)	0.002	< 0.001	< 0.001

MI – patients with acute myocardial infarction, MI Follow-up – visit in 6 months after MI

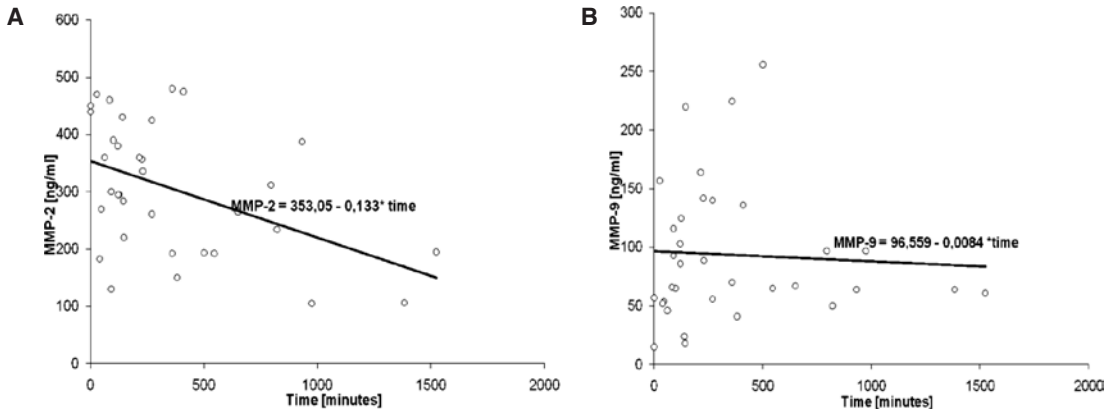

Fig. 1. Dependence of MMP-2 (A) and MMP-9 (B) on the time point of sample collection

lected genes did not exhibit statistical or clinical significance as defined for our study.

Discussion

The metabolism in ECM is regulated by a balance of MMP and their inhibitors. We evaluated the levels of MMP-2 and MMP-9 in samples collected within 24 h from the first signs of acute MI and in samples collected after a 6-month follow-up period. The results obtained from patients were compared with the results from controls. Webb et al. (2006) evaluated the plasma concentration of MMPs and their inhibitors in a 6-month period after MI. They reported decreasing values of MMP-2 and increasing MMP-9 values. This is in contradiction to our findings. We observed that the MMP-9 levels decreased significantly in six months after MI, whereas the levels of MMP-2 were almost equal to the post-infarction ones. This leads to the assumption that it is not possible to characterize the MMP-2 and MMP-9 levels after MI in general since a wide variety of factors can influence the remodelling processes and changes in the myocardium.

Four enrolled patients died of cardiovascular cause within six months from the initial cardiac event. The levels of both MMPs were found to be significantly higher in these patients. The mean MMP-2 value was 435.00 ± 55.83 ng/ml and the mean MMP-9 value was 166.25 ± 41.07 ng/ml. Previous studies reported that the activation of MMPs has been linked to adverse left ventricular remodelling post myocardial infarction and heart failure (Matsunaga et al., 2005; Wagner et al., 2006). Kelly et al. (2007) reported significant correlation between serum MMP-9 levels measured early after MI and left ventricular remodelling, which may occur in the post infarction period. The increased serum levels of MMPs indicate higher proteolytic activity and extensive tissue degradation in these patients.

Blood samples were collected immediately after admission, within 24 h from the first signs of cardiac event in all patients. We focused on the MMP concentrations in the course of 24 h after MI because the majority of patients are brought to the hospital within this time frame and a sample of blood can be collected. The increased MMP-9 concentration was stable within 24 h from the cardiac event, unlike increased serum MMP-2, which at that time was variable. These results were confirmed when we compared the mean serum MMP-2 and MMP-9 levels in patients divided into groups based on the time of sample collection. Since the high serum MMP-9 level is stable in the course of 24 h after MI, it could be a suitable marker for diagnostics. Possible subgrouping in MMP-9 would be a perspective for the future.

Diverse changes in plasma MMP-9 and MMP-2 after MI have been reported (Kaden et al., 2003; Webb et al., 2006), but the mechanism is not yet clear. Several possible factors may be considered. The diverse changes may be caused by different transcription regulation or

Table 4. Serum MMP [ng/ml] level based on the time of sample collection

Marker	MIO6 (Mean ± SD)	MIO24 (Mean ± SD)	P value	
			t-test	F-test
MMP-2	337.90 ± 94.05	252.77 ± 126.25	0.032	0.236
MMP-9	89.90 ± 53.60	99.46 ± 67.44	0.650	0.351

Table 5. Results from gene expression analysis (comparing MI vs. controls)

Gene symbol	Average expression	BH adjust. P value (q value)	Log-FC	Probability of differential expression
MMP8	5.89	-0.523	0.5374	0.1125
SPP1	5.86	0.313	0.7132	0.0405

Average expression – average value of gene expression intensities, BH adjust. P value – Benjamini-Hochberg's adjustment, Log-FC – $\log_2$ change of intensities of samples from MI patients vs. from controls

by the source of the particular MMPs. Although MMP-9 can be produced by myocytes and fibroblasts, the most important source are neutrophils. In contrast, MMP-2 is mainly produced by fibroblasts in the myocardium. Tayebjee et al. (2005) evaluated the effects of age, gender, ethnicity and diurnal variation on the circulating levels of MMP-2, MMP-9, and their tissue inhibitors (TIMPs). They reported a significantly lower MMP-9 level in the Far Eastern group compared to other ethnical groups. Age, gender and diurnal variation had a modest effect on circulating concentrations of MMPs.

Furthermore, the diverse and controversial results may be caused by using a different set of MMP kits. The principle of each kit can differ in many ways because some applied antibodies can react differently with relevant active and inactive MMP molecules and with the MMP-TIMPs complex.

The final and probably one of the most important limitations of serum MMP profiling is that of the actual source of these enzymes. MMPs are synthesized within a wide variety of tissues, and therefore, changes in the serum MMP levels may not reflect the changes occurring within the tissue of the myocardium. For example, patients with rheumatologic disorders or cancer have increased serum MMP levels as well. This is why we took this in consideration while setting up the inclusion and exclusion criteria for the study. Interestingly, in their large-scale study Sundstorm et al. (2004) did not set up rigorous exclusion criteria and they postulated that certain changes in plasma MMP levels remain predictive of myocardial remodelling or cardiovascular events. Thus, it is likely that a certain portfolio of plasma MMP and TIMP measurements may be more specific and sensitive to myocardial remodelling processes. Identification of such a set of plasma MMPs could be essential for identification of ongoing myocardial remodelling processes in patients with multiple chronic diseases. Since it is becoming apparent that serum MMP profiling may hold relevance with respect to diagnosis and prognosis, standardization of analytical methods and establishment of reference normal concentrations for subpopulations of patients would be an important step to further research.

MMPs play a significant role in the pathogenesis of various cardiovascular diseases. Our previous study has reported that the serum levels of MMP-2 and MMP-9 were significantly elevated in patients that underwent surgery because of aortic valve disease compared to the controls. Ten % of the cases had concomitant ascending aortic dilatation. However, the serum levels of MMP-2 and MMP-9 were not statistically different in patients with the dilatation. These results indicate that there may be some changes in the extracellular matrix of heart. Still, there is the question regarding the regulation of specific upstream signalling and pathways involved in the remodelling process in aortic valve and ascending aorta (Šímová et al., 2013).

The majority of the genes selected for microarray analysis demonstrated very low intensity and low expression among other genes. As a result, they were eliminated from further statistical analysis. Only two genes – *MMP8* (gene for MMP-8) and *SPP1* (gene for osteonectin) – showed adequate intensity to be further analysed. The results of the statistical analysis did not prove significant statistical or clinical differences in the expression levels of these two genes. The set of selected genes did not exhibit statistical or clinical significance as defined for our study.

Fang et al. (2010) employed two superarrays (ECM and adhesion molecules, and common cytokines; 84 genes included in each array) to screen gene expression profiles by peripheral blood mononuclear cells (PBMCs) in five patients with acute MI and five controls. A total of 42 genes were differentially expressed in patients with acute MI compared to controls. MMP-9 expression by PBMCs was increased, but MMP-2 was not expressed by PBMCs. Using microarray analysis we evaluated the expression level of 21 genes that are in relationship to the MMP. Based on our results, we do not consider this type of microarray analysis to be an appropriate method for detection of expression of MMPs and related factors from peripheral blood samples. In comparison to serological testing this method is long-lasting and dependent on advanced and expensive equipment.

The pathogenesis of myocardial infarction is likely to involve multiple factors acting on many levels. It is clear that the pathological alterations in the structure and composition of the myocardial extracellular matrix are associated with the adverse left ventricular remodelling. Still, there is the question regarding the regulation of specific signalling and pathways involved in the remodelling process in myocardium. A simple marker that can allow early risk stratification would benefit patients in need of intensive therapy. It would be an important advancement if measurement of MMP levels could serve as biochemical indicators for diagnosis and prognosis of cardiovascular diseases.

We conclude that measurement of serum MMP levels in patients with myocardial infarction is a simple and relatively rapid laboratory test, which can be easily obtained even in the primary health care setting. Although the levels of MMP-2 and MMP-9 are not specific and cannot provide a definite clinical diagnosis, they could be a part of a potential set of biochemical indicators that would be predictive for identification of myocardial remodelling processes.

This is an initial study and although a causative link has not been shown, these data are very important for further research on the role of extracellular matrix components in relation to cardiovascular risk-associated myocardial infarction.

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