# Circulating matrix metalloproteinases and tissue inhibitors of metalloproteinases levels in pediatric patients with congenital heart disease: Relationship to cardiac functions

*Zübeyir Kılıç, Birsen Uçar, Gökmen Özdemir, Ömer Çolak*, Cengiz Bal**, Türkan Ertuğrul[1]*

Departments of Pediatric Cardiology, *Biochemistry, **Biostatistics, Faculty of Medicine, Eskişehir Osmangazi University; Eskisehir-*Turkey*
[1]Department of Pediatric Cardiology, Faculty of Medicine, İstanbul University; İstanbul-*Turkey*

## Abstract

**Objective:** The pathological effects of matrix metalloproteinases and their tissue inhibitors in cardiovascular diseases are of considerable interest. In our study, we aimed to determine and evaluate the potential significance of circulating matrix metalloproteinases-2 and 9, tissue inhibitors of matrix metalloproteinases-1 and 2 levels in four patient subgroup of pediatric cardiology field and expose pathophysiologic differences between these groups.

**Methods:** Eighty-seven patients with the diagnosis of congenital heart disease and 47 healthy controls were enrolled in the study. The study group was stratified to 4 subgroups; 14 patients with right ventricular volume overload, 30 patients with left ventricular volume overload, 19 patients with left to right shunt who developed pulmonary hypertension and 24 patients with cyanotic congenital heart disease. For evaluation of the relationships between serum matrix metalloproteinases and their tissue inhibitors levels with cardiac structures and functions; complete blood count, arterial oxygen saturation, detailed echocardiographic measurements (including tissue Doppler) in all patients and hemodynamic parameters of the patients who went to cardiac catheterization were recorded. Serum matrix metalloproteinase levels were determined by ELISA test. Statistical evaluations were performed with SPSS 16.0. For parameters showing normal distribution, comparisons were made with t-test and ANOVA test. However, for parameters without normal distribution, groups were compared with Mann-Whitney U test and Kruskal-Wallis test.

**Results:** We demonstrated that serum tissue inhibitors of matrix metalloproteinases-1 levels of patients with pulmonary hypertension secondary to congenital heart diseases were significantly higher than the patients with left to right shunt without pulmonary hypertension and controls ($p<0.01$). Although serum matrix metalloproteinases and their tissue inhibitors levels in patients with cyanotic congenital heart diseases and patients with right or left ventricular volume overload were found to be altered when compared with controls but not significant.

**Conclusion:** Our data may suggest the possible role of matrix metalloproteinases and their tissue inhibitors on myocardial remodeling in congenital heart defects and especially in patients who developed pulmonary hypertension. *(Anadolu Kardiyol Derg 2014; 14: 531-41)*

**Key words:** matrix metalloproteinases, tissue inhibitors of metalloproteinases, congenital heart diseases, pulmonary hypertension

## Introduction

Matrix metalloproteinases, or matrixins, are a large group of zinc-dependent proteases that are responsible for cleaving and rebuilding connective tissue components such as collagen, elastin, gelatin and casein (1-3). Tissue inhibitors of metalloproteinases are specific inhibitors of matrixins that participate in controlling the local activities of matrix metalloproteinases in tissues (4, 5). In the pathological conditions with unbalanced matrix metalloproteinases activities, changes in tissue inhibitors of matrix metalloproteinases levels are considered important. The pathological effects of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in cardiovascular disease processes that involve vascular remodeling, atherosclerotic plaque instability, and left ventricular remodeling after myocardial infarction are of considerable interest (6-8).

Gelatinases, a subgroup of metalloproteinases, include matrix metalloproteinase-2 and matrix metalloproteinase-9 proteins. Matrix metalloproteinase-2 is known to cleave native collagen type I (1-3). Therefore, gelatinases play an important role

**Address for Correspondence:** Dr. Birsen Uçar, Eskişehir Osmangazi Üniversitesi Tıp Fakültesi, Pediyatrik Kardiyoloji Bilim Dalı, Eskişehir-*Türkiye* Phone: +90 506 397 85 85 E-mail: drbucar@yahoo.com
**Accepted Date:** 24.12.2013 **Available Online Date:** 25.06.2014

*DOI:10.5152/akd.2014.4950*

in vascular remodeling. In human and animal studies, it was demonstrated that increased matrix metalloproteinase-2 and matrix metalloproteinase-9 activity are related with the destruction of the elastic lamina of arteries and the aneurysm formation (9-11). In patients with hypertrophic cardiomyopathy, it have been suggested that modifications of matrix metalloproteinase-2 and tissue inhibitors of matrix metalloproteinase-2 release and activity can be related or responsible for cardiac remodeling mechanisms (12). Also, in patients with idiopathic pulmonary arterial hypertension, imbalance between matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases levels has been reported (13).

Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases have been demonstrated to influence left ventricular properties and found that increased plasma matrix metalloproteinase levels correlate with increased left ventricular volumes and reduced left ventricular ejection fraction in patients with congestive heart failure (14). In contrast to adult patients, pediatric patients almost have a congenital heart defect that play major role on body with different mechanisms. We assumed that congenital heart diseases have to be divided to four subgroups by their major influences on body. These groups are left to right shunt causing left or right ventricular volume overload, cyanotic congenital heart diseases causing hypoxia and congenital heart diseases causing pulmonary hypertension. In our study, we aimed to determine and evaluate the potential significance of circulating matrix metalloproteinase-2, matrix metalloproteinase-9, tissue inhibitors of matrix metalloproteinase-1 and tissue inhibitors of matrix metalloproteinase-2 levels in four patient subgroup of pediatric cardiology field and expose pathophysiologic differences between these groups.

## Methods

### Ethics statement

Written informed consent was obtained from all participating parents of patients, as required by the institutional review board under an approved protocol (Ethic Committee of Eskişehir Osmangazi University, Faculty of Medicine, and Number 2007/124).

### Patients and controls

From March 2007- June 2009, 87 patients (46 males and 41 females) evaluated at Eskişehir Osmangazi University Medical Faculty, Department of Pediatric Cardiology with the diagnosis of congenital heart disease were enrolled in the study. The patients' age were between 3 months-18 years (mean 57.1±6.2 months). The control group of the present study consisted of 47 healthy subjects who were age and sex matched with patients group and presented for innocent murmur.

After a complete physical examination, complete blood count and arterial oxygen saturations of all subjects were studied; telecardiograms and electrocardiograms were evaluated. A diagnostic transthoracic echocardiography was performed to all cases. Moreover, for evaluation of the relationships between serum matrix metalloproteinase, tissue inhibitors of matrix metalloproteinase levels and cardiac structures and functions, detailed echocardiographic measurements were recorded.

The study group was stratified to four subgroups:

Group 1: Fourteen patients with congenital heart disease leading to right ventricular volume overload (atrial septal defect),

Group 2: Thirty patients with congenital heart disease leading to left ventricular volume overload (ventricular septal defect or patent ductus arteriosus),

Group 3: Nineteen patients with left to right shunt (atrial septal defect, ventricular septal defect or patent ductus arteriosus) who developed pulmonary hypertension,

Group 4: Nineteen patients with cyanotic congenital heart disease (Tetralogy of Fallot or complex cardiac defects including transposition of great arteries or truncus arteriosus, tricuspid atresia, pulmonary atresia, total anomalous pulmonary venous connection, along with associated defects).

Patients with pulmonary hypertension were not included in Groups 1 and 2. Patients with right and left ventricular volume overload (Group 1 and 2) were compared with each other and with controls in terms of matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase levels for demonstrating the roles of these molecules on left and right ventricular remodeling. In this respect, we also calculated matrix metalloproteinase / tissue inhibitors of matrix metalloproteinase ratios to find out corruption of balance between matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase.

In order to investigate the effect of pulmonary hypertension, group 1 and 2 cases were compared with group 3 cases whose shunt led to pulmonary hypertension. The mean pulmonary artery pressure detected during cardiac catheterization more than 25 mm Hg was accepted as pulmonary hypertension. Also patients with a cyanotic (group 1 and 2) and cyanotic (group 4) congenital heart diseases were compared with each other and controls.

In all patient groups, the relevance of matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase levels with left and right ventricular systolic and diastolic functions were investigated in all patients, however the relevance with hemodynamic parameters (atrial mean pressure, systolic and diastolic pressures, pulmonary artery and aorta systolic, diastolic and mean pressures, pulmonary and systemic blood flows (Qp and Qs, respectively) and the flows ratio (Qp/Qs), pulmonary and systemic resistance values (Rp and Rs) and the resistances ratio (Rp/Rs), the volumes of left to right and right to left shunts) were investigated in selected patients who went to cardiac catheterization. In addition, the relationship of matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase levels with the degree of hypoxia was also questioned in cya-

notic patients by investigating their correlations with the levels of hemoglobin, hematocrit, mean corpuscular volume, red-cell distribution width and arterial oxygen saturation values.

**Echocardiographic evaluation**

Hewlett Packard Sonos 5500 echocardiography device with four - or 8-MHz broadband probe was used for echocardiographic studies. After standard diagnostic evaluation with echocardiography, left ventricular systolic functions were evaluated by M-mode study through the level of the papillary muscles in the parasternal long axis position. The values of ejection fraction, fractional shortening, the left ventricular end-diastolic internal diameter, left ventricular end-systolic internal diameter, diastolic left ventricular mass and systolic left ventricular mass were recorded.

Left ventricular diastolic functions were calculated from mitral early (E) and second (A) peak flow velocities, E/A ratio, deceleration time and isovolumetric relaxation time with pulsed-wave Doppler method. By tissue Doppler method, the cursor placed through regions of lateral mitral annulus and lateral tricuspid annulus, the left and right ventricular systolic and diastolic peak flow velocities were recorded: ventricular ejection during systole ($S_m$ for mitral, $S_t$ for tricuspid), the rapid passive ventricular filling during diastole ($E_m$ for mitral, $E_t$ for tricuspid), and ventricular filling by atrial contraction ($A_m$ for mitral, $A_t$ for tricuspid). Also myocardial performance index for both ventricles were calculated by the method which was described previously by Tei et al. (15). The relationships between matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase levels with all calculated echocardiographic parameters were evaluated.

**Biochemical measurements**

Venous blood samples were collected from the subjects of all groups and then the sera were separated and stored at -70°C until analysis. The levels of matrix metalloproteinase-2, matrix metalloproteinase-9, tissue inhibitors of matrix metalloproteinase-1 and tissue inhibitors of matrix metalloproteinase-2 were analyzed by ELISA method using commercially available assays RayBiotech Kits (RayBiotech, Inc. Norcross, GA, USA).

**Statistical analysis**

Statistical evaluations were performed with SPSS 16.0 for Windows package program. Suitability of the normal distribution of variables was assessed with the Shapiro-Wilk test. For parameters showing normal distribution, comparison between the two groups was made with t-test and multiple comparisons were made with ANOVA test. In case of ANOVA test yield difference, assessment of the difference between the groups posthoc tests (Tukey or Tamhan test) were used. However, for parameters without normal distribution, two groups were compared with Mann-Whitney U test and multiple groups with Kruskal-Wallis test. Chi-square test was used in the analysis of cross tables. Pearson's correlation test was used for the evaluation of parameters showing normal distribution and the Spearman's test was used for not normally distributed parameters.

A p value of <0.05 was considered significant.

## Results

The baseline characteristics of the subjects are shown in Tables 1 and 2. There were no significant differences between the all of the study and control groups for age and gender.

The mean serum levels of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases of left to right shunt patients (Group 1 and 2) and the control group are shown in Table 3. Although serum levels of matrix metalloproteinase-2

**Table 1. Age and sex distribution of the subjects◆**

| Groups | n | Gender, Female/Male | Age, month | |
|---|---|---|---|---|
| | | | Mean | Upper-lower limits |
| Right ventricular volume overload (Group 1) | 14 | 5/9 | 59.1±11.9 | 4.5-132 |
| Left ventricular volume overload (Group 2) | 30 | 16/14 | 69.5±10.2 | 3.5-192 |
| Left to right shunt leading to pulmonary hypertension (Group 3) | 19 | 12/7 | 45.8±12.3 | 2.5-192 |
| Cyanotic congenital heart disease (Group 4) | 24 | 8/16 | 49.3±13.9 | 3-216 |
| Controls | 47 | 21/26 | 78.4±8.3 | 3-216 |

◆: *P*>0.05 for comparison of all groups ($\chi^2$ test for comparison of gender and Kruskal-Wallis test for comparison of age)

**Table 2. Detailed diagnosis of patients**

| Groups | Diagnosis | n |
|---|---|---|
| CHD with left to right shunt without PHT (n=44) | ASD | 14 |
| Right ventricular volume overload (Group 1) | VSD | 17 |
| Left ventricular volume overload (Group 2) | PDA | 13 |
| CHD with left to right shunt with PHT (n=19) (Group 3) | ASD | 3 |
| | VSD | 5 |
| | PDA | 4 |
| | VSD+PDA | 7 |
| Cyanotic CHD (n=24) (Group 4) | Tetralogy of fallot | 10 |
| | Complex cardiac defects (TGA, t runcus arteriosus, tricuspid atresia, pulmoner atresia, TAPVC etc.) | 14 |

ASD - atrial septal defect; CHD - congenital heart disease; PDA - patent ductus arteriosus; PHT - pulmonary hypertension; TAPVC - total anomalous of pulmonary venous connection; TGA - transposition of great arteries; VSD - ventricular septal defect

were higher in group 1 than group 2 and controls, the difference between the groups was not significant (p>0.05). In the same way, both matrix metalloproteinase-9 and tissue inhibitors of matrix metalloproteinase-1 levels measured higher in group 2 than group 1 and the controls, tissue inhibitors of matrix metalloproteinase-2 levels were lower in both group 1 and 2 than the controls, the differences found were not significant.

To demonstrate the balance between matrix metalloproteinases and tissue inhibitors of matrix metalloproteinase in congenital heart diseases, matrix metalloproteinases/tissue inhibitors of matrix metalloproteinases ratios were examined between any of the study and control groups, but no significant differences were found (p>0.05). Matrix metalloproteinase-2 and matrix metalloproteinase-9 levels in patients with pulmonary hypertension (group 3) were higher than the group 1 & 2 (patients with left to right shunt but without pulmonary hypertension) and the control group but it was not significant (p>0.05) (Table 4). Serum tissue inhibitors of matrix metalloproteinase-1 levels in patients with pulmonary hypertension were found significantly higher than group 1 & 2 and the controls (respectively 905.3±191.2; 370.1±76.7 and 344.1±76.3 pg/mL; Fig. 1) (p<0.01). Although the calculated mean matrix metalloproteinase-2/tissue inhibitors of matrix metalloproteinase-1 and matrix metalloproteinase-9/tissue inhibitors of matrix metalloproteinase-1 ratios of patients with pulmonary hypertension were found quite lower than patients without pulmonary hypertension and the control groups, the difference between these groups was not significant (p>0.05).

**Table 3. Comparison of serum MMP (matrix metalloproteinases), TIMP (tissue inhibitors of matrix metalloproteinases) levels (pg/mL) and MMP/TIMP ratios between patients with volume overload and controls***

| | Right ventricular volume overload (Group 1) | Left ventricular volume overload (Group 2) | Controls |
|---|---|---|---|
| n | 14 | 30 | 47 |
| MMP2 | 2797.9±407.5 | 2567.1±279.9 | 2477±215.9 |
| MMP9 | 697.2±65.9 | 743.9±64.7 | 723.8±62.9 |
| TIMP1 | 357.4±137.5 | 376.0±94.0 | 344.1±76.3 |
| TIMP2 | 89.7±22.9 | 108.9±23.2 | 125.5±20.6 |
| MMP2/TIMP1 | 116.7±39.3 | 109.5±53.6 | 140.2±41.5 |
| MMP2/TIMP2 | 47.1±8.2 | 36.4±5.1 | 35.2±3.8 |
| MMP9/TIMP1 | 26.2±9.5 | 27.4±10.4 | 31.5±8.6 |
| MMP9/TIMP2 | 11.2±1.5 | 10.4±1.3 | 10.3±1.1 |

*$P$>0.05 for comparison of between all groups (comparisons were evaluated by Kruskal-Wallis test).
To demonstrate the balance between MMPs and TIMPs in congenital heart diseases, MMPs/TIMPs ratios were examined between group 1 and 2 and controls but the differences found were not significant ($P$>0.05).

The mean matrix metalloproteinase-2/tissue inhibitors of matrix metalloproteinase-1 ratio was lower when compared with controls especially in cyanotic congenital heart disease group, but the difference between the groups was not significant (p>0.05). It was also valid for other measurements and ratios of cyanotic congenital heart disease group in comparison with acyanotic patients and the control subjects.

Correlations between serum matrix metalloproteinase, tissue inhibitors of matrix metalloproteinase levels (pg/mL), matrix metalloproteinase/tissue inhibitors of matrix metalloproteinase ratios and echocardiographic parameters, hemodynamic parameters and hypoxia (Table 5 and Fig. 2-5):

**Group 1**

Echocardiographic evaluation: In patients with atrial septal defect, matrix metalloproteinase-2 levels were correlated negatively with only the right ventricular myocardial performance index and matrix metalloproteinase-9 levels were correlated negatively with only the left ventricular myocardial performance index. However, tissue inhibitors of matrix metalloproteinase-2

**Table 4. Serum MMP (matrix metalloproteinases), TIMP (tissue inhibitors of matrix metalloproteinases) levels (pg/mL) and MMP/TIMP ratios of the patients with left to right shunt with and without pulmonary hypertension, cyanotic CHD and the control groups**

| | CHD with left to right shunt (without PHT) (group 1&2) | CHD with left to right shunt (with PHT) (group 3) | Cyanotic CHD | Controls |
|---|---|---|---|---|
| n | 44 | 19 | 24 | 45 |
| MMP2 | 2640.6±228.6 | 3013.3±524.0 | 2224.1±408.9 | 2477±215.9 |
| MMP9 | 729.1±48.5 | 954.2±104.8 | 698.9±107.2 | 723.8±62.9 |
| TIMP1 | 370.1±76.7 | 905.3±191.2[a,b] | 513.3±196.7 | 344.1±76.3 |
| TIMP2 | 102.8±17.3 | 110.4±16.4 | 111.1±20.6 | 125.5±20.6 |
| MMP2/TIMP1 | 111.9±37.7 | 30.0±14.8 | 70.5±21.9 | 140.2±41.5 |
| MMP2/TIMP2 | 39.8±4.4 | 33.3±6.3 | 38.4±12.0 | 35.2±3.8 |
| MMP9/TIMP1 | 27.0±7.5 | 8.0±3.3 | 27.1±12.0 | 31.5±8.6 |
| MMP9/TIMP2 | 10.7±1.0 | 11.5±1.7 | 9.8±2.2 | 10.3±1.1 |

CHD - congenital heart disease; PHT - pulmonary hypertension
[a,b]$P$<0.01 for comparison between group 3 with group 1 and 2 and controls (Kruskal-Wallis test was used to evaluate the differences between the groups)

**Table 5. Correlations between serum matrix metalloproteinase, tissue inhibitors of matrix metalloproteinase levels (pg/mL), matrix metalloproteinase/ tissue inhibitors of matrix metalloproteinase ratios and echocardiographic parameters, hemodynamic parameters and hypoxia (r values)**

| | | |
|---|---|---|
| Right ventricular volume overload (Group 1) | Echocardiographic parameters | MMP-2 - RV MPI: -0.547* |
| | | MMP-9 - LV MPI: -0.639* |
| | | TIMP-2 - LVPWd: -0.697**, Am: -0.570* |
| | Hemodynamic parameters | MMP-9 - Qp/Qs: -0.785*, Rp: 0.901**, Rp/Rs: 0.964**, L-R shunt: -0.756* |
| | | TIMP-1 - Qp: -0.786*, Rp: 0.847*, Rp/Rs: 0.786*, L-R shunt: -0.893** |
| | | TIMP-2 - RVP (s): -0.829* |
| Left ventricular volume overload (Group 2) | Echocardiographic parameters | TIMP-1 - DT: 0.460*, Em: -0.439* |
| | | TIMP-2 - E/A: -0.452* |
| | Hemodynamic parameters | MMP-2 - AoP (s): 0.486*, AoP (d): 0.509* |
| | | TIMP-2 - LVP (d): 0.802*** |
| Left to right shunt leading to pulmonary hypertension (Group 3) | Echocardiographic parameters | MMP-2 - Sm: 0.469* |
| | | TIMP-1 - RV MPI: 0.465* |
| | Hemodynamic parameters | MMP-2 - Qp: -0.472*, Qp/Qs: -0.572**, Rp/Rs: 0.463* |
| | | MMP-9 - LAP: 0.679* |
| | | TIMP-2 - RVP (d): 0.602** |
| | Hypoxia related parameters | No correlation |
| Cyanotic congenital heart disease (Group 4) | Echocardiographic parameters | MMP-9 - Et: -0.415*, E/At: -0.0483* |
| | Hemodynamic parameters | MMP-9 - LAP: 0.714*, RAP: 0.583*, Qs: 0.770**, Rp: -0.786*, Rs: -0.881** |
| | | TIMP-1- RVP (s): -0.713**, Qs: 0.778**, Rs: -0.786** |
| | Hypoxia related parameters | TIMP-2 - MCV: 0.457* |

*$P$<0.05; **$P$<0.01; ***$P$<0.001 (Pearson's correlation test was used for the evaluation of parameters showing normal distribution and the Spearman's test was used for not normally distributed parameters).
(Non-correlated data have not been shown in table)
Am - late diastolic phases with tissue Doppler (mitral); AoP (s) - aortic systolic pressure; AoP (d) - aortic diastolic pressure; DT - deceleration time; E/A - mitral ratio of peak early to late diastolic filling velocity; Em - early diastolic phases with tissue Doppler (mitral); L - R shunt-left to right shunt; LV - left ventricle; LVP (d) -LV diastolic pressure; LVPWd - LV posterior wall diameter; MCV - mean corpuscular volume; MPI - myocardial performance index; Qp - pulmonary blood flow; Qs - systemic blood flow; RV - right ventricle; Rp - pulmonary resistance; Rs - systemic resistance; RVP (s) - RV systolic pressure

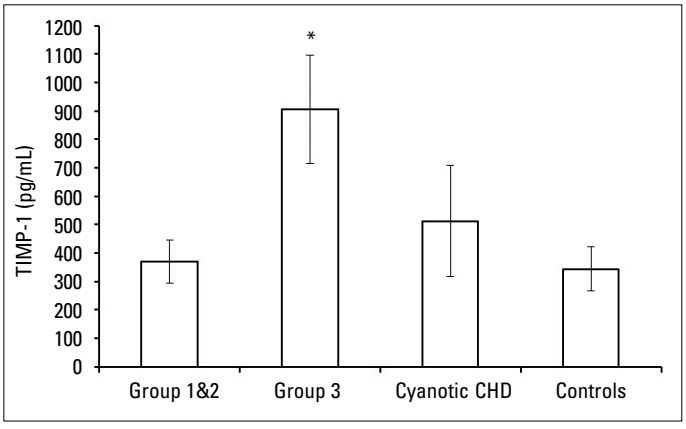


**Figure 1. Serum tissue inhibitors of matrix metalloproteinases (TIMP-1) levels of the patients with left to right shunt with and without pulmonary hypertension, cyanotic CHD and the control groups.**
*p<0.01 for comparison between group 3 with group 1&2 (mean TIMP-1 levels are 905.3±191.2 and 370.1±76.7 pg/mL, respectively) and p<0.01 for comparison between group 3 with controls (mean TIMP-1 levels are 905.3±191.2 and 344.1±76.3 pg/mL, respectively).

levels were negatively correlated with diastolic left ventricular posterior wall diameter and $A_m$ values measured by tissue Doppler method.

Hemodynamic evaluation: Both matrix metalloproteinase-9 and tissue inhibitors of matrix metalloproteinase-1 levels were found to be negatively correlated with left to right shunt amount and a positively correlated with Rp and Rp/Rs values. Also, matrix metalloproteinase-9, tissue inhibitors of matrix metalloproteinase-1 and tissue inhibitors of matrix metalloproteinase-2 levels showed a negative correlation with Qp/Qs, Qp and RVPs values, respectively.

**Group 2**

**Echocardiographic evaluation:** Tissue inhibitors of matrix metalloproteinase-1 levels positively correlated with deceleration time and negatively correlated with $E_m$ measured by tissue Doppler method. Tissue inhibitors of matrix metalloproteinase-2 levels were correlated negatively with only mitral E/A ratio.

**Hemodynamic evaluation:** Matrix metalloproteinase-2 levels correlated poorly with aortic systolic and diastolic pressures. Tissue inhibitors of matrix metalloproteinase-2 levels were strongly positively correlated with left ventricular diastolic pressure.

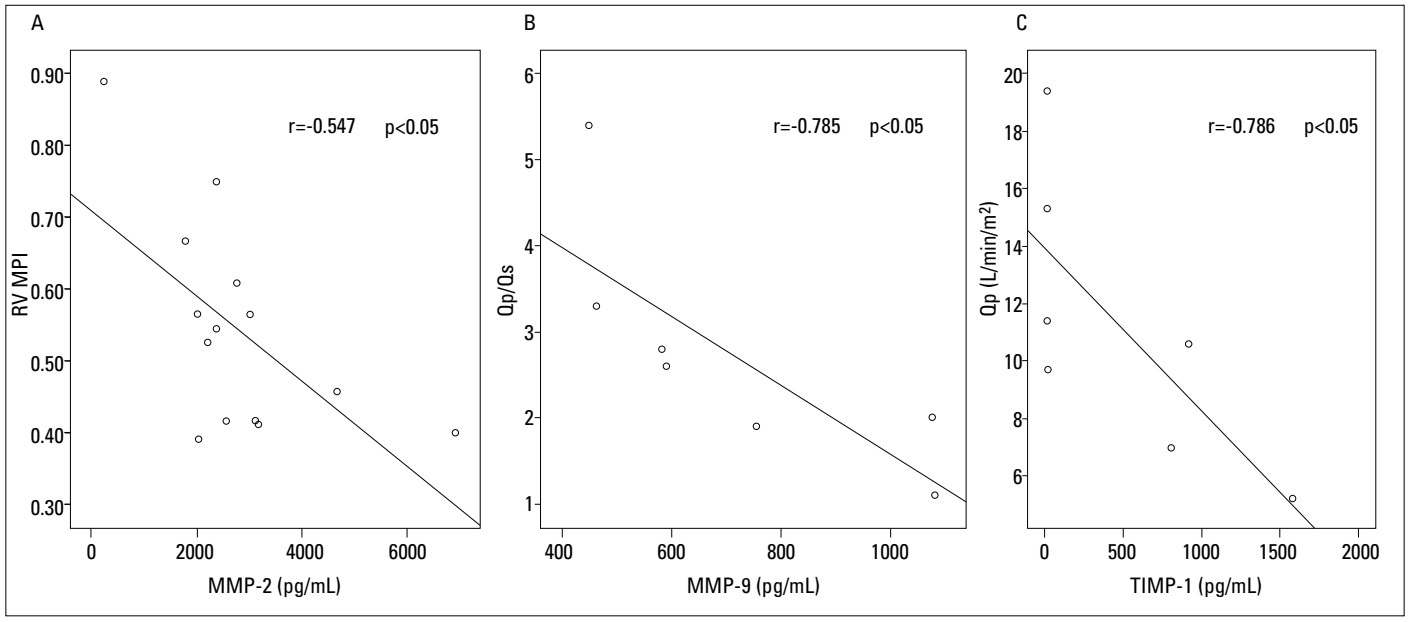


**Figure 2. In patients with right ventricular volume overload, correlations between MMP-2 levels and RV MPI (A), MMP-9 levels and Qp/Qs (B), TIMP-1 levels and Qp (C)**

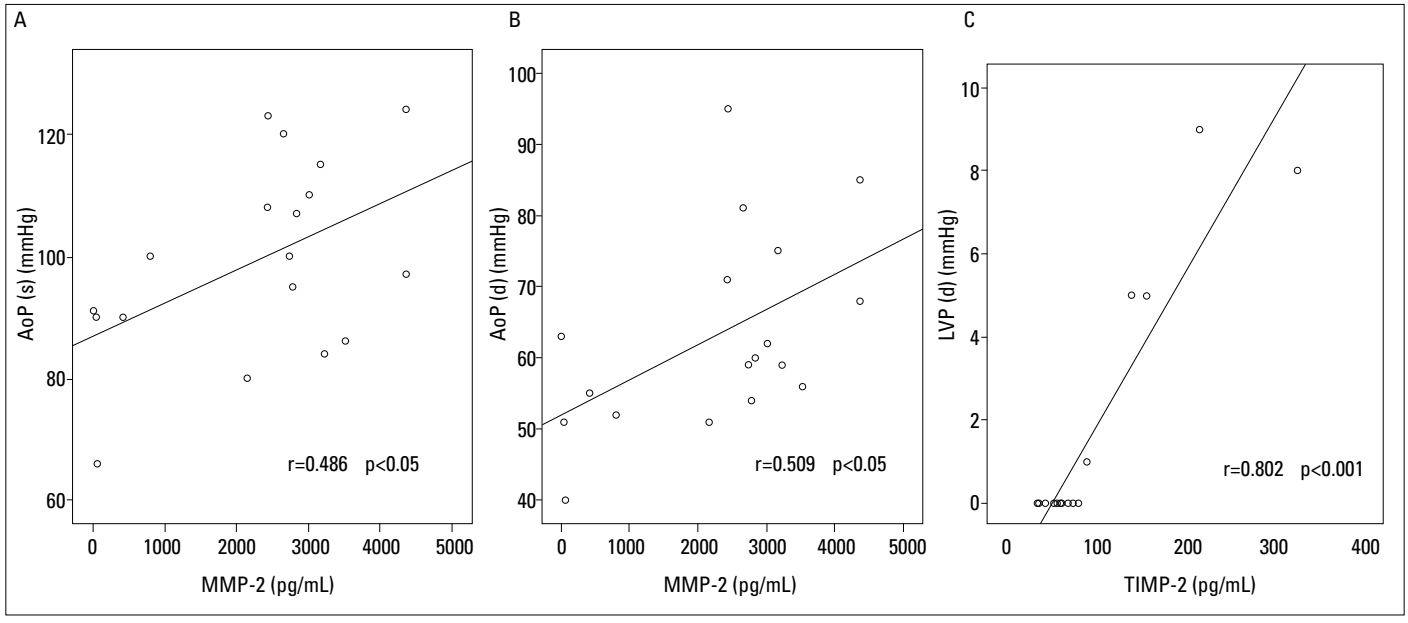


**Figure 3. In patients with left ventricular volume overload, correlations between MMP-2 levels and aortic systolic and diastolic pressures (A and B), and between TIMP-2 levels and left ventricular diastolic pressures (C)**

**Group 3**

**Echocardiographic evaluation:** Matrix metalloproteinase-2 levels positively correlated with $S_m$ value measured by tissue Doppler method and tissue inhibitors of matrix metalloproteinase-1 levels were found positively correlated with the right ventricular myocardial performance index.

**Hemodynamic evaluation:** Matrix metalloproteinase-2 levels showed negative correlation with Qp and Qp/Qs values and positive correlation with Rp/Rs values. Matrix metalloproteinase-9 and tissue inhibitors of matrix metalloproteinase-2 levels were positive correlated with left atrial pressure and right ventricular diastolic pressure, respectively.

There was no correlation between the parameters associated with hypoxia and matrix metalloproteinase, tissue inhibitors of matrix metalloproteinase levels.

**Group 4**

**Echocardiographic evaluation:** Matrix metalloproteinase-9 levels negatively correlated with $E_t$ and $E/A_t$ values measured by tissue Doppler method.

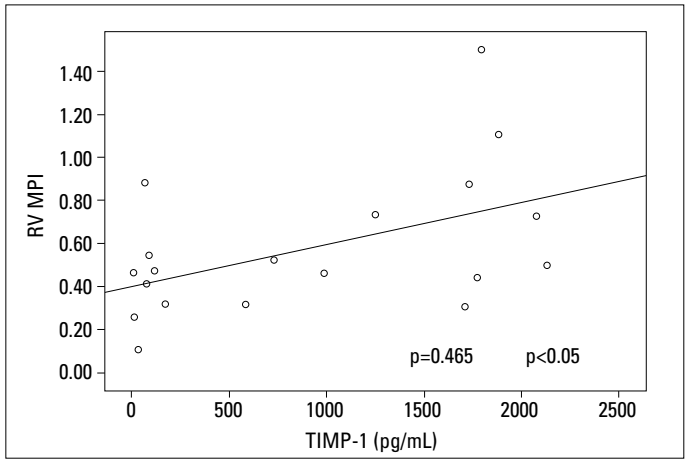


**Figure 4. In patients with left to right shunt leading to pulmonary hypertension, correlations between TIMP-1 levels and RV MPI**

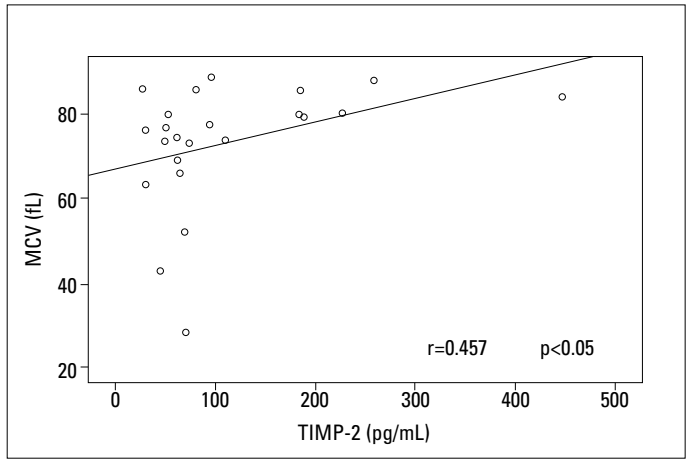


**Figure 5. In patients with cyanotic congenital heart disease, correlations between TIMP-2 levels and MCV**

**Hemodynamic evaluation:** Matrix metalloproteinase-9 levels showed negative correlation with Rp/Rs values and positive correlation with left and right atrial pressures and Qs value. Tissue inhibitors of matrix metalloproteinase-1 levels were positive correlated with Qs value and negatively correlated with RVPs and Rs values.

Among the parameters associated with hypoxia, only mean corpuscular volume levels positively correlated with tissue inhibitors of matrix metalloproteinase-2 levels.

## Discussion

The current study examined the relationship between serum matrix metalloproteinases and their inhibitors with echocardiographic and hemodynamic parameters in subjects with various congenital heart defects for the first time.

### Matrix metalloproteinases and tissue inhibitors in the heart with volume overload

In our study, serum matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase levels in patients with the right ventricular or left ventricular volume overload did not show a significant difference with control group or the other study groups but matrix metalloproteinase-2 levels in the group with right ventricular volume overload, matrix metalloproteinase-9 and tissue inhibitors of matrix metalloproteinase-1 levels in the group with left ventricular volume overload were higher than the control group, tissue inhibitors of matrix metalloproteinase-2 levels in both groups with right and left ventricular volume overload were lower than the controls (p>0.05).

It was claimed that metalloproteinases which selectively degrade components of the extracellular matrix during developmental stages of tissue act on remodelling of myocardium during congestive heart failure and cardiomyopathy (12, 14). Many types of matrix metalloproteinases are regarded as particularly being related with myocardial remodeling (8, 16). For that reason, matrix metalloproteinase inhibitors have been suggested as a potential therapeutic approach in the prevention of cardiovascular diseases.

Chronic ventricular volume overload leads to changes in ventricular weight, end-diastolic volume and functions. For the formation of these characteristic myocardial remodeling, some changes might occur interstitial collagen matrix (17). In ventricular remodelling created by chronic volume overload or overdrive pacing, partial destruction of extracellular matrix has been shown mainly related with the increase in matrix metalloproteinase activity (18, 19).

In a rat model, chronic biventricular volume overload created with an aorto-caval fistula it was observed that matrix metalloproteinase activity and collagen volume fraction remained normal all along the compensated hypertrophy phase when left ventricular mass, size and compliance was continuing to decrease (17, 20). However matrix metalloproteinase activity increased in the decompensated phase, and also severe fibrosis, left ventricular enlargement, increase in compliance and decrease in contractility were determined. Nagatomo et al. (21) generated mitral insufficiency leading acute volume loading due to chordal rupture in dogs and reported that matrix metalloproteinase activity increased more than threefold during acute volume overload but decreased to the levels of control group. Also, they reported that proportion of matrix metalloproteinase activity to total amount of matrix metalloproteinase increased more than fourfold in the acute volume overload and, therefore, indicated the loss of inhibitory control. On the other hand, Spinale et al. (22) and McElmurray et al. (23) observed that inhibition of matrix metalloproteinase attenuated the degree of left ventricular dilatation, but led to an increase in interstitial collagen and an abnormal in myocardial stiffness. They thought that these negative effects are resulted from the inhibition of the normal breakdown of collagen. In contrast, Peterson et al. (24) stated that a reduction in myocardial fibrosis in rats with hypertensive heart failure treated with matrix metalloproteinase inhibitors.

As we have seen, there are conflicting studies about alterations of matrix metalloproteinases and tissue inhibitors in the

heart with volume overload. All these studies are experimental that conducted in animal models and In our study, findings may be compatible with chronic volume overload because our patients generally are sustained to chronic volume load but yet not entered the decompensated phase.

**Matrix metalloproteinases and tissue inhibitors in pulmonary hypertension**

In our study, serum tissue inhibitors of matrix metalloproteinase-1 levels of patients with pulmonary hypertension secondary to congenital heart diseases, were significantly higher than controls and the patients with left to right shunt without pulmonary hypertension ($p<0.01$). Also it was observed that matrix metalloproteinase-2 and matrix metalloproteinase-9 levels were higher in patient with pulmonary hypertension associated congenital heart diseases, although it was not significant. We think that significantly elevated tissue inhibitors of matrix metalloproteinase-1 levels in cases with pulmonary hypertension either contributes to the development of remodeling seen in pulmonary hypertension or to form a protective effect against the development of pulmonary hypertension by inhibiting matrix metalloproteinases.

In experimental models with pulmonary arterial hypertension, it was searched whether matrix metalloproteinases contribute to the progression of pulmonary hypertension and migration of smooth muscle cell or not. In some studies, matrix metalloproteinases were found to contribute to the development of pulmonary arterial hypertension in animals but differences in the effects of matrix metalloproteinase inhibitors were observed between studies. Knowledge about matrix metalloproteinase/ tissue inhibitors of matrix metalloproteinase balance in humans with idiopathic pulmonary arterial hypertension is limited (25-29).

Lepetit et al. (13) studied secretion and activity of the collagenase matrix metalloproteinase-1, matrix metalloproteinase-3, matrix metalloproteinase-7, tissue inhibitors of matrix metalloproteinase-1 and tissue inhibitors of matrix metalloproteinase-2 in the lung tissues of patients with idiopathic pulmonary arterial hypertension who underwent lung transplantation. The authors found tissue inhibitors of matrix metalloproteinase imbalance in cultured pulmonary artery smooth muscles, with increased tissue inhibitors of matrix metalloproteinase-1 and decreased matrix metalloproteinase-3. An increase in total matrix metalloproteinase-2 and proportion of active matrix metalloproteinase-2 were observed and matrix metalloproteinase-2 is found to be particularly in smooth muscle cells and elastic fibers. Furthermore, a matrix metalloproteinase-3/ tissue inhibitors of matrix metalloproteinase-1 imbalance was found. MT1-matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase-2 amounts in idiopathic pulmonary arterial hypertension cells were similar with control cells. Our finding that serum tissue inhibitors of matrix metalloproteinase-1 levels of the patients with pulmonary hypertension was found 3 times higher than control group without pulmonary hypertension ($p<0.05$). Although Lepetit et al. (13) supports the result of a slight increase in matrix metalloproteinase-2 levels found in patient with pulmonary hypertension, compared to the other groups, there was not an obvious difference between them.

In idiopathic pulmonary hypertension, disruption of internal elastic lamina, disorganization of extracellular matrix and migration of smooth muscle cells are strong evidences that supporting direct role of matrix metalloproteinase-2. This enzyme not only degrades neurofibrillary collagen but also breaks down collagen (30). It has been shown that elastin degradation is an early pulmonary vascular abnormality in the patients with congenital heart disease associated with pulmonary arterial hypertension (31). Frisdal et al. (29) identified increased matrix metalloproteinase-2 expression and activity in rats with pulmonary hypertension by exposure to chronic hypoxia or monocrotaline. The authors enounced that matrix metalloproteinase expression might be modulated by cytokines, most notably interleukin-1$\alpha$, which are induced by monocrotaline and hypoxia related inflammatory processes. The authors also suggested another possible mechanism might involve physical forces. They thought that the increased pressure/stretch might affect gelatinase expression since they found a correlation between gelatinase activity and severity of pulmonary hypertension.

In a rat model, Palei et al. (32) observed that inhibition of matrix metalloproteinases with doxycycline, reduces the hemodynamic changes of acute pulmonary embolism. They found an overt increase in matrix metalloproteinase-2 and matrix metalloproteinase-9 activity of the lung in acute pulmonary embolism and then treatment with L-arginine brought relief in pulmonary hypertension and caused reduction of matrix metalloproteinase-2 and matrix metalloproteinase-9 activity. On the same way, batimastat, a specific matrix metalloproteinase inhibitor, were tested by different researchers and batimastat formed a similar beneficial effects in chronically hypoxic rats with pulmonary hypertension (33).

In the studies which were generally conducted in experimental animal models and in scattered human studies with the different etiologies of pulmonary hypertension, matrix metalloproteinase-2 activity, usually, was found to be higher (13, 29, 34). However, results related to matrix metalloproteinase-9 activity are generally conflicted (29, 31).

**Matrix metalloproteinases and tissue inhibitors in cyanotic congenital heart disease**

In this present study, patients with cyanotic congenital heart disease compared with acyanotic patients and control group and significant difference was not found between the groups ($p>0.05$). However, serum matrix metalloproteinase-2 and matrix metalloproteinase-9 levels in cyanotic group were lower whereas tissue inhibitors of matrix metalloproteinase-1 levels were higher than the controls and acyanotic group. Tissue inhibitors of matrix metalloproteinase-2 levels were lower in the acyanotic and cyanotic groups than the controls.

These findings suggest that tissue inhibitors of matrix metalloproteinase-1 increment in cyanotic congenital heart diseases is in the forefront and perhaps, matrix metalloproteinase-2 and matrix metalloproteinase-9 reduction may be due to inhibition by tissue inhibitors of matrix metalloproteinase-1. However, these findings need to be assessed by further studies. We could not find any study comparing serum matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase levels in a cyanotic and cyanotic congenital heart disease.

**Correlation of matrix metalloproteinases and tissue inhibitors levels with echocardiographic, hemodynamic and hypoxia-related parameters**

In patients with right ventricular volume overload the correlations showing that increments in left to right shunt, pulmonary blood flow and right ventricular function impairment were related with matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase level reduction were striking. However, in patients with left ventricular volume overload, matrix metalloproteinase-2 levels correlated positively with systolic and diastolic pressures in the aorta and tissue inhibitors of matrix metalloproteinase-2 levels with left ventricular diastolic pressure values. In patients with left to right shunt who developed pulmonary hypertension, tissue inhibitors of matrix metalloproteinase-1 levels showed a positive correlation with right ventricular myocardial performance index. This correlation showing that tissue inhibitors of matrix metalloproteinase-1 levels increased parallel to right ventricular dysfunction in those patients with pulmonary hypertension showing higher tissue inhibitors of matrix metalloproteinase-1 levels is seem to support each other. Matrix metalloproteinase and tissue inhibitors of matrix metalloproteinase levels and the hematological parameters reflecting hypoxia were not correlated with each other. Exceptionally, tissue inhibitors of matrix metalloproteinase-2 levels showed a negative correlation with mean corpuscular volume values.

Finally it is seen that pharmacological inhibitors of matrix metalloproteinases such as doxycycline, zinc chelators and batimastat have been used as diagnostic and therapeutic tools in cancer, autoimmune and cardiovascular diseases (32, 33). Therefore, the following question comes to mind for our study, wonder if the medications used by patients had impact on the matrix metalloproteinases levels? Although it is very difficult to answer, we think that this is mostly valid for adult studies. Unlike adult cardiac diseases and heart failure, pediatric cardiac problems generally corrected or improved in clinical status by surgical or transcatheter intervention. When our study groups were analyzed, it would be seen that all the recruited patients had cardiac defects that should require a prompt intervention. Therefore long-term drug therapy is not suitable for these patients. In our patient groups, mainly, angiotensin converting enzyme inhibitors (ie. enalapril), diuretics and digoxin were used. Theoretically, some antihypertensive drugs may alter the levels of matrix metalloproteinases but it was found that enalapril has no effect on matrix metalloproteinase levels (35). Moreover, it has been reported that spironolactone treatment for 24 weeks found partially reverse the dysregulation in collagen metabolism (36).

Also, it should be noted that best procedure of blood sampling to optimize the analysis of both matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases is a matter of strong controversy (37). For studying the function and role of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in different conditions, some authors used plasma and the others used serum samples, not considering the impact of blood collection influencing both quantitative and qualitative level (37-40). Gerlach et al. (40) declared positive correlations between serum and plasma matrix metalloproteinase levels but Jung et al. (41) found some methodological weakness in Gerlach's study. However, at a recent study (42), the authors have emphasized that serum levels of matrix metalloproteinase-8, not plasma levels, were significantly associated with patients' clinical outcome.

## Study limitations

Lack of prior research studies on the topic and relatively small number of patients included are challenging to interpret our results. Additional large and prospective clinical studies are required to demonstrate the importance of serum matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases levels in clinical practice and their predictive role as biomarkers of congenital heart diseases. The majority of our findings was not significant. Therefore, our results need to be supported by more comprehensive studies.

## Conclusion

We demonstrated that serum tissue inhibitors of matrix metalloproteinase-1 levels of patients with pulmonary hypertension secondary to congenital heart diseases were significantly higher than those of the patients with left to right shunt without pulmonary hypertension and controls. Also serum matrix metalloproteinases and their tissue inhibitors levels in patients with cyanotic congenital heart diseases and patients with right or left ventricular volume overload were found to be altered when compared with controls although the data was not significant. Our data may suggest the possible role of matrix metalloproteinases and their tissue inhibitors on myocardial remodeling in congenital heart defects.

**Acknowledgments:** The study was funded by Eskişehir Osmangazi University Scientific Research Projects Commission (contract 20061108).

**Ethics statement:** Written informed consent was obtained from all participating parents of patients, as required by the institutional review board under an approved protocol (Ethic Committee of Eskişehir Osmangazi University, Faculty of Medicine, Number 2007/124).

**Patients and controls:** From March 2007- June 2009, 87 patients (46 males and 41 females) evaluated at Eskişehir Osmangazi University Medical Faculty, Department of Pediatric Cardiology with the diagnosis of congenital heart disease were enrolled in the study.

**Conflict of interest:** None declared.

**Peer-review:** Externally peer-reviewed.

**Authorship contributions:** Concept - Z.K., B.U.; Design - Z.K., B.U., Ö.Ç.; Supervision - T.E.; Material - Z.K., B.U., G.Ö.; Data collection &/or processing - Z.K., B.U., Ö.Ç.; Analysis &/or interpretation - Z.K., B.U., C.B.; Literature search - B.U., G.Ö.; Writing - Z.K., B.U., G.Ö.; Critical review - T.E.

## References

1. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ Res 2003; 92: 827-39. [CrossRef]
2. Zitka O, Kukacka J, Krizkova S, Huska D, Adam V, Masarik M, et al. Matrix metalloproteinases. Current Medicinal Chemistry 2010; 17: 3751-68. [CrossRef]
3. Kuzuya M, Iguchi A. Role of matrix metalloproteinases in vascular remodelling. J Atheroscler Thromb 2003; 10: 275-82. [CrossRef]
4. Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. Eur J Cell Biol 1997; 74: 111-22.
5. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000; 1477: 267-83. [CrossRef]
6. Creemers EJM, Cleutjens JP, Smits JF, Daemen MJ. Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? Circ Res 2001; 89: 201-10. [CrossRef]
7. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002; 90: 251-62.
8. Spinale FG, Janicki JS, Zile MR. Membrane-associated matrix proteolysis and heart failure. Circ Res 2013; 112: 195-208. [CrossRef]
9. Marchesi C, Dentali F, Nicolini E, Maresca AM, Tayebjee MH, Franz M, et al. Plasma levels of matrix metalloproteinases and their inhibitors in hypertension: a systematic review and meta-analysis. J Hypertens 2012; 30: 3-16. [CrossRef]
10. Mohamed SA, Noack F, Schoellermann K, Karluss A, Radtke A, Schult-Badusche D, et al. Elevation of matrix metalloproteinases in different areas of ascending aortic aneurysms in patients with bicuspid and tricuspid aortic valves. ScientificWorldJournal 2012; 2012: 806261. [CrossRef]
11. Siefert SA, Sarkar R. Matrix metalloproteinases in vascular physiology and disease. Vascular 2012; 20: 210-6. [CrossRef]
12. Noji Y, Shimizu M, Ino H, Higashigata T, Yamaquchi M, Nohara A, et al. Increased matrix metalloproteinase-2 in patients with hypertrophic cardiomyopathy with systolic dysfunction. Circ J 2004; 68: 355-60. [CrossRef]
13. Lepetit H, Eddahibi S, Fadel E, Frisdal E, Munaut C, Noel A, et al. Smooth muscle cell matrix metalloproteinases in idiopatic pulmonary arterial hypertension. Eur Respir J 2005; 25: 834-42. [CrossRef]
14. Yamazaki T, Lee JD, Shimizu H, Uzui H, Ueda T. Circulating matrix metalloproteinase-2 is elevated in patients with congestive heart failure. Eur J Heart Fail 2004; 6: 41-5. [CrossRef]
15. Tei C, Ling L, Hodge D, Bailey KR, Oh JK, Rodeheffer RJ, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function-a study in normals and dilated cardiomyopathy. J Cardiol 1995; 26: 357-66.
16. Wilson EM, Spinale FG. Myocardial remodelling and matrix metalloproteinases in heart failure: turmoil within the interstitium. Ann Med 2001; 33: 623-34. [CrossRef]
17. Brower GL, Janicki JS. Contribution of ventricular remodelling to pathogenesis of heart failure in rats. Am J Physiol 2001; 280: 674-83.
18. Spinale FG, Coker ML, Thomas CV, Walker JD, Mukherjee R, Hebbar L. Time-dependent changes in matrix metalloproteinase activity and expression during the progression of congestive heart failure: relation to ventricular and myocyte function. Circ Res 1998; 82: 482-95. [CrossRef]
19. Janicki JS, Brower GL, Henegar JR, Wang L. Ventricular remodeling in heart failure: the role of myocardial collagen. Adv Exp Med Biol 1995; 382: 239-45. [CrossRef]
20. Brower GL, Henegar JR, Janicki JS. Temporal evaluation of the left ventricular remodeling and function in rats with chronic volume overload. Am J Physiol 1996; 271: 2071-8.
21. Nagatomo Y, Carabello BA, Coker ML, McDermott PJ, Nemoto S, Hamawaki M, et al. Differential effects of pressure or volume overload on myocardial MMP levels and inhibitory control. Am J Physiol 2000; 278: 151-61.
22. Spinale FG, Coker ML, Krombach SR, Mukherjee R, Hallak H, Houck WV, et al. Matrix metalloproteinase inhibition during the development of congestive heart failure: effects on left ventricular dimensions and function. Circ Res 1999; 85: 364-76. [CrossRef]
23. McElmurray JH 3rd, Mukherjee R, New RB, Sampson AC, King MK, Hendrick JW, et al. Angiotensin-converting enzyme and matrix metalloproteinase inhibition with developing heart failure: comparative effects on left ventricular function and geometry. J Pharmacol Exp Ther 1999; 291: 799-11.
24. Peterson JT, Hallak H, Johnson L, Li H, O'Brien PM, Sliskovic DR, et al. Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. Circulation 2001; 103: 2303-9. [CrossRef]
25. Tan X, Chai J, Bi SC. Involvement of matrix metalloproteinase-2 in medial hypertrophy of pulmonary arterioles in broiler chickens with pulmonary arterial hypertension. Vet J 2012; 193: 420-5. [CrossRef]
26. Vieillard-Baron A, Frisdal E, Eddahibi S, Deprez I, Baker AH, Newby AC, et al. Inhibition of matrix metalloprotienases by lung TIMP-1 gene transfer or doxycycline aggrevates pulmonary hypertension in rats. Circ Res 2000; 87: 418-25. [CrossRef]

27. Cowan KN, Jones PL, Rabinovitch M. Elastase and matrix metalloproteinase inhibitors induce regression, and tenascin-C antisense prevents progression, of vascular disease. J Clin Invest 2000; 105: 21-34. [CrossRef]
28. Chelladurai P, Seeger W, Pullamsetti SS. Matrix metalloproteinases and their inhibitors in pulmonary hypertension. Eur Respir J 2012; 40: 766-82. [CrossRef]
29. Frisdal E, Gest V, Vieillard-Baron A, Levame M, Lepetit H, Eddahibi S, et al. Gelatinase expression in pulmonary arteries during experimental pulmonary hypertension. Eur Respir J 2001; 18: 838-45. [CrossRef]
30. Overall CM, Lopez-Otin C. Strategies for MMP inhibition in cancer: innovations for the post-trial era. Nat Rev Cancer 2002; 2: 657-72. [CrossRef]
31. Rabinovitch M, Bothwel TH, Hayakawa BN, Williams WG, Trusler GA, Rowe RD, et al. Pulmonary artery endothelial abnormalities in patients with congenital heart defects and pulmonary hypertension. A correlation of light with scanning electron microscopy and transmission electron microscopy. Lab Invest 1986; 55: 632-53.
32. Palei AC, Zaneti RA, Fortuna GM, Gerlach RF, Tanus-Santos JE. Hemodynamic benefits of matrix metalloproteinase-9 inhibition by doxycycline during experimental acute pulmonary embolism. Angiology 2005; 56: 611-7. [CrossRef]
33. Herget J, Novotna J, Bibova J, Povysilova V, Vankova M, Hampl V. Metalloproteinase inhibition by Batimastat attenuates pulmonary hypertension in chronically hypoxic rats. Am J Physiol Lung Cell Mol Physiol 2003; 285: 199-208.
34. Souza-Costa DC, Zerbini T, Palei AC, Gerlach RF, Tanus-Santos JE. L-arginine attenuates acute pulmonary embolism-induced increases in lung matrix metalloproteinase-2 and matrix metalloproteinase-9. Chest 2005; 128: 3705-10. [CrossRef]
35. Fontana V, Silva PS, Izidoro-Toledo TC, Biagi C, Oliveira EB, Gerlach RF, et al. Comprehensive evaluation of the effects of enalapril on matrix metalloproteinases levels in hypertension. Cardiovasc Drugs Ther 2012; 26: 511-9. [CrossRef]
36. Li MJ, Huang CX, Okello E, Yanhong T, Mohamed S. Treatment with spironolactone for 24 weeks decreases the level of matrix metalloproteinases and improves cardiac function in patients with chronic heart failure of ischemic etiology. Can J Cardiol 2009; 25: 523-6. [CrossRef]
37. Mannello F, Tonti GA, Canestrari F. The 'never-ending story' of the influence of blood specimen collection methods affecting the concentration, the zymographic profile and the usefulness of matrix metalloproteinases and their tissue inhibitors in multiple sclerosis diagnosis/prognosis: a landmark for limiting the misuse of serum samples. Mult Scler 2007; 13: 687-90. [CrossRef]
38. Lomholt AF, Frederiksen CB, Christensen IJ, Brunner N, Nielsen HJ. Plasma tissue inhibitor of metalloproteinase-1 as a biologic marker? Pre-analytical considerations. Clin Chim Acta 2007; 380: 128-32. [CrossRef]
39. Jung K. Impact of blood sampling on circulating tissue inhibitors of metalloproteinases. Clin Cancer Res 2006; 12: 2648-9. [CrossRef]
40. Gerlach RF, Meschiari CA, Marcaccini AM, Palei AC, Sandrim VC, Cavalli RC, et al. Positive correlations between serum and plasma matrix metalloproteinase (MMP)-2 or MMP-9 levels in disease conditions. Clin Chem Lab Med 2009; 47: 888-91. [CrossRef]
41. Jung K, Wu CY. Methodological weakness in using correlation coefficients for assessing the interchangeability of analyte data between samples collected under different sampling conditions-the example of matrix metalloproteinase 9 determined in serum and plasma samples. Clin Chem Lab Med 2010; 48: 733-6. [CrossRef]
42. Fertin M, Lemesle G, Turkieh A, Beseme O, Chwastyniak M, Amouyel P, et al. Serum MMP-8: a novel indicator of left ventricular remodeling and cardiac outcome in patients after acute myocardial infarction. PLoS One 2013; 8: e71280. [CrossRef]