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Contribution of Plasma Matrix Metalloproteinases to Development of Left Ventricular Hypertrophy and Diastolic Dysfunction in Hypertensive Subjects

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Saglam, M., Karakaya, O., Esen, A.M., Barutcu, I., Dogan, S., Karavelioglu, Y., Karapinar, H., Akgun, T., Esen, O., Ozdemir, N., Turkmen, S. and Kaymaz, C. *Contribution of Plasma Matrix Metalloproteinases to Development of Left Ventricular Hypertrophy and Diastolic Dysfunction in Hypertensive Subjects*. Tohoku J. Exp. Med., 2006, **208** (2), 117-122 —— Matrix metalloproteinases (MMPs) are involved in the regulation of the extracellular matrix (ECM) of the myocardium and thus the pathogenesis of vascular and cardiac hypertrophy. In this study, we investigated contribution of plasma matrix metalloproteinases to development of left ventricular hypertrophy (LVH) and diastolic dysfunction in hypertensive subjects. Hypertensive patients with (n = 27) and without LVH (n = 23) were included. All participants underwent a complete transthoracic echocardiographic examination, including recordings of the mitral annular early, late, systolic and diastolic velocities by Doppler imaging. Plasma concentrations of MMP-3 and MMP-9 were determined by the one-step sandwich enzyme immunoassay method. Plasma MMP-3 and MMP-9 concentrations were significantly higher in patients with LVH than those without LVH (2.4 ± 1.2 vs 1.5 ± 0.7 ng/ml, p = 0.006 and 5.2 ± 2.8 vs 3.3 ± 1.7 ng/ml, p = 0.003, respectively). MMP-3 and MMP-9 levels were also correlated with left ventricular posterior wall thickness and Doppler indices of diastolic dysfunction. Our findings have suggested that increased MMP levels may contribute to LVH and left ventricular diastolic dysfunction. Therefore, treatment of hypertension with MMP lowering drugs, such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers, may have favorable effects on LVH and left ventricular diastolic dysfunction. ———— matrix metalloproteinases; left ventricular hypertrophy; diastolic dysfunction

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Arterial hypertension is associated with cardiovascular remodeling process, such as myocardial fibrosis, hypertrophy and diastolic dysfunction. Changes in the structure of the cardiac extracellular matrix (ECM) can impede left ventricular filling during diastole, and possibly contribute to symptomatic diastolic dysfunction. Indeed, it has been reported that circulating matrix metalloprotienase-9 level and tissue inhibitor matrix metalloprotienase-1 levels are higher in hypertensive subject than controls, and tissue inhibitor matrix metalloprotienase-1 levels are associated with left ventricular hypertrophy (LVH) and left ventricular diastolic dysfunction (LVDD) in some but not all studies (Laviades et al. 1998; Li-Saw-Hee et al. 2000; Lindsay et al. 2002; Timms et al. 2002; Tayabjee et al. 2004).

The matrix metalloproteinases (MMPs) are a family of molecules that are associated with the breakdown of collagens and other constituents of the extracellular matrix (ECM), such as gelatin, elastin and fibronectin (Yang and Liu 1998; Sasaki et al. 2004). Both MMPs and their inhibitors are involved in the regulation of the ECM of a variety of tissues, including the arterial wall and myocardium and it is therefore likely that they are involved in the pathogenesis of vascular and cardiac hypertrophy (Dollery et al. 1995; Weber 1995; Nakamura et al. 2000; Kadoglou et al. 2005). Giving the possible link between MMPs and LVH and LVDD we wanted to test whether LVH coexisting with LVDD leads to further MMPs release. Therefore, in the present study we attempted to measure plasma MMP-3 and MMP-9 levels and to investigate contribution of plasma MMPs to development of LVH and LVDD in hypertensive subjects.

Methods

The study population included 50 patients with essential hypertension. The patients were grouped into those with LVH (n = 27) {(left ventricular posterior wall thickness in diastole [LVPWd] ≧ 12 mm)} and those without LVH (n = 23) (LVPWd < 12 mm). Hypertension was confirmed by elevated supine blood pressure of > 140/90 mmHg on at least three separate clinical visits. Patients with any of the following conditions were excluded: secondary causes of hypertension, clinical history or biochemical evidence of renal disease, chronic liver disease or excessive alcohol use, lung or connective tissue disease, previous myocardial infarction, documented coronary artery disease, cardiomyopathy and congestive heart failure. The study protocol was approved by ethic committee of Kosuyolu Heart Education and Research Hospital and all participants gave their informed consent.

All participants underwent a complete transthoracic echocardiographic examination (two-dimensional, pulse wave Doppler recording of the transmitral, and recordings of the mitral annular velocity by tissue Doppler imaging, respectively) with 3.5-MHz sector transducer (Vivid System Five, GE Vingmed, Horten, Norway). Two-dimensional and Doppler echocardiographic studies were performed at the left lateral decubitus position with the conventional views (parasternal long and short axis, apical four chamber). An electrocardiogram was recorded simultaneously with the M-mode and Doppler tracings in the same monitor. LV septal thickness in diastole, LVPWd, LV end-diastolic and end-systolic dimensions, and left atrial dimensions were measured in the parasternal long-axis view. Mitral inflow velocity was recorded from the apical four-chamber view by pulsed-wave Doppler sample during diastole. Peak early (E) and late (A) mitral inflow velocity, and E/A ratio were obtained. Tissue Doppler imaging was also applied in the pulsed-Doppler mode of the mitral annulus velocity at its lateral corners with the same echocardiographic unit. Systolic (S), early diastolic (é), and late diastolic (á) velocity and é/á ratio were measured. All echocardiographic parameters were averaged over three cardiac cycles and measurements were performed by one of two observers blinded to the patient clinical details. Both inter-and intra observer variation in echocardiographic measurement was < 5%.

Measurement of plasma MMPs concentrations

Blood samples were taken from peripheral veins after overnight fast and collected in ice-cold vacuum glass tubes containing ethylene-diamino-tetraacetic acid (EDTA). Next, the plasma was separated by centrifugation at 1,000 G for 10 min at 4°C. These samples were immediately frozen and stored at –80°C. Plasma concentration of MMP-3 was determined by one-step sandwich enzyme immunoassay (EIA) method using commercially available kits with monoclonal antibodies against the substance (Biosource International

Immunoassay Kits, Camarillo, CA, USA) according to the instructions provided by the manufacturer to determine plasma MMP-levels. The EIA system for MMP-3 is capable of measuring both pro-MMP-3 and active forms of MMP-3 as well as the forms of MMP-3 complexed with tissue inhibitor of metalloproteinases (TIMPs), but MMP-3 existed only as the precursor from in human blood. The EIA system for MMP-9 detects free pro-MMP-9, intermediate 83 kDA MMP, and MMP-9 in complexes with TIMP-1.

MMP-3 and MMP-9 have different proteolytic activity. MMP-9 belongs to gelatinases group and readily digests the denatured collagens, gelatins. However, MMP-3 is a member of stromelysins. Among the stromelysins group, both MMP-3 and MMP-10 have similar substrate specificity, but MMP-3 has a higher proteolytic efficiency than that of MMP-10 (Visse and Nagase 2003). In hypertensive subjects, plasma MMPs and their TIMPs were increased and associated with LVH and LVDD (Lindsay et al. 2002; Timms et al. 2002; Tayabjee et al. 2004). Therefore, we selected two different members of MMPs family to investigate their role in hypertensive LVH and LVDD. The mean intra-assay coefficient of variation and mean inter-assay coefficient of variation for both the assays were < 5% and < 10%, respectively. The lower limit of detection for MMP-3 and MMP-9 were 0.1 ng/ml and 0.3 ng/ml, respectively.

Statistical analysis

Statistical analysis was performed with SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± S.D. For continuous variables student's *t*-test and for categorical changes chi-square test was used. To test distribution of normality of values Levene's test was used and for non-parametrically distributed values nonparametric tests was used where appropriate. Correlation analyses were performed by Sperman correlation method and linear regression analysis where appropriate. A p value < 0.05 was considered to indicate statistical significance.

Results

On echocardiographic examination no significant valvular disorders and wall motion abnormality were detected among the study subjects. In patients with LVH, 10 patients were taking antihypertensive treatment, including calcium channel blockers (5 patients), beta-blocker (3 patients), and diuretics (2 patients). In patients without LVH, 11 patients were taking antihypertensive treatment, including calcium channel blockers (6 patients), beta-blocker (2 patients), and diuretics (3 patients). There was no difference between the two groups with regard to history of antihypertensive use. Also, there was no significant difference between patients with and without LVH in demographics of age, sex, heart rate, and blood pressure; however, plasma MMP-3 and MMP-9 concentrations were significantly higher in patients with LVH than those without LVH (Table 1). On correlation analysis, no correlation was detected between the MMP-3 and MMP-9 levels and LVPWd, or Doppler indices of diastolic dysfunction. However, when only the patients with LVH were included, MMP-3 and MMP-9 levels were correlated with LVPWd (Figs. 1 and 2), and conventional and tissue Doppler indices of diastolic dysfunction, including E velocity, E/A ratio, é, á velocity and é/á ratio, respectively (Table 2).

Discussion

In the present study we have shown that plasma MMP 3 and MMP-9 levels are higher in hypertensive patients with LVH when compared to those without LVH. In addition, MMP 3 and MMP-9 levels are positively correlated with LVPWd and correlated with tissue Doppler indices of LVDD.

Previously, the relationship between MMPs and their inhibitors and LV function has been extensively studied, although conflicting results have been reported. Sundstrom et al. (2004) reported that plasma TIMP-1 level was positively correlated with LV mass, wall thickness, relative wall thickness, end-systolic diameter, and left atrial diameter and the risk of having increased LV end-diastolic diameter or increased wall thickness, and negatively correlated with fractional shortening. Soejima et al. (2003) showed a significant negative correlation between the concentration of serum MMP-1 and LV ejection fraction. Likewise, Noji et al. (2004) showed that MMP-2 and TIMP-2 were negatively correlated with fractional shortening and positively with left ventricular diameters in patients with hypertrophic cardio-

TABLE 1. *Baseline demographic and echocardiographic parameters in patients with and without left ventricular hypertrophy*

Variable	LVH (+) patients	LVH (-) patients	*p* values
Age (years)	63 ± 9	62 ± 10	0.9
Gender (male/female)	18/9	16/7	0.77
Body mass index (kg/m^2)	27 ± 6	28 ± 5	0.79
Number of smokers	4	5	0.6
History of diabetes	5	4	0.4
History of hyperlipidemia	4	5	0.7
Calcium channel blocker use	5	6	0.4
Beta blocker use	3	2	0.5
Diuretic use	2	3	0.6
Systolic blood pressure (mmHg)	150 ± 18	149 ± 14	0.65
Diastolic blood pressure (mmHg)	91 ± 12	90 ± 10	0.7
Mean heart rate beat/min	80 ± 15	83 ± 16	0.5
LVDd (cm)	5.1 ± 0.5	5.0 ± 0.4	0.4
LVDs (cm)	3.1 ± 0.6	3.0 ± 0.4	0.66
EF %	65 ± 4	63 ± 5	0.1
LVPWDd (cm)	1.3 ± 0.1	1.0 ± 0.1	< 0.0001
IVSd (cm)	1.3 ± 0.2	1.1 ± 0.1	< 0.0001
LA (cm)	3.9 ± 0.7	3.8 ± 0.5	0.08
E peak (cm/s)	58 ± 14	67 ± 12	0.09
A peak (cm/s)	90 ± 19	84 ± 15	0.18
E/A ratio	0.6 ± 0.2	0.8 ± 0.2	0.009
S (cm/s)	7.5 ± 1.7	8.6 ± 2.3	0.19
é (cm/s)	6.6 ± 1.4	7.2 ± 1.4	0.19
á (cm/s)	11.0 ± 1.6	10.2 ± 2.4	0.18
é/á ratio	0.61 ± 0.18	0.72 ± 0.15	0.03
MMP-3 (ng/ml)	2.4 ± 1.2	1.5 ± 0.7	0.006
MMP-9 (ng/ml)	5.2 ± 2.8	3.3 ± 1.7	0.003

LVH, left ventricular hypertrophy; LVDd, left ventricle diastolic diameter; EF, ejection fraction, LVPWDd, left ventricular posterior wall thickness in distole; IVSd, interventricular septum systolic diameter; LA, left atrium; E, early; A, late; S, systolic velocity; é, early diastolic; á, late diastolic.

myopathy. In contrast, Li Saw-Hee et al. (2000) observed no relationship between LV mass, Doppler indices of diastolic function, blood pressure, left ventricular mass index and either MMP-9 or TIMP-1 levels. On the other hand, Lindsay et al. (2002) observed that TIMP-1 was correlated with markers of LV diastolic filling, indicating essential hypertension is characterized by an increase in collagen synthesis, degradation, and inhibition of degradation. Those authors concluded that TIMP-1 could be a potential noninvasive marker of fibrosis. Timms et al. (2002) showed a correlation between TIMP-1 and LV mass index and left ventricular posterior wall diameter, which is consistent in part with our results. Tayebjee et al. (2004) found that only TIMP-1 but not MMP-9 levels were correlated with LV mass, LV mass index, and tissue Doppler parameters of diastolic dysfunction, including é, á, and E/é. These results were in part conflicting

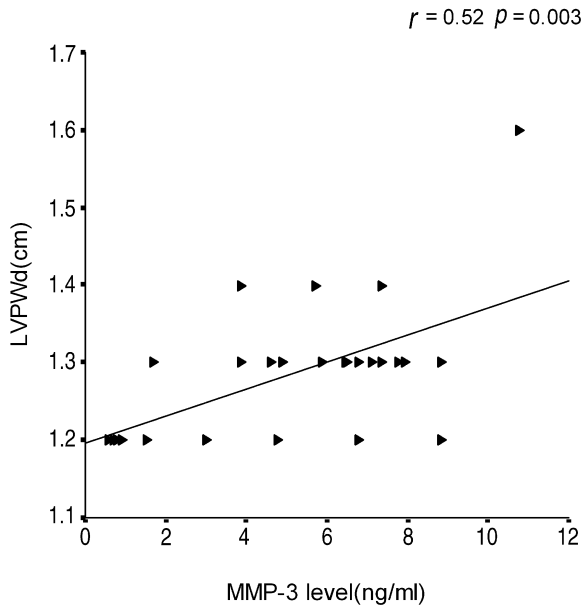

Fig. 1. Correlation of left ventricular posterior wall thickness in diastole (LVPWd) and plasma MMP-3 concentration.

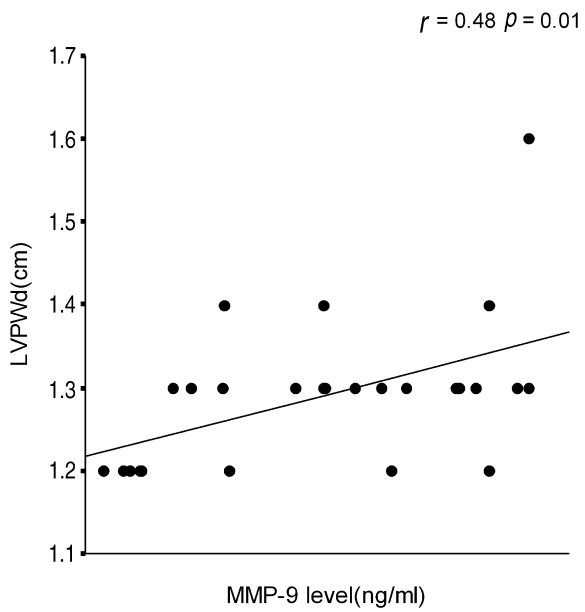

Fig. 2. Correlation of left ventricular posterior wall thickness in diastole (LVPWd) and plasma MMP-9 concentration.

with our study, because we found a correlation between MMP levels and Doppler indices of diastolic dysfunction. However, this discrepancy might have resulted from the selected patient population, since we found a correlation between MMP levels and Doppler indices of diastolic dysfunction only in those with LVH but not in all hypertensive population.

TABLE 2. *Correlation between echocardiographic parameters and MMP-3 and MMP-9 levels*

	MMP-3	MMP-9
E	$r = -0.72$	$r = -0.59$
	$p = 0.001$	$p = 0.002$
A	$r = -0.24$	$r = -0.23$
	$p = 0.3$	$p = 0.1$
E/A	$r = -0.75$	$r = -0.70$
	$p = 0.0001$	$p = 0.0003$
é	$r = -0.51$	$r = -0.44$
	$p = 0.003$	$p = 0.002$
á	$r = 0.50$	$r = 0.54$
	$p = 0.004$	$p = 0.002$
é/á	$r = -0.78$	$r = -0.69$
	$p = 0.0001$	$p = 0.0001$

The composition of ECM turnover plays a predictably important role in the vascular changes in hypertension in both the heart and vascular tree. The ECM is vital for maintaining tissue integrity and consistency over and above any changes associated with hypertrophy and hyperplasia of cardiac or smooth muscle. LVH is an established risk factor for all the squeal of coronary artery disease with a three-to five fold increase in cardiovascular mortality. The pathologic basis for LVH is a combination of myocyte hypertrophy and increased collagen deposition within the myocardium. Theoretically, elevated collagen levels may be due to an increase in collagen synthesis or a decrease in collagen degradation, or both (Lavaides et al. 1994). Certainly, alterations in the composition of collagen or the ratio of collagen composition can have a major impact on left ventricular dynamics. Changes in the composition of cardiac ECM are likely to play a major role in response to changing cardiac contractile function in both systole and diastole. Accordingly, the relationship between MMP-3 and MMP-9 levels and Doppler indices of diastolic dysfunction may suggest the role of circulating

MMPs level in chronic changes in LV mechanics in hypertensive heart disease. Progressive activation of MMPs might be expected to lead to progressive degradation of ECM, which then lead to mural realignment of myocyte bundles and/or individual myocyte within LV wall, and thus may account for LVH and LVDD.

Study limitations

There are several potential limitations in the current study that may influence the observed results. Sample size was small and the biochemical analysis of the study was limited. Obviously, the fact that the other family members of MMPs were not studied is the main limitation. In addition, due to strict inclusion criteria and also to minimize effects of confounding factors on results we included a limited number of subjects. On the other hand, lack of Doppler examination of mitral inflow during Valsalva maneuver (delta E/A) and of pulmonary venous inflow parameters for measurements of left ventricular diastolic dysfunction is the other limitation of the study. Certainly, our overall findings should be confirmed by large scale studies.

In conclusion, we have noted that MMP levels are correlated with LV posterior wall thickness and Doppler indices of diastolic dysfunction. These findings suggest that MMPs may also have a role in development of hypertrophy and diastolic dysfunction through definition of the ventricular matrix composition in hypertensive subjects. Therefore, treatment of hypertension with MMP lowering drugs, such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers, may have favorable effects on LVH and LVDD.

References

Dollery, C.M., McEwan, J.R. & Henney, A.M. (1995) Matrix metalloproteinases and cardiovascular disease. *Circ. Res.*, **77**, 863-868.

Kadoglou, N.P., Daskalopoulou, S.S., Perrea, D. & Liapis, C.D. (2005) Matrix metaloproteinases and diabetic vascular complications. *Angiology,* **56**, 173-189.

Lavaides, C., Mayor, G. & Diez, J. (1994) Treatment with lisinopril normalizes serum concentrations of PIIIP in patients with essential hypertension. *Am. J. Hypertens.*, **7**, 52-58.

Laviades, C., Varo, N., Fernandez, J., Mayor, G., Gil, M.J., Monreal, I. & Diez, J. (1998) Abnormalities of the extracellular degradation of collagen type I in essential hypertension. *Circulation,* **98**, 535-540.

Lindsay, M., Maxwell, P. & Dunn, F. (2002) TIMP-1, a marker of left ventricular diastolic dysfunction and fibrosis in hypertension. *Hypertension,* **40**, 136-141.

Li-Saw-Hee, F.L., Edmunds, E., Blann, A.D., Beevers, D.G. & Lip, G.Y. (2000) Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 levels in essential hypertension. Relationship to left ventricular mass and antihypertensive therapy. *Int. J. Cardiol.,* **75**, 43-47.

Nakamura, M., Tachieda, R., Niinuma, H., Ohira, A., Endoh, S., Hiramori, K. & Mikata, S. (2000) Circulating biochemical marker levels of collagen metabolism are abnormal in patients with abdominal aortic aneurysm. *Angiology*, **51**, 385-392.

Noji, Y., Shimizu, M., Ino, H., Higashikata, T., Yamaguchi, M., Nohara, A., Horita, T., Shimizu, K., Ito, Y., Matsuda, T., Namura, M. & Mabuchi, H. (2004) Increased circulating metrix metalloproteinase-2 in patients with hypertrophic cardiomyopathy with systolic dysfunction. *Circ. J.*, **68**, 355-360.

Soejima, H., Owaga, H., Sakamoto, T., Miyamoto, S., Kajiwara, I., Kojima, S., Hokamaki, J., Sugiyama, S., Yoshimura, M., Suefuji, H., Miyao, Y., Fujimoto, K., Miyagi, H. & Kishiwaka, H. (2003) Increased serum matrix metalloproteinase-1 concentration predicts advanced left ventricular remodeling in patients with acute myocardial infarction. *Circ J.*, **67**, 301-304.

Sundstrom, J., Evans, J.C., Benjamin, E.J., Levy, D., Larson, M.G., Sawyer, D.B., Siwik, D.A., Colucci, W.S., Wilson, P.W. & Vasan, R.S. (2004) Relations of plasma total TIMP-1 levels to cardiovascular risk factors and echocardiographic measures: the Framingham heart study. *Eur. Heart J.*, **25**, 1509-1516.

Tayabjee, M.H., Nadar, S.K., MacFayden, R.J. & Lip, G.Y. (2004) Tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9 levels in patients with hypertension. Relationship to tissue Doppler indices of diastolic relaxation. *Am. J. Hypertens.*, **17**, 770-774.

Timms, P.M., Wright, A., Maxwell, P., Campbell S, Dawnay, A.B. & Srikanthan, V. (2002) Plasma tissue inhibitor of metalloproteinase-1 levels are elevated in essential hypertension and related to left ventricular hypertrophy. *Am. J. Hypertens.*, **15**, 269-272.

Sasaki, A., Sasaki, K., Konttinen, Y.T., Santavirta, S., Takahara, M., Takei, H., Ogino, T. & Takagi, M. (2004) Hyaluronate inhibits the interleukin-1 β-induced expression of matrix metalloproteinase (MMP)-1 and MMP-3 in human synovial cells. *Tohoku J. Exp. Med.,* **204**, 99-107.

Visse, R. & Nagase, H. (2003) Matrix metalloprotienses and tissue inhibitors of metalloproteinases. Structure, function and biochemistry. *Circ. Res.*, **92**, 827-839.

Weber, K.T., Sun, Y., Guarda, E., Katwa, L.C., Ratajska, A., Cleutjens, J.P. & Zhou, G. (1995) Myocardial fibrosis in hypertensive heart disease: an overview of potential regulatory mechanisms. *Eur. Heart J.*, **16**, 24-28.

Yang, R.-S. & Liu, T.-K. (1998) Immunohistochemical analysis of matrix proteolytic enzymes in the periprosthetic tissue in the patients with loosening prostheses. *Tohoku J. Exp. Med.,* **184**, 99-111.