The Effects of Quinapril on Plasma Level of Tissue Inhibitor Metalloproteinase 1, Left Ventricular Hypertrophy and Diastolic Functions in Hypertensive Patients

Hypertensive Patients Kliniklerinin Metalloproteinaz 1 Doku İnihilitörünün Plazma Düzeyi, Sol Ventrikül Hipertrofisi ve Diyalistik Fonksiyonlara Etkileri

ABSTRACT Objective: Plasma level of tissue inhibitor metalloproteinase type-1 (TIMP-1) reflecting extent of extracellular fibrosis was found to be associated with left ventricular hypertrophy (LVH) and left ventricular diastolic dysfunction (LVDd). We aimed to investigate the effect of quinapril therapy on TIMP-1 levels, LVH and LVDd in hypertensive patients. Material and Methods: A total of 30 hypertensive patients with LVH (14 male, mean age 64.3 years) were enrolled to this study. The initial daily dose of quinapril was 20 mg/day. The dose was uptitrated to 40 mg/d and hydrochlorothiazide was added if necessary. LV mass index (LVMI) was calculated and left ventricular diastolic parameters were evaluated by transthoracic echocardiography. Transmitial and tissue Doppler pulse wave velocities were measured and, E/A and Em/Am ratios were calculated. Systolic and diastolic blood pressures, LVMI, LV diastolic functions and plasma TIMP-1 levels were evaluated at baseline and six months after the therapy. Results: After quinapril therapy TIMP-1 levels were also significantly reduced after therapy (619.9 ± 108.1-485.6 ± 105.9 ng/dl; p< 0.001). There were no significant correlations between the TIMP-1 levels, LVH and LVDD in hypertensive patients. Conclusions: Quinapril therapy reduced TIMP-1 levels, independently from regression of LVH and reduction of blood pressures in hypertensive patients

Key Words: Hypertension; tissue inhibitor of metalloproteinase-1; echocardiography, doppler; hypertrophy, left ventricular; angiotensin-converting enzyme Inhibitors

ÖZET Amaç: Hücre dışı matrisde fibrozis gelişimini yansıtan Metalloproteinaz 1 doku inhibitörü (TIMP-1) ve sol ventrikül hipertrofisi (SVH) ve sol ventrikül diyastolik disfonsiyonu (SVDD) ile ilişkilidir. Bu çalışmada, hipertansif hastalarda, bir ACE inhibitörü olan Kinapril’in TIMP-1 düzeyi, SVH ve SVDD üzerine olan etkilerini araştırmayı amaçladık. Gerçekleştirmenin hedefi, HH’lari olan 30 hipertansif hasta (14 erkek, ortalama yaş: 64.3 ± 8.5 yıl) çalışmayla dahil edildi. Kinapril tedavisi tüm hastalara 20 mg/gün dozunda başlandı, kan basıncı kontrol altına alınmaya kadar doz CO verilerek 40 mg/gün’a artırıldı ve gerekirse hidroklorotiazider ile tedavi ekledi. Transtorasik ekokardiografi ile sol ventriküllü kitle indesi (SVKI) hesaplandı, sol ventrikül diyalistik fonksiyonları (SVDF) değerlendirildi. Transmitial ve doku Doppler PW hızları ile ölçüldü, E/A ve Em/Am oranları hesaplandı. Sistolik ve diyalistik kan basınlar TIMP-1 düzeyleri SVKI, SVDF, tedavi öncesi ve altı aylık tedavi sonrası değişenlerdir. Bulgular: Tedavi sonrasında ortalama sistolik kan basını (p< 0.001), diyalistik kan basını (p< 0.001) ve SVKI (p< 0.001) ve transmitial deselarasyon zamanı (p< 0.017) anlamlı olarak azaldı. Septal duvar Em/Am oranında anlamlı artış saptandı. Plazma TIMP-1 düzeyi tedavi sonrası, tedavi öncesine göre anlamlı olarak azaldı (619.9 ± 108.1-485.6 ± 105.9 ng/dl; p< 0.001). TIMP-1 düzeyindeki azalma, sistolik kan basını (r= 0.12 p= 0.51) diyalistik kan basını (r= 0.10 p= 0.58), Δ deselarasyon zamanı (p= 0.98), ve E/A ve Em/Am (p= 0.76) oranındaki değişimlerle ilişkili bulunmadı. Sonuç: Hipertansif hastalarda Kinapril tedavi ile kan basını ve sol ventrikül hipertrofisindeki azalmanın bağımsız olarak TIMP-1 düzeyleri azalmaktadır.

Anahtar Kelimeler: Hipertansiyon; metalloproteinaz-1’in doku inhibitörü; ekokardiografi, doppler; hipertrofi, sol ventriküler; anjiyotensin dönüştürücü enzim inhibitörleri

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Hypertension (HT) is a serious problem that increases the cardiovascular mortality and morbidity. Left ventricular hypertrophy (LVH) developed due to HT is associated with increased risks regard with coronary heart disease, heart failure, and ventricular arrhythmia. LVH may cause diastolic dysfunction and ultimately leads to diastolic heart failure. Diastolic dysfunction is a complex situation, which characterized by impaired ventricular filling due to the reduced ventricular compliance.

LVH developed secondary to hypertension is found to be associated with progressive interstitial fibrosis. The change of extracellular matrix (ECM) composition plays an important role in the genesis of left ventricular hypertrophy in hypertension. Matrix metalloproteinases (MMPs) are a family of molecules that are responsible for breakdown of collagen and other extracellular matrix elements including elastin and fibronectin. MMP-1 is responsible for degradation of type 1 collagen, has also a natural inhibitor, tissue inhibitors of matrix metalloproteinase (TIMP-1). TIMP-1 inhibits MMP-1 and therefore leads to increase in type I collagen content of myocardium and contributes to development of LVH in hypertension. Proportional increase in type I collagen compared to type III contributes to be high tensile strength and rigidity due to having thicker strands.

A limited number of studies showed that TIMP-1 levels are increased in hypertension and this situation may reflect an increased deposition of collagen within the cardiac extracellular matrix and cardiac fibrosis. Some studies suggest that TIMP-1 levels increased in patients with LVH. Moreover it was also found to be correlated with parameters reflecting the diastolic dysfunction. Some of other studies did not confirm these findings and similar TIMP-1 levels were reported.

Antihypertensive therapy was shown to regress in LVH in hypertensive patients. Medications that make blockage of Renin-Angiotensin-Aldosterone system (RAAS) were found to make a more effective regression of LVH. Clinical and experimental evidence suggest that RAAS has clearly associated with structural remodelling and fibrosis. The prevention of cardiac fibrosis with antihypertensive therapy may be important in terms of protection of diastolic function and reduction of arrhythmia risk. In experimental studies, medications with angiotensin converting enzyme inhibitors (ACEi) and aldosterone antagonists were reported to be effective for reducing the cardiac collagen and elastin density and prevent the development of fibrosis. The cardiac fibrosis was shown to be regressed by Captopril and Quinapril treatment.

TIMP-1 was suggested to be a surrogate mediator of fibrosis and may reflect the change of LV diastolic function. The effect of antihypertensive therapy on TIMP-1 levels was not clearly known. Conflicting results were reported. Plasma TIMP-1 levels was found to be lowered by lisinopril after one year treatment whereas, other studies did not confirm this finding with enalapril treatment.

In this study we aimed to investigate to effect of quinapril, a strong tissue inhibitor of ACE, on TIMP-1 levels in hypertensive patients. We also planned to assess to role of this treatment on left ventricular hypertrophy and diastolic functions.

**MATERIAL AND METHODS**

All hypertensive patients admitted to cardiology unit in Dokuz Eylül University School of Medicine between February 2005 and March 2006 were screened and those which had not use any RAAS blocking agents for last 3 months were selected to the study. Transthoracic echocardiography was performed in all patients in order to assess LVH and diastolic functions. Patients who were detected LVH and diastolic dysfunction at echocardiography were included to the study. Exclusion criteria is as follows: secondary hypertension, presence of chronic obstructive lung disease, hepatic dysfunction, renal dysfunction (serum creatinine level > 1.5 mg/dl), presence of acute coronary syndromes, previous myocardial infarction, past history of pulmonary fibrosis, connective tissue diseases, left ventricular systolic dysfunction and malignancy.

The blood samples were drawn for TIMP-1 levels and echocardiographic measurements were obtained and blood pressure measurements were...
made. After baseline assessment, quinapril treatment was given to all patients. The dose of Quinapril was 20 mg/day, this dose was up-titrated to 40 mg/day at the 1st visit, if the blood pressure was not below the target level (140/90 mmHg). If the blood pressure was still uncontrolled, a hydrochlorothiazide treatment at a 12.5 mg/day was added at 2nd month visit. The treatment was continued at 6 months. At the end of follow-up measurements were repeated in all patients.

MEASUREMENTS OF TIMP-1
Blood samples were centrifuged at 4000 g and 4°C for 10 min, divided into aliquots and frozen at -80°C until analysis. Hemolytic and lipemic samples were not included in the study. Plasma TIMP-1 levels were measured with a commercially available enzyme linked immunosorbent assay (Human TIMP-1, Biosource International, Immuon assay kit, USA). A monoclonal antibody specific for human TIMP-1 has been coated onto the wells of the microtiter strips by the manufacturer. Plasma samples (1/20 dilution), standards of known TIMP-1 content (ranging from 1.56-25 ng/mL) and control specimens were pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody. After the first incubation, excess second antibody was removed and streptavidin-peroxidase was added. Again after a second incubation and washing, a substrate solution was added. The intensity of colored end-product was measured at 450 nm. The analytical sensitivity of method was < 1 ng/mL. The intra-assay and inter-assay coefficient of variation (CV) were 6.1% and 9.3%, respectively.

MEASUREMENTS OF BLOOD PRESSURES
Blood pressure measurements were made with OMRON 705 IT semi-automated device (Omron Healthcare Milton Keynes, UK) in a quite room with a sitting position after at least 5 minute period of rest. Three consecutive measurements were made and mean of these measurements was taken.

ECHOCARDIOGRAPHY
Echocardiography was performed with Hewlett Packard Sonos 4500 machine. All measurements were made with the same investigator (EO). M-Mode images were obtained in parasternal long axis view for LV measurements. LV mass was calculated according to formula described by Devereux.18 LV mass= 0.8 X 1.04 [(IVSd + LVIDd + LVPWd)3] + 0.6, where, IVSd is int ventricular septum thickness, LVID: left ventricular internal diameter, LVPW: left ventricular posterior wall. All measurements are made at the end of diastole. LV mass index was (LVMi) calculated by correcting LV mass with body surface area. LVH was accepted if LVMi > 104 gr/m² for women and 116 gr/m² for men. Pulsed wave Doppler flow was obtained in the apical 4 chamber view, with a cursor placed near the mitral inflow and aortic outflow, so it’s possible to get both aortic and mitral flow in the same heart. The E wave which was the 1st positive wave and A wave which was the 2nd positive wave was recorded, Deceleration time was measured. The isovolumetric relaxation time was measured from the end of aortic jet to beginning of E wave. For tissue Doppler studies (TDI) images were recorded apical 4 chamber view, the pulsed wave Doppler signal were obtained from mitral annulus both at the septal and lateral wall. The systolic (Sm), early (Em), and late (Am) diastolic velocities were analyzed. Diastolic dysfunction was defined as the presence of 2 factors including E/A ratio < 1 for transmirtal flow and Em < Am < 1 for TDI. All measurements were averaged five cycles and repeated at 6th months.

Written informed consent was obtained in all participants and the study was approved by the ethics committee of our local institution.

STATISTICAL ANALYSIS
Continuous variables were presented as a mean with SD. Paired sample t test was used for comparison of values baseline and after treatment. The comparison of TIMP-1 levels was made with Wilcoxon test due to the nonparametric condition. A Pearson and Spearman correlation analysis was used for the correlation between TIMP-1 and other parameters. All tests were two sided. A p value < 0.05 was considered significant. SPSS 11.0 for Windows was used for the statistical analysis.
RESULTS

The study population consisted of 30 hypertensive patients (mean age: 64.1 ± 8.5 years) with LVH. The demographic and clinic properties of patients are shown in Table 1. Previous antihypertensive medications were discontinued in 7 patients one week before the assignment (washout period) period. Remaining 23 patients were selected among new treated patients. Quinapril treatment was begun at a 20 mg/day dose in all patients. In order to reach the target blood pressure (< 140/90 mmHg), the dose was up-titrated to 40 mg/day in 7 patient and 4 out of them receive also a hydrochlorothiazide treatment.

BLOOD PRESSURE OBJECTIVES

The target blood pressure objectives were achieved in all patients (Figure 1). Mean systolic blood pressure significantly declined (152.2 mmHg-132.4 mmHg) after quinapril therapy (p<0.0001) (Table 2). Mean diastolic blood pressure also significantly decreased (91.6 mmHg-79.7 mmHg) (p<0.0001)

CHANGE OF TIMP-1 LEVELS WITH THERAPY

Mean baseline TIMP–1 level was 619.9 ± 108.1 ng/ml. After quinapril treatment, mean TIMP-1 levels was found to be significantly decreased (485.6 ± 105.9 ng/ml; p<0.0001). Quinapril treatment reduced TIMP-1 levels in 25 of 30 hypertensive patients (83.3%) (Figure 2).

CHANGE OF LVMI AFTER QUINAPRIL TREATMENT

Mean baseline LVMI (137.8 ± 31.5 g/m²) lowered after treatment (130.7 ± 32.4 g/m², p<0.0001) (Table 1, Figure 2).

CHANGES OF THE DIASTOLIC PARAMETERS AFTER QUINAPRIL TREATMENT

Some of diastolic Doppler parameters significantly improved after therapy. Deceleration time significantly decreased (242 ± 38.8-227 ± 39.7) and
Em/Am ratio obtained at septal mitral annulus significantly increased (0.70±0.15-0.78±0.11) after therapy. There was trend to increased transmitial E/A ratio (0.73±0.14 - 0.80±0.15), but the difference did not reach the significance (p= 0.076). Other diastolic Doppler parameters did not change significantly after the Quinapril treatment (Table 2).

CORRELATION ANALYSIS

Baseline TIMP-1 levels did not correlate with any other variables including SBP, DBP and Doppler findings (Table 3).

The change of TIMP–1 levels (D TIMP–1) was not found to be significantly correlated neither the change of systolic (r= 0.12) nor diastolic blood pressures (r= 0.10). The changes of other diastolic Doppler parameters were also not correlated with the D TIMP–1 levels (Table 3).

DISCUSSION

The regression of LVH, especially cardiac fibrosis is important in hypertension in terms of improve the diastolic functions and decrease the risk of diastolic heart failure. The increased collagen content produced by fibroblasts or decreased breakdown of those through RAAS activation causes accumulation of abnormal collagen fibril in myocardium. Progressive fibrosis developed due to increased type 1 collagen which has a high tensile strength may leads to myocardial stiffness. Matrix metalloproteinase system plays a key role in formation of extracellular matrix.

Improving left ventricular diastolic function and failing left ventricular hypertrophy were shown by several antihypertensive treatments. Previous experimental studies revealed the importance of the inhibition of RAAS in attempt to prove that reduced cardiac collagen content and fibrosis. Comparing the felodipin, enalapril provided a more reduction in collagen content as well as LVMI in genetically hypertensive rats. Additionally two studies revealed a regression of cardiac fibrosis with Captopril and quinapril in spontaneous hypertensive rats. The identification of cardiac fibrosis is difficult. Recent studies suggested that TIMP-1 may reflect the cardiac fibrosis and may be key mediator of LV diastolic dysfunction through definition of ventricular matrix composition.

TIMP-1 is a protease inhibitor; make an inhibition of collagen breakdown, especially inhibits matrix metalloproteinase1 (MMP-1) type 1 which responsible for breakdown of type 1 collagen.
Thus tissue collagen content increases in conjunction with the increase in TIMP-1. Angiotensin has been found to induce TIMP-1 production by vascular cells. So inhibition of RAAS is reasonable to prevent collagen accumulation in extracellular matrix beyond its antihypertensive effects. Except of one study, a majority of previous studies showed increased circulating TIMP-1 levels in hypertensive patients compared to healthy controls. Additionally, TIMP-1 levels was shown to be correlated with LVMI, however conflicting results were also reported.

Although elevated TIMP-1 levels were shown in hypertension, it’s not clear whether TIMP-1 level is reduced with antihypertensive treatment. Laviades et al reported that TIMP-1 levels decreased (848 ± 33 ng/ml - 714 ± 41 ng/ml) after 1 year of lisinopril treatment. However this finding was not confirmed other two studies. TIMP-1 levels were not found to decrease after 2 months of antihypertensive treatment using enalapril or losartan. These results may be explained by relatively shorter treatment duration and higher blood pressure objectives (160/90 mmHg). Thus neither LVMI nor parameters of diastolic functions improved after treatment. Moreover, on the contrary of previous studies, baseline TIMP-1 and MMP-9 levels were also lower in hypertensive patients than healthy control subjects. Other interesting result was reported with a subgroup analysis from Anglo–Scandinavian Cardiac Outcomes Trial. In this study, although both baseline MMP-9 and TIMP-1 levels are found to be increased in hypertensive patients compared to normotensive controls, interestingly on the contrary the previous studies, plasma TIMP-1 levels increased (400 ng/ml) (415 ng/ml) and MMP-9 levels decreased after antihypertensive treatment. No specific antihypertensive treatment was described in this study. The possible explanation of this surprising finding reported to fact that increased TIMP-1 levels may reflect positive remodeling in vascular and other target organs during treatment or it may be related to intensive blood pressure control.

Conflicting results observed with previous studies are thought to be due to complex relationship between metalloproteinase and inhibitors in hypertension. Moreover these studies were not specifically designed to investigate the role of an ACEi treatment on TIMP-1 levels.

In this prospective study, we specifically investigated the role of quinapril therapy on TIMP-1 levels in hypertension. A homogenous study population including patients who have both left ventricular hypertrophy and left ventricular diastolic dysfunction were selected. Quinapril was preferred due to strong tissue ACE inhibition properties. Although the healthy control group was not exist, mean baseline TIMP-1 level was high in our hypertensive study group (mean: 619.89 ng/ml) comparing to a given healthy population reported in literature (99-330 ng/ml).

We observed that plasma TIMP-1 level decreased in addition to decrease in LVMI and blood pressures after quinapril treatment. Additionally some of the diastolic parameters also improved after this therapy. Our finding is consistent with the hypothesis based on possible reduction of deleterious vascular remodeling and myocardial fibrosis after an ACEi treatment. It can be suggested that quinapril stimulates the breakdown of collagen by reducing TIMP-1 levels. This study confirmed findings reported with Laviades et al. The absence of beneficial effect on TIMP-1 levels with other ACE inhibitors such as enalapril, as well as other antihypertensive treatment may raise to possibility the class effect of lisinopril and quinapril.

However baseline TIMP-1 levels was not found to be correlated any other parameters including blood pressures, and any of diastolic functions. Thereby, we also did not observe any association between the reduction of TIMP-1 levels and change of these parameters. Neither improvements of left ventricular diastolic functions nor the reduction of blood pressures were found to be associated with the decrease of TIMP-1 levels. This finding is consistent with the Laviades et al.’s results. Our study suggests that decrease of TIMP-1 levels with quinapril may be independent with the decrease of blood pressures or antihypertensive efficacy of...
drug. Thus, a different mechanism other than hemodynamic factors may be responsible in the ability of quinapril to modify MMP-1/TIMP system. Another plausible mechanism is the fact that biochemical changes reflecting the fibrosis may not truly reflect the structural changes of tissues. The distribution of TIMP-1 levels was not homogenous and standard deviation of TIMP-1 was high. Some of the extreme values may affect the outcomes.

The relationship between plasma TIMP-1 levels and diastolic parameters were assessed at several studies. A strong correlations was reported between TIMP-1 levels and transmitral E/A ratio and the cut-off value for TIMP in order to reverse the E/A ratio was determined as 500 ng/ml.6,11 Lindsay et al suggested that TIMP-1 levels are associated with LV diastolic parameters rather than LVH or blood pressures. In this study, TIMP-1 levels in hypertensive patients with normal diastolic functions did also not increase compared to healthy controls.7 We confirmed the previous studies suggesting the improvement of diastolic functions and LVH with antihypertensive treatment in hypertension.23-25 However we did not find any relationship between TIMP-1 levels and other parameters. All patients in our study was already had a left ventricular hypertrophy and diastolic dysfunction. This may leads to small differences among patients regarding to LVMI and diastolic parameters and this situation may obviate the baseline correlations between TIMP-1 and other parameters.

LIMITATIONS OF THE STUDY

The absence of healthy control groups might limits the outcome of the study; however we did not aim to investigate whether TMP-1 levels increased or not in hypertensive patients. Additionally we could not use a control group who had leaved without antihypertensive medications for 6 months for ethical reason. Further controlled studies with other ACEi drugs on TIMP-1 levels are required.

Low sample size and relatively shorter follow-up duration might limit the outcome of study. However sample size of this study was similar to the numbers of patients in previous study, and we obtained a significant decrease in surrogate markers, so these may obviate the limitation.

The determination of MMP-9 or other matrix metalloproteinase levels may aid understand the mechanism of change of TIMP-1 levels in hypertension. The absence of assessment of these markers may limit the outcomes. We also did not measure central blood pressure which seems to be more relevant to LV mass.

In conclusion our study suggests that quinapril treatment reduce plasma TIMP-1 levels reflecting the cardiac fibrosis beyond to regression of left ventricular hypertrophy and improvements of diastolic functions in patients with hypertension. However the absence of association between the regression of TIMP-1 and decreased blood pressure and improvement of diastolic functions is a interesting finding. TIMP-1 may be a promising marker to determine the regression of fibrosis in hypertension.

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REFERENCES


