Smyrna Tıp Dergisi *Araştırma Makalesi*

Levels of Metalloproteinase 9 and Specific Metalloproteinase Inhibitor-1 in Acute Coronary Syndrome and Stable Angina Pectoris

Akut Koroner Sendrom ve Stabil Angina Pektorisde Metalloproteinaz-9 ve Spesifik Metalloproteinaz Doku İnhibitörü-1 Düzeyleri

Mehmet Burak Aktuğlu[1], Özgür Altun[2], Eray Atalay[3], Mesut Ayer[1], Namık Yiğit[1], Zeynep Karaali[4], Yeşim Gürkan[1], Aylin Ayer[1], Makbule Ulusoy[1]

[1] Uz.Dr., Haseki Eğitim Araştırma Hastanesi Dahiliye Kliniği, İstanbul, Türkiye
[2] Uz.Dr., Okmeydanı Eğitim Araştırma Hastanesi Dahiliye Kliniği, İstanbul, Türkiye
[3] Uz.Dr., Kafkas Üniversitesi Tıp Fakültesi, İç Hastalıkları Anabilim Dalı, Kars, Türkiye
[4] Doç.Dr., Haseki Eğitim Araştırma Hastanesi Dahiliye Kliniği, İstanbul, Türkiye

Abstract

Objective: Globally, the major cause of death is ischemic heart disease. Atherosclerotic plaque is the major cause of ischemic heart disease. Matrix Metalloproteinase (MMPs) are proteolytic enzymes responsible from extracellular matrix destruction. Especially Metalloproteinaz-9 (MMP-9) has an important role in the formation of atherosclerotic plaque and also in the matrix destruction of plaque capsule then. Specific Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) is the specific inhibitor of MMP-9. The balance between these two is very important. In this study, MMP-9, TIMP-1, hsCRP levels of the patients with acute myocardial infarction, unstable angina pectoris (USAP) and stable angina pectoris were investigated.

Material and Method: 20 patients (4 female, 16 male) with AMI who have high levels of troponin; 19 patients (4 female and 15 male) with USAP who have negative or very low levels of troponin; 28 patients (10 female, 18 male) with stable angina pectoris and 20 individuals (3 female, 17 male) for control group; were included . Their white blood cell (WBC) count, MMP-9, TIMP-1, hsCRP levels were analyzed with SPSS-13 statistic program.

Results: MMP-9 and TIMP-1 levels were statistically higher ($p<0,05$) in patients with Troponin (+) AMI, troponin (-) USAP and stable angina pectoris in contrast of control group. Also, positive co-relation between MMP-9 and WBC count in atherosclerotic patients were detected; they were related with high levels of hsCRP.

Conclusion: MMP-9 and TIMP-1 can be diagnostic markers for the diagnosis of atherosclerotic heart diseases. In this context; they can be useful in the early diagnosis of ACS. The positive relation between MMP-9 and WBC levels proves the role of macrophages with lipid content and the importance of their interaction with MMP-9 in atherosclerotic heart disease.

Key Words: Angina, atherosclerosis, metalloproteinase, plaque.

Özet

Amaç: Dünya çapında en önemli ölüm nedeni iskemik kalp hastalığıdır. İskemik kalp hastalığının ana nedeni aterosklerotik plaklardır. Matriks metalloproteinaz (MMPs) proteolitik bir enzimdir ve ekstraselüler matriksin yıkımından sorumludur. Özellikle MMP-9 aterosklerotik plak oluşumunda öncelikli rol oynar; ayrıca plak kapsülünün matriks yıkımında da etkindir. Doku metalloproteinaz inhibitörü-1 (TIMP-1) de, MMP-9'un özgün inhibitörüdür. Bu ikisi arasındaki denge önemlidir. Bu çalışmada akut myokard infarktüsü, stabil olmayan angina pektoris (USAP) ve stabil angina pektorisli hastalarda MMP-9, TIMP-1, hsCRP düzeyleri araştırıldı.

Gereç ve Yöntem: Çalışmaya troponin düzeyi yüksek 20 AMI'li hasta (4 kadın, 16 erkek), 19 çok düşük yada negatif troponin'li USAP'lı hasta (4 kadın, 15 erkek), 28 stabil angina pektoris'li (10 kadın ve 18 erkek) ve kontrol grubu olarak 20 kişi (3 kadın, 17 erkek) alındı. Bu hastalarda lökosit düzeyi, MMP-9, TIMP-1 ve hsCRP düzeyleri ölçülerek sonuçlar SPSS-13 istatistiksel analiz programı ile değerlendirildi.

Bulgular: Kontrol grubuyla karşılaştırınca troponin pozitif AMİ'li hastalarda, troponin negatif USAP'lılarda ve stabil angina pektoris'lilerde MMP-9 ve TIMP-1 istatistiksel olarak anlamlı yüksek bulundu ($p<0,05$). Ayrıca aterosklerotik hastalığı olanlarda MMP-9 ile beyaz küre (WBC) sayısı arasında pozitif bir ilişki bulundu; bu değerler yüksek hsCRP değerleri ile de ilişkilendirildi.

Sonuç: MMP-9 ve TIMP-1 aterosklerotik kalp hastalıklarının tanısında belirteç olarak kullanılabilir. Bu bağlamda akut koroner sendromun (AKS) erken tanısında yararlı olabilirler. MMP-9 ile WBC arasındaki pozitif ilişki; aterosklerotik kalp hastalığında lipid yüklü makrofajların rolünü ve bunların MMP-9 ile ilişkisini kanıtlar.
Anahtar kelimeler: Anjina, ateroskleroz, metaloproteinaz, plak.

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Introduction

In contrast of all the progresses in cardiovascular medicine, unstable angina pectoris (USAP), non-ST elevation myocardial infarctus (NSTEMI) and ST-elevation myocardial infarctus (STEMI) are still the leading causes of mortality and morbidity all over the world. Main lesion underlying angina pectoris and acute coronary syndrome is vulnerable atherosclerotic plaque in one or more coronary artery. Recently; new markers are found to identify the rupture of fragile atherosclerotic plaques thus leading to acute coronary syndrome. Vulnerable plaques cause pathologies leading to first, stable angina pectoris then NSTEMI and finally acute coronary syndrome due to rupture.

Nowadays new biomarkers are identified for this progress. Coronary arterial inflammation is widely seen in acute coronary syndrome (1). The level of serum inflammatory markers are parallel with the presence of atherosclerosis and the prevalance of ruptured plaques (1). The evalution of these inflammatory markers is important in diagnosis and risk scoring (MMP-9 and its physiologic inhibitor TIMP-1) . Metalloproteinase is a family of neutral, Zn and Ca dependent endoproteinase, responsible for the destruction of extracellular matrix components. These enzymes have an essential position in the extracellular matrix (ECM) turnover, tissue remodelling, angiogenesis, morphogenesis and evaluation (2).

Figure 1. *Classification of metalloproteinases*

Group	Member	MMP number	Main Substrats
COLLAGENASES	Interstitial collagenases	MMP-1	Fibrillary collagens
	Neutrophil collagenase	MMP-8	Fibrillary collagens
	Collagenase 3	MMP-13	Fibrillary collagens
	Collagenase 4	MMP-?	Unknown
GELATINASES	Gelatinase A	MMP-2	Gelatin,Collagen IV-V,fibronectin
	Gelatinase B	MMP-9	Gelatin,Collagen IV-V,fibronectin
STROMELYZINS	Stromelyzin 1	MMP-3	Laminin,non-fibrillary collagen,fibronectin
	Stromelyzin 2	MMP-10	Laminin,non-fibrillary collagen,fibronectin
	Matrylsin	MMP-7	Laminin,non-fibrillary collagen,fibronectin
	Stromelyzin 3	MMP-11	Serpin
Mt-MMI 'S	Mt-1 MMP	MMP-14	Pro-MMP-2,collagens,gelatine
	Mt-2 MMP	MMP-15	Pro-MMP-2,collagens,gelatine
	Mt-3 MMP	MMP-16	Pro-MMP-2,collagens,gelatine
	Mt-4 MMP	MMP-17	Pro-MMP-2,collagens,gelatine
OTHERS	Metalloelastase	MMP-12	Elastine
	Enalmelsin	MMP-?	Unknown
	Xenopus	MMP-?	Unknown
	Unknown	MMP-19	Aggrecan

Recently some autors published studies that showed the importance of MMP's augmentation in activities cause cardiac diseases, atherosclerosis, periodontal sickness, tumor cell metastase and arthritis (3). Molecular weight of MMP's is between 19- 92 kDa (4).

Gelatinases
MMP-9 (Gelatinase B)

In 1974 Sapota and Dancemicz described it as a gelatinolytic enzyme, secreted from polymorphonuclear neutrophils (PMN). It is the biggest member of MMP's. It is secreted as a molecul of 924 kDa weight. Fibronectin-like part facilitates the connection to collagen and gelatines (13). MMP-9 is very specific to kazeine (5).

Relationship between AMI and MMP

It is accepted that, in the early phases of atherosclerotic process, MMP enzymes have roles in the hyperplasia of arterial intima and rupture of atherosclerotic vulnerable plaques. It is well known that myocardial MMP's are produced in fibroblast-like cells, inflamatory cells and cardiomyocytes. MMP's are often in latest form and their activities and expressions augments in a lot of cardiac pathologies.

Augmentation in MMP-9 levels can be better detected in coronary plaques of patients with USAP than those with stable angina pectoris and also MMP level is high in AMI but TIMP-1 is low.

MMP's facilitate centripedal remodelling of arterial wall caused by external laminal degradation. This, at the beginning, is helpful because it protects the width of lumen but it also diminishes the mechanic resistance of arterial wall thus leading to risk of plaque rupture. It can mean that macrophage derived MMP's have important role in vulnerable areas of atherosclerotic plaque. Interestingly lipid lowering agents like HMG-coA reductase inhibitors diminish the MMP expression in macrophages as in vascular cells (14). It is known that peroxinitrite, naturally derived from nitric oxide and superoxide, activates inactive MMP and leads to destruction of TIMP-1 (14).

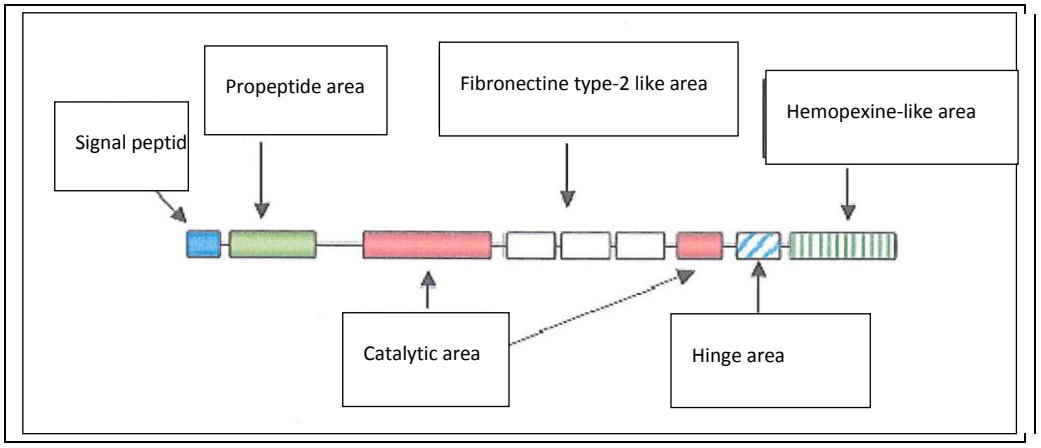
Secretion of MMP-9 is closely related with inflamation. Human monocytes and macrophages secrete MMP-9. Interaction between endothelial cells and monocytes leads to MMP-9 up-regulation.

Inflamatory cytokines like IL-1, IL-4 and TNF induce the production of MMP-1,3 and 9 from vascular smooth muscle cells (VSMC). MMP causes basal membrane migration and proliferation in VSMC (15). Interestingly; MMP-9 transcription is observed in atherosclerotic plaque areas and endothelial cell line (15). Kai firstly discovered the augmentation of MMP-2 and MMP-9 in serum of patients with vulnerable atherosclerotic plaque (16).

Structure and function of matrix metalloproteinase tissue inhibitors (TIMP)

Proteolytic activities of MMP's can be inhibited with non-specific (alfa-2 macroglobulin, alfa-1 antiproteinase, serum C-reactive protein (CRP) which has a anticollagenase activity) and also specific inhibitors such as tissue metalloproteinase inhibitors (TIMP) (5).

Graphic 1. *Matrix metalloproteinase-9*



There are 4 groups of TIMP (6).

TIMP-1:

TIMP-1 attaches non-covalently and irreversibly to MMP's (7). TIMP-1 attaches to proMMP-9 (92 kDa, member of gelatinase family). Pro-MMP/TIMP-1 complex inhibits all active MMP's and creates the more active form proMMP-9/TIMP-1/ MMP (8).

TIMP-2:

First isolated from melanoma cells, it is non-glycolized, 21 kDa weight. TIMP-2 inhibites all MMP's but MMP-9. TIMP-2 deacreases the proteolytic leekage of blood-brain barrier (9).

TIMP-3:
Isolated from breast cancer cells (10), it inhibits MMP-1,2,3,9 and 13 (11). It is a non-glycolized MMP inhibitor of 21 kDa weight. It facilitates the separation of transformed cells from extracellular matrix and starts morphologic changes (9).

TIMP-4:
It inhibits MMP-2,7,9 (12). It is the latest member of TIMP family and it is derived from human heart. It inhibits tumor invasion and metastase (11). Balance between MMP-9 and TIMP-1 is important in cardiovascular system. In recent studies, MMP-9/ TIMP-1 levels were high in stable angina pectoris, USAP and ACS.

Material and Methods

Study population
We studied on 20 AMI patients with high levels of troponin (4 females,16 males), 19 USAP patients with negative or very low levels of troponin (4 females, 15 males) and 28 patients with stable angina pectoris (10 females,18 males). Their informed consent forms and institutional ethic approval was obtained.

For AMI patients; WHO's criteria for MI (≥2 is accepted as positive) (ischemic type chest pain, typical ECG changes peculiar to AMI, serum cardiac maker elevation) was used. For USAP patients; chest pain of unstable type with no elevation of serum cardiac markers and/or accompanying ECG changes, was used. For patients with stable angina pectoris; ischemic type chest pain induced with activity and positive treadmill stress test, was the main criteria.

Evaluation of MMP-9 and TIMP-1 levels
Competitive ELISA method (Bender Med Human MMP-9 ELISA; KAT no BMS 2016/2) for MMP-9 and competitive ELISA method (Bender Med Human TIMP-1 ELISA; KAT no: BMS 2018) for TIMP-1 level evaluation was used.

Results

Basal characteristics
- There was no sex difference between groups (p>0,05).
- There was no age and antropometric condition related difference between groups (p>0,05).
- There was no statistically significant change between biochemical values of the groups (p>0,05).
- There was no statistically significant change between HDL and LDL levels of groups (p>0,05).
- There was no statistical difference of ejection fraction between groups.
- Interestingly trigliseride levels of troponin negative patients were higher than troponin positive ones (p<0,05).
- hsCRP levels of Troponin positive group are significantly higher than troponin negative and stable angina group (p<0,001).
- Also troponin negative group's hsCRP levels are significantly higher than stable angina group (p<0,001).
- There is a moderate level of significant positive corelation between MMP-9 and White blood cell (WBC) count in USAP patients.

Figure 2. *Natural inhibitors of metalloproteinases*

TIMP	MA active form (kDa)	Function
TIMP-1	28	It inhibits PRO-mmp-9 and various active MMP's
TIMP-2	21	It inhibits Pro MMP-2 and active MMP-2
TIMP-3	21.6	Undefined

TIMP: Tissue inhibitor of metalloproteinases

Figure 3. *Corelation between MMP-9 and WBC for USAP patients.*

	Acute coronary syndrome				Troponin (+)				Troponin (-)			
	MMP-9		TIMP-1		MMP-9		TIMP-1		MMP-9		TIMP-1	
	R	p	r	p	r	P	R	p	r	p	r	p
Leucocyte	,401	,017*	-,056	,751	,216	,390	,025	,922	,633	,006**	-,127	,626
Fibrinogene	,001	,997	,025	,895	,302	,255	,304	,252	-,428	,112	-,323	,240
hsCRP	-,140	,312	-,026	,851	,042	,871	,217	,403	,408	,104	,302	,238

Discussion

Myocardial infaction is a disease characterized with the necrosis of myocardial cells and this is globally the most important cause of death. Underlying reason is; development and evolution of atherosclerotic plaque, rupture of this vulnerable atherosclerotic plaques and occlusion of vessel lumen. Balance between MMP's and TIMP-1 is very important in this process because MMP's facilitate smooth muscle cell's migration to internal lamina elastica during plaque evolution and is also responsible for extracellular matrix destruction. MMP-9 especially influence plaque stability relative with inflamatory cytokines. High levels of MMP-9 and low levels of TIMP-1 were demonstrated in patients with coronary syndrome, serious coronary stenosis and in cardiovascular mortality.

MMP-9 and TIMP-1 levels were significantly high in patients with acute coronary syndrome (ACS) and stable angina pectoris group versus control group. This means that MMP-9 and TIMP-1 levels are independent markers of atherosclerosis and acute coronary syndrome. Chen investigated MMP-9 and TIMP-1 levels in ACS patients versus control group. MMP-9/TIMP-1 ratio was found significantly high for ACS group than control group. And; they are convinced that it can be an independent risk factor for defining the seriousity of ACS (17).

Zeng B. et al. investigated MMP-9, MMP-2 and hsCRP levels for symptomatic coronary disease patients. They've found that in acute coronary syndrome, MMP-9 levels were higher than in stable angina pectoris and control group thus they have resulted that MMP-9 is a potential marker of plaque rupture. They have also determined a positive corelation between hsCRP and MMP-9 due to high levels of hsCRP that they have obtained. These results leaded to the tought that, MMP-9 can be a marker of ischemic inflamatory processes underlying the pathophysiology of acute coronary syndrome (18).

In this study, there wasn't a significant difference between MMP-9 and TIMP-1 levels of patients with ACS and stable angina pectoris so we cannot admit that MMP-9 is a marker of plaque rupture but as MMP-9 and TIMP-1 levels are higher than control group; we can say that it can be an independent risk factor for the presence of atherosclerotic plaque. As MMP-9 was also high as other cardiac markers for AMI patients, we can also consider that it can be a biochemical marker in the early stage of AMI.

HsCRP levels were higher for AMI patients versus stable angina pectoris and control group as Zeng B. et al. This means that higher MMP-9 levels for ACS is due to inflamation and for stable angina pectoris it can be due to atherom plaque. Dominic K. et al., in their study, have found that plaque rupture in ACS is related with high levels of MMP. They have also found that WBC count is an important source of high levels of MMP-9 due to positive corelation between them (19). In this study, we have noted a positive corelation for ACS especially in USAP patients, between WBC and MMP-9. This leads us to think that, in early stages of ACS, high levels of MMP-9 can be related to WBC levels.

Kai found that in AMI group, MMP-9 levels were double than control group; in USAP group it was 3 times higher than control group (16). These results were similar with our group. In our study also, there was a significant difference between USAP versus control group and AMI versus control group. We can conclude that in the early stages of AMI, MMP-9 and TIMP-1 can be diagnostic markers. But, in this study, as there was no significant difference between USAP and AMI for MMP-9 and TIMP-1, we can not say that they can predict the prognosis and seriousity of coronary disease because we expected that the balance between MMP- 9 and TIMP- 1 had to change as in the references and there wasn't in our study, more studies with more patients are to be performed to understand the reason of these results.

Conclusion

In this study, it was found that MMP-9 and TIMP-1 levels were higher for AMI patients with high troponin levels, USAP with negative troponin levels and patients with stable angina pectoris than in control group. It means that MMP-9 and TIMP-1 can be markers in atherosclerotic heart disease and in this context they can be useful in its early diagnosis and treatment. Also, a positive corelation between MMP-9 and WBC count for atherosclerotic patients was detected. They were related with high levels of hsCRP. This proves the role of macrophages with lipid content and the importance of their interaction with MMP-9. All these results strongly emphasize the essential role of MMP enzymes in the pathogenesis of atherosclerosis.

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Correspondance:
Fam. Med. Spec. M. Burak Aktuğlu.
Haseki Research and Education Hospital.
Clinic of Internal Medicine. Istanbul, Turkey
Tel: +90.212.5294400-1503
e-mail: lifeiner@yahoo.com