International Scientific Journal
Medical and Biological Sciences
http://bioscience.scientific-journal.com

MATRIX METALLOPROTEINASES AND THEIR INHIBITORS AND MMP3 POLYMORPHISM IN PERIPHERAL VASCULAR DISEASE

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Abstract—**Peripheral Vascular Disease (PVD) is a progressive and chronic disease resulting from atherosclerotic events. Atherosclerotic obstructions are consequence of chronic ischemia in the lower extremity arteries in patients with PVD. It is suggested that blood levels of Matrix Metalloproteinases (MMPs) and endogenous Tissue inhibitors of Metalloproteinases (TIMPs) have been associated with atherosclerosis. In this study, we aimed to demonstrate whether or not levels of MMPs and TIMPs and genetic variations in the matrixmetalloproteinase 3 (MMP3) -1171 5A/6A play a role in PVD. The study included 102 patients with PVD and 65 healthy controls. Serum MMPs and TIMPs level were determined by using ELISA method. Genotype analysis was performed for MMP3 -1171 5A/6A polymorphism in samples from peripheral blood. Polimerase chain reaction (PCR) was performed in DNA samples and then digested amplified DNA with Tth111 I enzyme by using Restriction fragment length polymophism (RFLP). We found that MMP-2 ($p<0.001$), MMP-9 ($p<0.001$) and TIMP-1 ($p<0.01$), TIMP-2 ($p<0.001$) levels in serum were significantly higher in patients with PVD as compared to the those of control group. In PVD group, We found the ratios of MMP-2/TIMP2 and MMP-9/TIMP-2 were increased in PVD group than those of controls (for both $p<0.001$). In addition, Pearson's correlation test was performed between MMPs and TIMPs levels in patients with PVD group. We found lineer correlation between MMP-2 and TIMP-2 ($r=0.504$ $p=0.000$), and TIMP-1 ($r=0.297$ $p=0.002$). In contrast, we found reverse correlation between MMP-2 and MMP-9 ($r=-0.504$ $p=0.000$). The 5A5A, 6A5A genotypes of MMP3 -1171 5A/6A were more frequent in patients with PVD than in controls. In contrast, 6A6A genotype ($p<0.001$) and 6A allele ($p<0.001$) were significantly decreased in patients as compared with controls. Our results showed that the changes in the levels of MMPs, TIMPs and the ratios of MMP-2/TIMP2 and MMP-9/TIMP-2 in serum and 6A6A genotype of MMP3 -1171 5A/6A are important in progression of PVD.**

Keywords-MMP; TIMP; PVD; polymorphism

INTRODUCTION

Peripheral vascular disease (PVD) is an chronic and an atherosclerotic procesess. It is consequence of chronic ischemia seen in the lower extremity arteries. MMP's are associated with vascular remodelling and regulating of angiogenesis that is a formation of new vessels in atherosclerotic proceseses. In appropriate remodeling underlies the pathogenesis of major cardiovascular disease such as atherosclerosis.

Matrix Metalloproteinases (MMPs) and their endogenous inhibitors (TIMPs) are key modulators of ECM turnover. The alterations in the synthesis and/or degradation of the arterial ECM have been associated with vascular disease. Recently it has been shown that the increased expression of MMPs and their contribution to weakening of the vascular wall by degrading the ECM is an important factor progressing of atherosclerotic procesess.

Increased levels of the specialized enzymes called MMPs and their inhibitors TIMPs have been reported in patients with PVD. Moreover, it has been suggestedthat both MMPs and TIMPs play an important role in the pathological remodelling of ECM in PVD. MMP3 genotype possibly could be an important factor in regulation of the ECM in PVD.

In the light of these findings, we aimed to demonstrate whether or not levels of MMPs and TIMPs are important factors in PVD. On the other hand, we investigated whether the genetic variations in the MMP3 -1171 5A/6A are contribution to progression of PVD.

Materials and Methods

The study included 102 patients with PVD (age: 62.78 ± 11.54) and 65 healthy controls (age: 59.66 ± 10.36). The study group were composed from patients which enrolled to the Department of General Surgery, Istanbul University, Istanbul Faculty of Medicine. The exclusion criteria for the control group were included obvious symptoms or signs of PAD who have an ankle-brachial index lower than 0.9 on clinical assessment. Patients with co-existing renal impairment, pernicious anemia, hypothyroidism, malignancy and patients taking medications such as thiazide diuretics were excluded from the study. The control group were consisted of healthy adults who had any accompanying disease such as hyperlipidemia,

hypertension. A written consent was obtained from each subject. Blood samples taken from the ante-bracheal vein after a 12-hour fast were collected in vacuum tubes. Serum was separated immediately and the levels of glucose, HbA1c (DPP modular systems, Roche) and hs-CRP (Cobas Integra 800, Roche) were measured. Serum MMP and TIMP levels were determined by using ELISA method. Genotype analysis was performed for MMP3 5A/6A -1171 5A/6A polymorphism in samples from extraction of DNA using by salting out method from peripheral blood. A 130-bp fragment was amplified from genomic DNA by using the following primers. The forward primer (-1201 to –1172, mismatch at nucleotide –1173) and reverse primer (-1072 to –1101) were 5-GGT TCT CCA TTC CTT TGA TGG GGG GAA AgA-3 and 5'-CTT CCT GGA ATT CAC ATC ACT GCC ACC ACT-3', respectively. The PCR product was digested with Tth111I for 3 hours at 65 ^{0}C. All digested products were analyzed on 3 % agarose gel stained with ethidium bromide and examined under UV transillumination.

Statistical analysis

Statistical analyses were performed using a standard SPSS 15.0 statistical package program. Continous variables were evaluated using student-t test. Distributions of genotypes and alleles were compared by chi-square test. Descriptive data are presented as means ± standard deviations. p values less than 0.05 were considered statistically significant.

Table 1. Clinical and biochemical characteristics of the patients and controls

Parameters	*Controls*	*Patients*	*P value*
BMI (kg/m^2) †	25.7 ± 2.3	27.27 ± 1.75	p<0.05
Hypertension n(%)	-	84 (79.2%)	
Diabetes mellitus n(%)	-	87 (82.1%)	
Fasting glucose (mg/dl) †	98.77 ± 12.96	155.3 ± 65.67	p<0.001
HbA1c (%) †	5.56 ± 0.45	6.49 ±1.54	p<0.001
hs-CRP (mg/L) †	2.19±2.96	71.13±70.86	p<0.001
MMP-2 (ng/ml) *	106 (139-79)	240 (285.5-183.5)	p<0.001
MMP-9 (ng/ml) *	1155 (1767.5-448.5)	2305 (3239.5-1815)	p<0.001
TIMP-1 (ng/ml) *	230 (620-160)	502 (692.5-381)	NS
TIMP-2(ng/ml)*	89.5 (116-68.5)	115 (157-84)	p<0.001
MMP-2/TIMP-1	0.46±0.40	0.45±0.26	NS
MMP-2/TIMP-2	1.27±0.53	2.21±1.79	p<0.001
MMP-9/TIMP-1	5.56±6.03	8.28±15.43	NS
MMP-9/TIMP-2	16.48±15.17	34.04±64.78	p<0.05

)

NS: Non significant

†: Mean ± Standard deviation

*: Median value and interquartile ranges (ranges were given in paranthesis).

Table 2. Distribution of MMP3 -1171 5A/6A genotypes in patients with PVD and controls

MMP3 -1171 5A/6A	*Controls*	*Patients*	*P value*
Genotypes			
6A6A	***75 (46.3)***	***24 (25.5)***	
6A5A	***61 (37.7)***	***46 (49)***	
5A5A	***26 (16)***	***24 (25.5)***	***< 0.001***
Alleles			
6A	***211 (65)***	***94 (50)***	
5A	***113 (35)***	***94 (50)***	***< 0.001***

DISCUSSION

MMPs are also an important factor in atherosclerotic processes due to the contribution of ECM degradation. It has been suggested that MMPs are directly synthesized in atheromatous plaques. MMps and their inhibitors are important in ECM turnoever. In this study we found that MMP2,-9, TIMP-1, -2 serum levels were significantly higher in PVD compared with the controls. We think that these findings could be associated with ECM turnover in patients with PVD. In a previous study, it has been suggested that the vascular atherosclerotic plaque is also a source of raised MMP-9 and TIMP-1. There is a balance between MMps and TIPMs in normal circumstances. But in atherosclerosis, this balance is disturbed. This imbalance causes changing of arterial function.

In this study, this imbalance in MMPs/TIMPs indicates arterial dysfunction due to the ECM remodelling. MMP-3 may play a role in degradation of extracellular matrix during the atherosclerotic processes. It has been suggested that polymorphisms are important factors in pathogenesis of PVD. It has been shown that MMP3 -1171 6A/5A polymorphism is related to PVD risk. In our study, 5A5A, 6A5A genotypes of MMP3 -1171 5A/6A were more frequent in patients with PVD than in controls. In according to our results, we think that patients with carrying 5A5A and 6A5A genotypes may have risk for PVD. These results are consistent with previous studies in literature.

In conclusion, further studies should be done for investigation between related genes and PVD progression.

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