Expression of *MMP2*, *MMP9*, *TIMP2* and *TIMP3* genes in aortic dissection

TUGBA KOSE[1], ARZU ANTAL[2] and TUBA GUNEL[1]

[1]Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, 34134 Istanbul, Turkey;
[2]Cardiovascular Surgery Clinic, Kartal Kosuyolu High Specialization Education and Research Hospital, 34865 Istanbul, Turkey

Received January 16, 2024; Accepted May 28, 2024

DOI: 10.3892/etm.2024.12649

Abstract. Thoracic aortic dissection (TAD) is a highly lethal disease occurring inside the aortic wall and is characterized by matrix degradation. Matrix metalloproteinases (MMPs) are members of a large endopeptidase family that function in the degradation of the extracellular matrix (ECM) proteins, the maintenance of the ECM, and the regulation of signaling in the aorta. MMPs are found in tissue with their natural inhibitors. Tissue inhibitors of metalloproteinases (TIMPs) are actively involved in both the activation and inhibition of MMPs. The present study was designed to determine the mRNA level gene expression differences of *MMP2, MMP9, TIMP2* and *TIMP3*, which are considered to have an essential role in TAD, in aortic tissue and circulating monocyte cells. For the purpose of the present study, aortic vascular tissue and peripheral blood-derived monocyte cells were obtained from 10 patients with TAD and 10 control individuals. The gene expression levels of targeted genes (*MMP2, MMP9, TIMP2* and *TIMP3)* were examined by droplet digital PCR. In research results, decreased expression of *MMP9, TIMP2* and *TIMP3* genes (P=0.043, P=0.009 and P=0.028, respectively) and increased ratio of *MMP2/TIMP3* (P=0.012) were obtained in the aortic tissue. No changes were observed in terms of gene expression in monocyte cells. When the results obtained were evaluated within the framework of TAD pathogenesis, it was concluded that expression changes in *MMP9, TIMP2* and *TIMP3* genes may provide a sensitive environment in aortic tissue and may be associated with TAD formation. In addition, since the expression ratios of MMPs and TIMPs may reflect disease development, it was considered that the evaluation of MMPs along with TIMPs may be an appropriate and informative approach for future studies.

Correspondence to: Professor Tuba Gunel, Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, 1 Bozdogankemeri Street, Fatih, 34134 Istanbul, Turkey
E-mail: gunel@istanbul.edu.tr

Key words: aortic dissection, matrix metalloproteinase, tissue inhibitors of metalloproteinases, gene expression, extracellular matrix

Introduction

Thoracic aortic dissection (TAD) is defined as the separation of the aortic layers along the vessel wall following a tear in the intima layer of the aorta (1). TAD is quite a life-threatening and fatal condition. Based on estimations, the incidence of TAD is between 3 to 5 cases per 100.000 individuals per year (2-4). The primary characterization finding for TAD is the degraded aortic media layer (1). Media is the middle layer of the aorta which is composed of smooth muscle cells (SMC), elastic and collagen fibers, proteoglycans, glycosaminoglycans, and some other proteins. The elastic fibers and supportive protein structures located in the media layer are the components of the extracellular matrix (ECM) which has a key role in maintaining the shape and strength of the aortic wall (1,5,6). In the dissected aorta, an excess amount of protease such as matrix metalloproteinases (MMPs) is also found, whose substrates are mainly the ECM components. The functions of MMPs are variable, thus they can either degrade or activate proteins in the ECM (7). Numerous studies have revealed that most of the MMPs exhibit differentiated expression in cases with TAD (8-10). In particular, it has been reported that MMP2 and MMP9 can degrade collagen types I, II and III that have been cleaved by MMP1 at the beginning of the process (11). In addition, it has been stated that both MMP2 and MMP9 can be regulated by transforming growth factor β (TGF-β) signaling, which is known to be involved in matrix synthesis and degradation (12).

MMPs, which have an important role in the formation of TAD (8-10), have natural inhibitors in the cell (13). The tissue inhibitors of matrix metalloproteinases (TIMPs) inhibit the MMP function by binding in the active site of MMPs and prevent matrix degradation (14). Although the inhibitory capacity of TIMP2 and TIMP3 varies, they act on all MMPs. TIMP2 has been reported to be highly effective in inhibiting MMP2. Additionally, TIMP3 has been found to inhibit MMP2 and MMP9 successfully (15,16). In cases with TAD it was shown that the expression levels of TIMPs can be both increased and decreased (8,17,18). Additionally, the MMP/TIMP gene expression ratio appears to direct the cell function into a proteolytic stage, which is a key factor in TAD (1,19).

On the site of the tear, an inflammation also occurs in TAD. Macrophages are one of the main players of inflammation in TAD. Since the circulating monocytes give rise to

the macrophages, they are also important components of the process (20,21). It was observed that the percentage of total $CD14^+$ monocytes was increased in patients with TAD, and there were expression changes in the genes managing the formation of TAD (22).

Most of the studies in literature are not focused on the gene expression changes at the mRNA level in patients with TAD. In such complex diseases such as TAD, mRNA levels of target genes both in aortic vascular tissue and in the circulating counterparts, may provide important knowledge in the understanding of the pathogenesis of the disease. Since MMP2 and MMP9 are closely related to the maintenance and degradation of the ECM and its components (7-10,12), they were predicted to make significant contributions to the investigation of the pathogenesis of TAD and were selected among other MMPs. In addition, the fact that TIMP2 and TIMP3 have an effect on all MMPs and are particularly effective in the regulation of MMP2 and MMP9 (15-18) are the reasons for their inclusion in the present study. A two-step study was conducted, in which gene expression analysis was performed both in aortic tissue and circulating monocyte cells in patients with TAD and controls. The mRNA levels of *MMP2*, *MMP9, TIMP2* and *TIMP3* genes were investigated to identify candidate factors driving the development of TAD.

Materials and methods

Study design and subjects. The present study included 20 aortic tissue samples and 20 whole blood samples obtained from 10 patients with TAD (2 women and 8 men) and 10 individuals with coronary artery disease (2 women and 8 men) considered as a control group. All the participants underwent a surgical intervention between August 2019 and February 2021. Patients with TAD who were enrolled in the present study were diagnosed by clinicians with acute Stanford Type A aortic dissection. Patients with aortic aneurysms or connective tissue disorders such as Marfan syndrome were excluded. Individuals who underwent coronary artery bypass grafting (CABG) were used as the control. The ascending aorta tissue from the site of the central anastomosis of the CABG was obtained (17,18,23). Whole blood samples were collected during surgery. The experimental flowchart is provided in Fig. 1. The present study was approved (approval no. 2019/525) by the Istanbul Medical Faculty Clinical Research Ethics Committee and the Institute of Graduate Studies in Sciences, Istanbul University (Istanbul, Turkey) and written informed consent was obtained from all the participants.

Monocyte enrichment and RNA isolation. Venous whole blood (10 ml) was drawn from each patient with TAD and the control group, and stored in EDTA tubes. The human monocyte cells were collected from the whole blood through negative selection by using RosetteSep Human Monocyte Enrichment Cocktail (cat. no. 15668; StemCell Technologies, Inc.) with the help of the Ficoll-Hypaque centrifugation method. To apply this method, a whole blood-Rosette mixture prepared according to the manufacturer's recommended protocol was added to a Falcon tube containing 15 ml Ficoll-Hypaque. The addition was performed by spreading the mixture slowly on the surface of the Ficoll to avoid mixing of the blood-Rosette mixture. The tubes were centrifuged at 1,200 x g for 20 min at 4˚C with the brake off. The desired cells were collected by pipette from the layer between the Ficoll and serum layer. All samples were processed for the RNA isolation using the PureLink RNA Mini Kit (cat. no. 12183018A; Thermo Fisher Scientific, Inc.). The obtained total RNA for each sample was reverse transcribed to cDNA with the iScript cDNA Synthesis Kit (cat. no. 1708890; Bio-Rad Laboratories, Inc.).

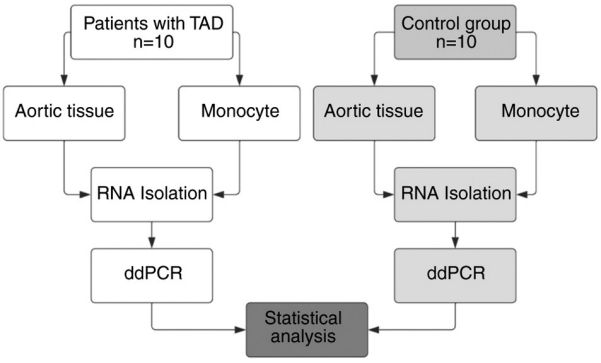

Figure 1. Flowchart of the experimental applications performed. TAD, thoracic aortic dissection; ddPCR, droplet digital PCR.

Gene expression analysis. To analyze the mRNA expression of target genes, the QX200 Droplet Digital PCR System (ddPCR) (Bio-Rad Laboratories, Inc.) was used. The PCR was set up with EvaGreen Supermix (Bio-Rad Laboratories, Inc.) in 20 μl total volume. The following primer sequences for *MMP2*, *MMP9, TIMP2, TIMP3* and *GAPDH* genes were used: *MMP2* gene forward, 5'-GCTACGATGGAGGCGCTA ATG-3' and reverse, 5'-GGGCAGCCATAGAAGGTGTTC-3'; *MMP9* gene forward, 5'-TTTGGTGTCGCGGAGCAC-3' and reverse, 5'-CGAGTTGGAACCACGACGC-3'; *TIMP2* gene forward, 5'-CTGGACGTTGGAGGAAAGAAGG-3' and reverse, 5'-CATCTGGTACCTGTGGTTCAGG-3'; *TIMP3* gene forward, 5'-GCAACTCCGACATCGTGATCC-3' and reverse, 5'-TGGTGAAGCCTCGGTACATCTTC-3'; *GAPDH* gene forward, 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse, 5'-TGGTGAAGACGCCAGTGGA-3'. The reaction mixture of each sample was partitioned into droplets with the QX200 droplet generator and transferred into a 96-well plate. After sealing, the plate was placed into a T100 Thermal Cycler (Bio-Rad Laboratories, Inc.) and the cycling protocol was activated. PCR cycling conditions were as follows: 1 cycle of preincubation at 94˚C for 180 sec, then 39 cycles of 3-step amplification at 94˚C for 40 sec, 60˚C for 40 sec and 72˚C for 50 sec, and 1 cycle of final extension at 72˚C for 300 sec. Since the EvaGreen is a fluorescent dye, the expression values of the target genes were obtained using the QX200 reader (Bio-Rad Laboratories, Inc.) which is the last component of the ddPCR system.

Statistical analysis. All statistical analyses were performed using GraphPad Prism version 9.3.1 (Dotmatics). The numerical and categorical data were presented as the mean, standard error of the mean (SEM), and percentage (%). To reveal whether the data were normally distributed or not,

Table I. Descriptive characteristics of patients with TAD and the control group.

Parameters	Patients with TAD (n=10)	Control group (n=10)	P-value
Mean age ± SEM, years	53.80±3.15	55.10±1.95	0.492
Mean height ± SEM, cm	167.30±2.26	167.60±2.08	0.896
Mean weight ± SEM, kg	84.20±3.60	80.80±2.24	0.245
Mean BMI ± SEM, kg/m^2	30.29±1.67	28.99±1.42	0.393
Smokers, %	60 (n=6)	80 (n=8)	0.639
Sex (female/male), %	20/80 (n=2/8)	20/80 (n=2/8)	0.999
Hypertension, %	100 (n=10)	80 (n=8)	0.478

Mann-Whitney U test was used for continuous variables, and Fisher's exact test was used for categorical variables. TAD, thoracic aortic dissection; SEM, standard error of mean; BMI, body mass index.

the Shapiro-Wilk test was used. For descriptive statistical analysis of the study group, Mann-Whitney U and Fisher's exact test were used for continuous and categorical variables, respectively. In the ddPCR method (24,25), the expression values were obtained as copy/ml for each sample using the QuantaSoft™ software (version 1.7.4; Quantosoft, s.r.o.) for the absolute quantification of the target genes. For each test subject, copy/1ng RNA values were calculated by considering the initial amount of RNA used for the individual reactions. In addition to the target genes, a reference gene (*GAPDH*) was also included in the study to perform the normalization. Since the data was not normally distributed, the expression levels of the target genes (*MMP2, MMP9, TIMP2* and *TIMP3*) between the patients with TAD and the control group were compared using the two-tailed Mann-Whitney U test. The correlation of the expression levels of target genes between aortic tissue and the monocyte cells was performed with the Spearman's correlation test due to the not normally distributed data type. Receiver operating characteristic (ROC) curve analysis was performed and area under the curve (AUC) values were calculated to investigate sensitivity and specificity. The results also revealed the differentiation degree between the patients with TAD and the control group samples and what the diagnostic values of the targeted mRNAs were. For the all-statistical analysis, P<0.05 was considered to indicate a statistically significant difference.

Results

Descriptive characteristics. The descriptive characteristics of the patients with TAD and the control group are revealed in Table I. There was no statistically significant difference in age, height, weight, body mass index (BMI), smoking status, sex and hypertension between the patients with TAD and the respective control group.

mRNA expression analysis. In the aortic wall tissue, the expression level of *MMP2* did not demonstrate a significant difference between patients with TAD and the control group (P=0.85). Expression levels of *MMP9*, *TIMP2* and *TIMP3* genes were revealed to be significantly lower in patients with TAD compared with the control group (P=0.043, P=0.009 and P=0.028, respectively). In the monocyte cells, while the expression level of *TIMP2* (P=0.248) increased, the expression levels of *MMP2* (P=0.148), *MMP9* (P=0.114) and *TIMP3* (P=0.370) slightly decreased in patients with TAD compared with the respective control groups (Table II). However, the observed changes in the expression level of any target genes were not statistically significant in monocyte cells (Fig. 2).

The gene expression level ratios of *MMP2/TIMP2, MMP2/TIMP3, MMP9/TIMP2* and *MMP9/TIMP3* were also calculated in both aorta samples and the circulated monocyte cells. In the aorta samples, *MMP2/TIMP3* (P=0.012) expression ratio was higher in the patients with TAD compared with the control group and this difference was statistically significant. There was no significant difference in *MMP2/TIMP2* (P=0.063), *MMP9/TIMP2* (P=0.248) and *MMP9/TIMP3* (P=0.796) gene expression ratios between the tested groups. In monocyte cells, none of the MMP/TIMP ratios of the examined target genes exhibited a significant difference between the patients with TAD and the respective control group. The following values were found: *MMP2/TIMP2* (P=0.447), *MMP2/TIMP3* (P=0.681), *MMP9/TIMP2* (P=0.796) and *MMP9/TIMP3* (P=0.279) (Fig. 3).

Findings of the ROC curve analyses of the target genes, which demonstrated statistically significant changes at the mRNA expression levels between the patients with TAD and the control group, are as follows. *MMP9* (P=0.041; AUC=0.77; 95% CI, 0.490-0.971), *TIMP2* (P=0.010; AUC=0.840; 95% CI, 0.633-1.00), *TIMP3* (P=0.028; AUC=0.800; 95% CI, 0.600-0.999), *MMP2/TIMP3* (P=0.013; AUC=0.830; 95% CI, 0.644-1.00). The data are presented in Fig. 4. The correlation analysis was performed to reveal whether there was a relationship between the obtained expression levels of the target genes in the aorta and the monocyte cells. Additionally, the correlation analysis was also performed to check the possible relationship between the expression levels of target genes in the same tissue. No significant correlation was identified in the expression levels of *MMP2* (r_s=-0.771, P=0.103), *MMP9* (r_s=0.267, P=0.493), *TIMP2* (r_s=0.503, P=0.144) and *TIMP3* (r_s=-0.524, P=0.197) genes between the aorta and the monocyte cells. In addition, a significant positive correlation between the expression of *MMP2* and *TIMP2* (r_s=0.93, P<0.001) genes in aorta was observed. All the r and P values are presented in Table III.

Table II. mRNA expression levels of target genes in aortic tissue and monocyte cells.

A, Aortic tissue

	MMP2		*MMP9*		*TIMP2*		*TIMP3*	
Groups	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value
TAD	0.0186±0.0045		0.0035±0.0013		0.23±0.035		0.058±0.018	
Control	0.0187±0.0041	0.853	0.0088±0.0029	0.043[a]	0.39±0.049	0.009[a]	0.180±0.048	0.028[a]

B, Monocyte cells

Groups	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value
TAD	0.028±0.025		0.0074±0.0027		0.12±0.053		0.00081±0.00024	
Control	0.0030±0.003	0.148	0.021±0.0067	0.114	0.11±0.022	0.248	0.0037±0.0027	0.370

Mann-Whitney U test was used to calculate the P-values. [a]P<0.05 indicates a statistically significant difference. MMP, matrix metalloproteinases; TIMP, tissue inhibitors of metalloproteinases; SEM, standard error of mean; TAD, thoracic aortic dissection.

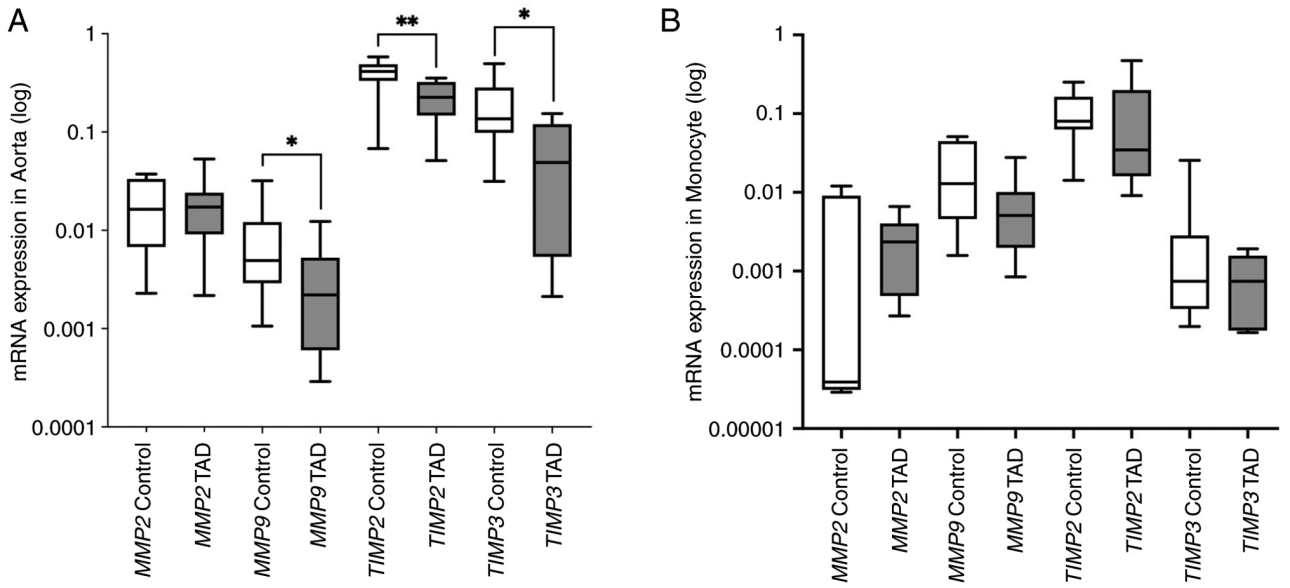

Figure 2. mRNA expression levels of target genes. *MMP2, MMP9, TIMP2* and *TIMP3* expression levels for both patients with TAD and controls in (A) aortic tissue and (B) monocytes cells. *P<0.05 and **P<0.01. MMP, matrix metalloproteinases; TIMP, tissue inhibitors of metalloproteinases; TAD, thoracic aortic dissection.

Discussion

TAD is a lethal condition that occurs when there is a tear in the intima and media layer of the aorta, and can result in death if not treated immediately. Since the aorta is a multilayered tissue, it is composed of numerous different types of cells and molecules. In TAD, a tear in the intima layer extends along the vessel, providing a new route for blood flow and weakening the aortic tissue (20). Although the details of the molecular background and pathogenesis of TAD have not been completely elucidated, MMPs are one of the most important players in TAD and are considered to be responsible for ECM degradation (26,27). Numerous studies have revealed that the expression levels of MMPs differ in cases of TAD (8,9,17,28). The importance of MMPs for the pathogenesis of TAD makes it reasonable to consider their tissue inhibitors as well (14). TIMPs regulate the proteolytic ability and activities of MMPs (29,30).

The present study aimed to investigate the mRNA gene expression levels of *MMP2, MMP9, TIMP2* and *TIMP3* genes in the aortic wall and circulating monocyte cells in patients with TAD. The mRNA expression level of the MMP2 gene both in the aortic wall and monocyte cells did not reveal a statistically significant change in patients with TAD and control subjects (P=0.85 and P=0.15, respectively). *MMP2* can be expressed by the SMC in the aorta. During formation of TAD, a considerable amount of SMC might be lost due to apoptosis (31). *MMP2* also has an important role in tissue

Table III. Spearman's correlation analysis of the target genes.

A, Gene expression in aortic tissue

	MMP2		*TIMP2*		*MMP9*		*TIMP3*	
Genes	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
MMP2	1.00	-	0.93	<0.001[a]	0.02	0.973	0.30	0.407
TIMP2	0.93	<0.001[a]	1.00	-	-0.03	0.946	0.33	0.349
MMP9	0.02	0.973	-0.03	0.946	1.00	-	0.04	0.918
TIMP3	0.30	0.407	0.33	0.349	0.04	0.918	1.00	-

B, Gene expression in monocyte cells

	MMP2		*TIMP2*		*MMP9*		*TIMP3*	
Genes	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
MMP2	-0.77	0.103	-0.60	0.242	0.14	0.803	-0.54	0.297
TIMP2	0.38	0.279	0.50	0.144	0.24	0.513	0.41	0.247
MMP9	-0.05	0.912	0.15	0.708	0.26	0.493	-0.60	0.097
TIMP3	-0.21	0.619	-0.07	0.882	0.17	0.703	-0.52	0.197

[a]P<0.01 indicates a statistically significant difference. r_s Spearman's correlation coefficient; MMP, matrix metalloproteinases; TIMP, tissue inhibitors of metalloproteinases.

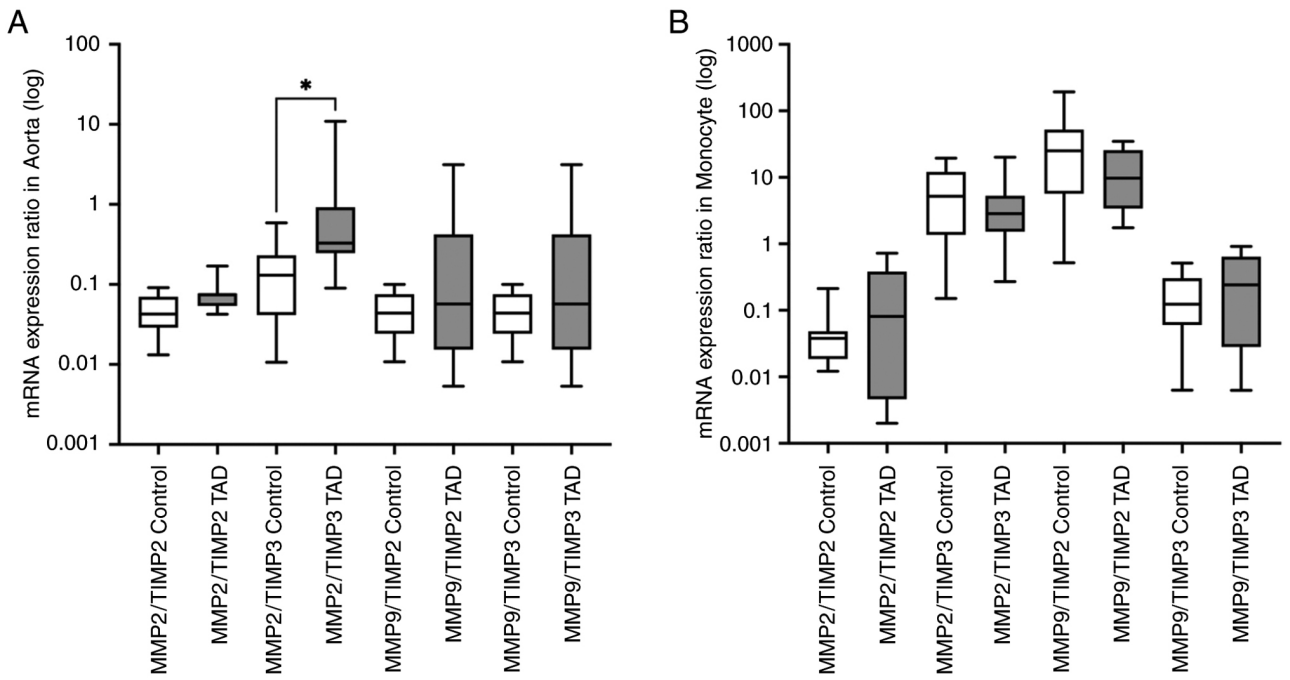

Figure 3. MMP/TIMP mRNA gene expression level ratios of target genes. MMP/TIMP gene expression ratios of target genes in patients with TAD and controls in (A) aortic tissue and (B) monocyte cells.*P<0.05. MMP, matrix metalloproteinases; TIMP, tissue inhibitors of metalloproteinases; TAD, thoracic aortic dissection.

remodeling in patients with TAD, which can be considered as an important part of the wound healing process. The MMP2 level starts to increase after the acute phase in TAD (32). It is clearly observed that MMP2 levels can vary, and can either be increased or decreased in the aortic tissues of patients with TAD, as revealed in previous studies (8,17). These inconsistencies with regard to the MMP2 levels may be due to the disease stage differences in patients with TAD (31). Additionally, the results of a meta-analysis study supported that there was no difference between patients with TAD and control groups

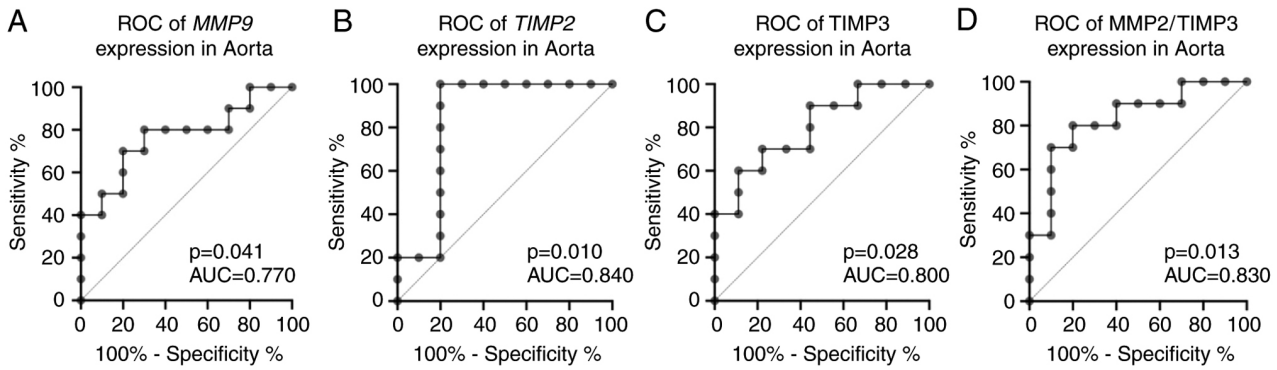

Figure 4. ROC curve analyses and AUC of *MMP9, TIMP2, TIMP3* and *MMP2/TIMP3* gene expression levels in patients with TAD and the control group in aorta. (A) *MMP9* (P=0.041; AUC=0.77; 95% CI, 0.490-0.971), (B) *TIMP2* (P=0.010; AUC=0.840; 95% CI, 0.633-1.00), (C) *TIMP3* (P=0.028; AUC=0.800; 95% CI, 0.600-0.999), (D) *MMP2/TIMP3* (P=0.013; AUC=0.830; 95% CI, 0.644-1.00). ROC, receiver operating characteristic; AUC, area under curve; CI, confidence interval; MMP, matrix metalloproteinases; TIMP, tissue inhibitors of metalloproteinases; TAD, thoracic aortic dissection.

for the circulating mRNA expression level of the MMP2 gene (10). The complex molecular background of TAD and the fact that MMPs often work cooperatively with other MMPs and TIMPs to exert different functions (degradation of ECM and remodeling of tissues) rather than working alone may explain the undifferentiated gene expression level of *MMP2* in cases of TAD.

In addition, it was further revealed in the present study that the mRNA gene expression level of the *MMP9* gene in aortic tissue was significantly decreased (P=0.043) in patients with TAD. Even though a decreased mRNA level for the *MMP9* gene was also detected in monocyte cells in cases with TAD, the result was not statistically significant (P=0.114). Moreover, the results of the *MMP9* ROC curve analysis in the aorta revealed that *MMP9* expression levels may be used to differentiate patients with TAD and the control group (AUC=0.770; P=0.041). It was revealed that the level of MMP9 did not present a statistically significant difference in patients with TAD (17). Since MMP9 is an important player in the wound-healing process in the intima layer of the aorta (33), it can be inferred that its measured amount might be affected by the rupture within the onset of the dissection. By contrast, there are a number of studies that conclude that the expression level of *MMP9* was increased in patients with TAD whether in aortic tissue or in plasma (8-10,17). It is known that *MMP9* is also stimulated in response to mechanical injury (34). The aortic region where the medial dissection begins may be the site of ECM alteration caused by hemodynamic stress (35,36). This may explain the changes in the expression level of *MMP9* in the patients with TAD (9). The decreased *MMP9* mRNA expression level obtained in patients with TAD in the present study expresses the level at a particular time point, not the time-dependent variation. It can be surmised that the aortic samples obtained during the surgical operation might not represent the exact same disease stage. The aortic tissue may still be under the influence of mechanical signaling or the wound may not have started the healing process. This difference may lead to different *MMP9* levels in patients with TAD. It can be concluded that further studies are required to confirm the importance of the findings on *MMP9* for improved understanding of the pathogenesis of TAD.

TIMPs are found in aortic tissue concurrently with MMPs. Any change in the expression level of a TIMP may change the MMP/TIMP ratio, resulting in a change in MMP activity (14). In the present study, it was revealed that there was a statistically significant decrease in the mRNA expression levels of *TIMP2* (P=0.009) and *TIMP3* (P=0.028) in the aorta of cases with TAD. Furthermore, ROC curve analyses of *TIMP2* (AUC=0.840; P=0.010) and *TIMP3* (AUC=0.800; P=0.028) in aortic tissue demonstrated that patients with TAD and control groups can be differentiated by using *TIMP2* and *TIMP3* mRNA expression levels in aorta. No significant difference was observed in terms of *TIMP2* and *TIMP3* gene expression in monocyte cells. In a previous study, in which ELISA was used, it was revealed that the expression level of TIMP2 was decreased in the aorta of cases with TAD. It is stated that the decreased expression level of TIMP2 might represent the pre-dissection situation rather than the dissection-related function such as wound healing (17). In addition, it was revealed that the mRNA level of *TIMP3* was decreased in aortic tissue in patients with TAD. It was shown that the decrease in TIMP3 level may have occurred as a result of stimulation by TNF-α and TGF-β signalling *in vitro* (37). Considering the role of TGF-β on ECM component regulation, fibrosis, and the regulation of *MMP2* and *MMP9* genes (1), it can be inferred that the decrease of TGF-β may directly affect the expression levels of *TIMP3*, *MMP2*, and *MMP9*. The present study has provided supporting results to the previous studies (1,17,37) for *TIMP2* and *TIMP3* gene expression in patients with TAD. Since TIMPs are the regulators of the MMPs, the decrease in their gene expression levels might be associated with the elevated MMP function or the elevated MMP/TIMP ratio, which may cause increased degradation in aorta tissue in patients with TAD.

MMP/TIMP ratio is as important as the individual expression changes of MMP and TIMP genes in explaining the pathogenesis of TAD. It has been reported that due to impaired MMP/TIMP balance, MMP functions in the cell are moving toward the proteolytic state (1,19). Decreased TIMP2/MMP2 results were also obtained in patients with TAD in a study by Manabe *et al* (17), and they supported the theory that an increased MMP/TIMP ratio has shifted the cell into the proteolytic stage. In the results of the present study, the increase

of *MMP2/TIMP2* (P=0.063), *MMP2/TIMP3* (P=0.012) gene expression ratios were revealed in aortic tissue. Based on these findings, it can be considered that increasing MMP/TIMP ratios may cause a tendency to increase aortic lysis. Moreover, ROC curve analysis of the MMP2/TIMP3 expression ratio revealed that MMP2/TIMP3 (AUC=0.830; P=0.013) may differentiate patients with TAD from the control group.

The aim of the present study was to also investigate whether there was a relationship between aortic tissue and monocyte cells in terms of the expression levels of target genes. As a result of the correlation analysis, it was revealed that gene expression levels of *MMP2* and *TIMP2* exhibited a positive correlation in the aortic tissue (r_s=0.93; P<0.001). This result supports the close functional relationship of *MMP2* and *TIMP2* genes with each other (14,15). Accordingly, the positive correlation of the mRNA levels of MMPs and TIMPs, which may alter the MMP/TIMP ratio, might support the importance of the MMP/TIMP ratio in cases with TAD. Further analysis may provide further insight with regard to the MMP-TIMP relationship in patients with TAD.

Additionally, a limitation of the present study is the absence of protein level analysis. It is clear that performing protein level analysis would contribute greatly to the validation of the gene expression data. However, protein analysis could not be included in the present study due to the limited amount of vascular material used as control tissue. Considering the risks of dividing the small amount of tissue into two parts, the obtained material was only used for RNA isolation in sufficient quantity.

In conclusion, the present study revealed that *MMP9, TIMP2* and *TIMP3* genes have differentiated gene expression in aortic vascular tissue of patients with TAD. Considering the role of MMPs in matrix maintenance, altered gene expression might be expected in TAD. However, the altered expression of MMP inhibitors (TIMPs) in cases with TAD are noteworthy. It can be acknowledged that TIMPs as well as MMPs have an important role in understanding the pathogenesis of TAD. Additionally, it was considered that examining MMPs along with TIMPs rather than alone is more informative in terms of understanding the disease pathogenesis. Considering the statistically significant differences in *MMP9, TIMP2* and *TIMP3* gene expression, as well as *MMP2/TIMP3* ratio, it can be inferred that future studies in both aortic tissue and circulation at the protein level by increasing the sample size may be an important step for the early diagnosis of TAD. Furthermore, considering the large number of members of the MMP and TIMP gene families, it is likely that they affect each other in various combinations. In accordance with this point of view, the possible cross-interference of all MMPs and TIMPs are planned to be analyzed in future studies.

Acknowledgements

Not applicable.

Funding

The funding of the present study was provided by the Scientific Research Projects Coordination Unit of Istanbul University (grant no. 34118).

Availability of data and materials

The data generated in the present study are not publicly available due to sensitivity reasons but may be requested from the corresponding author.

Authors' contributions

TK, TG and AA designed the study. AA provided the patient and control samples through the surgical process. TK performed the isolations and ddPCR applications. TK and TG performed the statistical analysis and prepared the manuscript. AA revised the final version of the manuscript. TK and TG confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. 2019/525) by the Istanbul Medical Faculty Clinical Research Ethics Committee and the Institute of Graduate Studies in Sciences, Istanbul University (Istanbul, Turkey). Written informed consent was obtained from all the participants. All the procedures and applications conducted in the present study adhere to the tenets of The Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Wu D, Shen YH, Russell L, Coselli JS and LeMaire SA: Molecular mechanisms of thoracic aortic dissection. J Surg Res 184: 907-924, 2013.
2. Clouse WD, Hallett JW Jr, Schaff HV, Spittell PC, Rowland CM, Ilstrup DM and Melton LJ: Acute aortic dissection: Population-based incidence compared with degenerative aortic aneurysm rupture. Mayo Clin Proc 79: 176-180, 2004.
3. Mészáros I, Mórocz J, Szlávi J, Schmidt J, Tornóci L, Nagy L and Szép L: Epidemiology and clinicopathology of aortic dissection. Chest 117: 1271-1278, 2000.
4. Golledge J and Eagle KA: Acute aortic dissection. Lancet 372: 55-66, 2008.
5. He R, Guo DC, Estrera AL, Safi HJ, Huynh TT, Yin Z, Cao SN, Lin J, Kurian T, Buja LM, *et al*: Characterization of the inflammatory and apoptotic cells in the aortas of patients with ascending thoracic aortic aneurysms and dissections. J Thorac Cardiovasc Surg 131: 671-678, 2006.
6. Ishii T and Asuwa N: Collagen and elastin degradation by matrix metalloproteinases and tissue inhibitors of matrix metalloproteinase in aortic dissection. Hum Pathol 31: 640-646, 2000.
7. Ra HJ and Parks WC: Control of matrix metalloproteinase catalytic activity. Matrix Biol 26: 587-596, 2007.
8. Koullias GJ, Ravichandran P, Korkolis DP, Rimm DL and Elefteriades JA: Increased tissue microarray matrix metalloproteinase expression favors proteolysis in thoracic aortic aneurysms and dissections. Ann Thorac Surg 78: 2106-2110; discussion 2110-2101, 2004.
9. Sangiorgi G, Trimarchi S, Mauriello A, Righini P, Bossone E, Suzuki T, Rampoldi V and Eagle KA: Plasma levels of metalloproteinases-9 and -2 in the acute and subacute phases of type A and type B aortic dissection. J Cardiovasc Med (Hagerstown) 7: 307-315, 2006.

10. Takagi H, Hari Y, Nakashima K, Kuno T and Ando T: Matrix metalloproteinases and acute aortic dissection: Et Tu, Brute? Interact Cardiovasc Thorac Surg 30: 465-476, 2020.
11. Wang X, LeMaire SA, Chen L, Shen YH, Gan Y, Bartsch H, Carter SA, Utama B, Ou H, Coselli JS and Wang XL: Increased collagen deposition and elevated expression of connective tissue growth factor in human thoracic aortic dissection. Circulation 114 (1 Suppl): I200-I205, 2006.
12. Chung AW, Yang HH, Radomski MW and van Breemen C: Long-term doxycycline is more effective than atenolol to prevent thoracic aortic aneurysm in marfan syndrome through the inhibition of matrix metalloproteinase-2 and -9. Circ Res 102: e73-e85, 2008.
13. Chow AK, Cena J and Schulz R: Acute actions and novel targets of matrix metalloproteinases in the heart and vasculature. Br J Pharmacol 152: 189-205, 2007.
14. Wang X and Khalil RA: Matrix metalloproteinases, vascular remodeling, and vascular disease. Adv Pharmacol 81: 241-330, 2018.
15. Bourboulia D and Stetler-Stevenson WG: Matrix metalloproteinases (mmps) and tissue inhibitors of metalloproteinases (timps): Positive and negative regulators in tumor cell adhesion. Semin Cancer Biol 20: 161-168, 2010.
16. Arpino V, Brock M and Gill SE: The role of timps in regulation of extracellular matrix proteolysis. Matrix Biol 44-46: 247-254, 2015.
17. Manabe T, Imoto K, Uchida K, Doi C and Takanashi Y: Decreased tissue inhibitor of metalloproteinase-2/matrix metalloproteinase ratio in the acute phase of aortic dissection. Surg Today 34: 220-225, 2004.
18. Lesauskaite V, Tanganelli P, Sassi C, Neri E, Diciolla F, Ivanoviene L, Epistolato MC, Lalinga AV, Alessandrini C and Spina D: Smooth muscle cells of the media in the dilatative pathology of ascending thoracic aorta: Morphology, immunoreactivity for osteopontin, matrix metalloproteinases, and their inhibitors. Hum Pathol 32: 1003-1011, 2001.
19. Chow BS, Chew EG, Zhao C, Bathgate RA, Hewitson TD and Samuel CS: Relaxin signals through a rxfp1-perk-nnos-no-cgmp-dependent pathway to up-regulate matrix metalloproteinases: The additional involvement of inos. PLoS One 7: e42714, 2012.
20. Shen YH and LeMaire SA: Molecular pathogenesis of genetic and sporadic aortic aneurysms and dissections. Curr Probl Surg 54: 95-155, 2017.
21. Samadzadeh KM, Chun KC, Nguyen AT, Baker PM, Bains S and Lee ES: Monocyte activity is linked with abdominal aortic aneurysm diameter. J Surg Res 190: 328-334, 2014.
22. Lu L, Tong Y, Wang W, Hou Y, Dou H and Liu Z: Characterization and significance of monocytes in acute stanford type B aortic dissection. J Immunol Res 2020: 9670360, 2020.
23. Zhang L, Yu C, Chang Q, Luo X, Qiu J and Liu S: Comparison of gene expression profiles in aortic dissection and normal human aortic tissues. Biomed Rep 5: 421-427, 2016.
24. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, *et al*: High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem 83: 8604-8610, 2011.
25. Hindson CM, Chevillet JR, Briggs HA, Gallichotte EN, Ruf IK, Hindson BJ, Vessella RL and Tewari M: Absolute quantification by droplet digital PCR versus analog real-time PCR. Nat Methods 10: 1003-1005, 2013.
26. Iyer RP, Patterson NL, Fields GB and Lindsey ML: The history of matrix metalloproteinases: Milestones, myths, and misperceptions. Am J Physiol Heart Circ Physiol 303: H919-H930, 2012.
27. Coady MA, Rizzo JA, Goldstein LJ and Elefteriades JA: Natural history, pathogenesis, and etiology of thoracic aortic aneurysms and dissections. Cardiol Clin 17: 615-635; vii, 1999.
28. Weis-Müller BT, Modlich O, Drobinskaya I, Unay D, Huber R, Bojar H, Schipke JD, Feindt P, Gams E, Müller W, *et al*: Gene expression in acute stanford type A dissection: A comparative microarray study. J Transl Med 4: 29, 2006.
29. Djuric T and Zivkovic M: Overview of MMP biology and gene associations in human diseases. In: The Role of Matrix Metalloproteinase in Human Body Pathologies. IntechOpen, London, 2017.
30. Brew K and Nagase H: The tissue inhibitors of metalloproteinases (timps): An ancient family with structural and functional diversity. Biochim Biophys Acta 1803: 55-71, 2010.
31. Zhang X, Wu D, Choi JC, Minard CG, Hou X, Coselli JS, Shen YH and LeMaire SA: Matrix metalloproteinase levels in chronic thoracic aortic dissection. J Surg Res 189: 348-358, 2014.
32. Akiyama M, Ohtani H, Sato E, Nagura H and Tabayashi K: Up-regulation of matrix metalloproteinase-2 and membrane-type 1-matrix metalloproteinase were coupled with that of type I procollagen in granulation tissue response after the onset of aortic dissection. Virchows Arch 448: 811-821, 2006.
33. Schneiderman J, Bordin GM, Adar R, Smolinsky A, Seiffert D, Engelberg I, Dilley RB, Thinnes T and Loskutoff DJ: Patterns of expression of fibrinolytic genes and matrix metalloproteinase-9 in dissecting aortic aneurysms. Am J Pathol 152: 703-710, 1998.
34. James TW, Wagner R, White LA, Zwolak RM and Brinckerhoff CE: Induction of collagenase and stromelysin gene expression by mechanical injury in a vascular smooth muscle-derived cell line. J Cell Physiol 157: 426-437, 1993.
35. Schlatmann TJ and Becker AE: Pathogenesis of dissecting aneurysm of aorta. Comparative histopathologic study of significance of medial changes. Am J Cardiol 39: 21-26, 1977.
36. Roberts WC: Aortic dissection: Anatomy, consequences, and causes. Am Heart J 101: 195-214, 1981.
37. Kimura N, Futamura K, Arakawa M, Okada N, Emrich F, Okamura H, Sato T, Shudo Y, Koyano TK, Yamaguchi A, *et al*: Gene expression profiling of acute type A aortic dissection combined with in vitro assessment. Eur J Cardiothorac Surg 52: 810-817, 2017.