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Serum matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in patients with familial Mediterranean fever

INTESTINE

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Original Article

ABSTRACT

Background/Aims: Serum matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) are well-known inflammatory biomarkers, with a diagnostic potential for various diseases. The aim of the present study was to determine the potential diagnostic applications of serum MMP-9 and TIMP-1 concentrations in patients with familial Mediterranean fever (FMF).

Materials and Methods: A total of 66 male FMF patients and 40 age-matched healthy subjects were included in this research. TIMP-1 and MMP-9 levels with conventional inflammation markers were determined. Pearson correlation analysis was used to determine the correlation between the characteristics of patients and the laboratory data.

Results: In patients with FMF, serum MMP-9 levels and MMP-9/TIMP-1 ratios were found to be significantly elevated in both acute episode and asymptomatic periods (p=0.0001 and p=0.0001, respectively). There was no significant difference between TIMP-1 levels. A significant negative correlation between patients' current age and TIMP-1 level in patients with acute episodes was detected (p=0.0008, r=–0.52). Moreover, a moderate negative correlation was noticed between erythrocyte sedimentation rate and TIMP-1 level in patients with acute episodes (p=0.01, r=–0.39). Additionally, a moderate negative correlation was found between the duration of colchicine use and MMP-9 and TIMP-1 levels during the attack period (p=0.04, r=–0.36 and p=0.02, r=–0.39, respectively).

Conclusion: Our findings demonstrate that a significant MMP-9/TIMP-1 imbalance exists in patients with FMF, which reflects an ongoing inflammation in both FMF periods. Thus, the increased MMP-9 levels observed in FMF patients could rationalize therapeutic targeting to MMPs.

Keywords: Familial Mediterranean fever, metalloproteinases, inflammation, subclinical inflammation, chronic inflammation

INTRODUCTION

The matrix metalloproteinases (MMPs) are a family of neutral endopeptidases that are involved in the degradation of the extracellular matrix (ESM). Under physiological conditions, the activity of MMPs is regulated by the transcription levels, activation of the zymogen precursors, and interaction with specific endogenous ESM components and endogenous inhibitors (tissue inhibitor of metalloproteinase; TIMP) (1-3). In normal tissue components, MMPs and TIMPs are expressed at low levels and are involved in many biological processes. If the MMP/TIMP balance shifts towards MMP, then it leads to an uncontrolled matrix collapse and predisposes to the formation of pathophysiologic events (3-6).

Familial Mediterranean fever (FMF) is the most frequently encountered periodic fever syndrome and is characterized by mutations in the MEFV (Mediterranean Fever) gene, which encodes pyrin (7-9). With recurrent FMF serositis attacks, permanent tissue destruction and repair takes place. Although what causes and ends FMF attacks is not completely understood, it is currently suggested that the increased activation of inflammasome and hypersecretion of interleukin 1-β (IL-1β) has a piv-

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otal role in the pathogenesis of FMF (10-13). Moreover, there is an increasing number of evidence in the literature regarding an ongoing subclinical inflammation in FMF, such as the existence of high cytokine levels even during the asymptomatic period (14-16).

Based on these proven data, we conducted a crosssectional study to assess serum MMP-9 and TIMP-1 levels and their correlation with conventional inflammation parameters in patients with FMF. Moreover, we determined whether these endogenous molecules are associated with recurrent attacks during the course of the disease.

MATERIALS AND METHODS

Patients

Prior to enrolling participants in the current research, ethical approval was received and patients signed an informed consent form. In total, 77 male patients with FMF and 40 age-matched healthy males who attended our Gastroenterology Outpatient Clinic between August 2011 and March 2012 were randomly included in the study. Eleven patients who had attacks within the last 10 days were excluded from the study to prevent selection bias. Therefore, we conducted the research with 66 male FMF patients and 40 healthy controls, i.e., a total of 106 participants. Thirty-seven of 66 (56.07%) patients were evaluated only during the asymptomatic period, 10 (15.15%) were evaluated only during an acute episode, and the remaining 19 patients (28.78%) were followed in both periods. The mean age of patients was 21.65±0.47 (min=19, max=32) years, and the mean age of the healthy controls was 21.20±0.3 (min=19, max=28) years.

All FMF patients were diagnosed based on the Tel-Hashomer criteria with further genetic verification for the presence of MEFV mutations. Acute episodes and asymptomatic periods were differentiated by clinical and biochemical analysis. The asymptomatic period was identified as the period at least 10 days after an attack, with an absence of signs and symptoms of an acute episode. Blood samples of 39 patients with acute episodes were taken within 12 h of the beginning of fever and abdominalgia. Patients who used non-steroidal anti-inflammatory drugs, steroids, or other immune suppressor/immune-modulating drugs and those with conditions such as vasculitis, malignancy, cardiovascular disease, metabolic syndrome, chronic liver disease, amyloidosis, and FMF arthropathy were excluded from the study.

Laboratory examinations

The parameters were studied using the following devices: Erythrocyte sedimentation rate, Vacuette Sed Rate Screener 100 (Greiner Bio-One GmbH, Austrija) with the precipitation method; Fibrinogen, Architect ci8200 (Abbott Diagnostics Division, Santa Clara, CA, USA); complete blood count, Cell-Dyn Sapphire (Abbott Diagnostics Division, Santa Clara, CA, USA); and MEFV mutation analysis (M694V, M694I, M680I, V726A, E148Q), Stratagene MX3000 (Agilent technologies, Santa Clara, California,USA) using the real-time PCR method. MMP-9 and TIMP-1 were tested at the end of the research period on separated serum, which had been stored at –80°C. They were measured with a Rayto ELISA reader (Rayto Life and Analytical Sciences Co., Ltd., Shenzen, China) using Adipobioscien brand kits (Cat no: SK00160-02 and SK00039-02, respectively).

Statistical analysis

All statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) (Version 15.0, SPSS Inc; Chicago, IL, USA). The parameters of patients and controls were calculated as mean values and value ranges. To check for normal distributions, we used the Kolmogorov–Smirnov test. Laboratory data of patients in the asymptomatic periods and acute episodes were compared using the paired sample t-test or the Wilcoxon signed-rank test. Student's t test or the Mann–Whitney U-test was used to compare laboratory data of patients, in both asymptomatic periods and acute episodes, and the healthy controls. The ROC analysis was performed to determine the specificity and sensitivity of MMP-9 and TIMP-1. Pearson correlation analysis was used to determine the correlation between characteristics of patients and the laboratory data. A "p" value of less than 0.05 was regarded as statistically significant for the current FMF study.

RESULTS

MMP-9 levels and MMP-9/TIMP-1 levels of FMF patients during both periods were significantly higher than those of the healthy controls (p=0.0001 and p=0.0001, respectively) (Table 1). For patients in the asymptomatic period, the mean erythrocyte sedimentation rate was 9.04±1.06 mm/h, mean fibrinogen level was 296.94±13.33 mg/dL, and mean WBC count was $8.61\pm0.41\times10^3$/μL. In patients in the asymptomatic period, the median C-reactive protein (CRP) levels were 4.18 mg/dL (2.4–23.10 mg/dL). During the acute episode period, the median CRP levels were 84.9 mg/dL (20–371 mg/dL) (p<0.001).

In this study, MEFV mutation analysis was performed for a total of 42 patients, and FMF gene mutation results are given in Table 3. There was no statistically significant difference between patients with one mutation and those with more than one mutation in both periods. In addition, there was no statistically significant difference between patients with and without the homozygous M694V mutation. Moreover, no significant results were found when the data were analyzed for the other FMF gene mutations.

TIMP-1 levels were significantly higher in patients with FMF whose age of onset was less than 8 years than in those with an onset after 8 years, particularly in asymptomatic periods (p=0.03). However, this relationship was not demonstrated in patients experiencing acute episodes. When patients with a daily colchicine dose of <1.5 mg or ≥1.5 mg were compared, no significant differences were found in either period.

Table 1. Demographic features and laboratory values of FMF patients and healthy controls

	FMF Patients			
	Asymptomatic (n=56)	**Attack period (n=29)**	**Controls (n=40)**	**p**
Gender (F/M)	M	M	M	NS
Fibrinogen (g/dL)	296.94±13.33	523.47±19.67	-	p<0.001
WBC ($mm^3 \times 10^3$)	8.61±0.41	12.53±0.60	-	p<0.001
ESR (mm/h)	9.04±1.06	35.88±2.90	-	p<0.001
MMP-9 (ng/mL)	861.54 (±73.73)	1011.69 (± 126.11)	364.99 (±52.33)	p<0.001
TIMP-1 (ng/mL)	316.50 (±10.90)	300.93 (±20.05)	319.81 (±18.78)	NS
MMP-9/TIMP-1 ratio	2.97 (±0.35)	3.43 (±0.38)	1.35 (±0.21)	p<0.001
Age of onset (years)	7.9±1.4	7.5±1.6	-	NS

NS: not significant; MMP-9: matrix metalloproteinase-9; TIMP-1: tissue inhibitor of metalloproteinase-1
*Data are presented as mean±SD.

Table 2. Overall accuracy and ROC analyses of MMP and MMP-9/TIMP-1 ratio among patients with FMF

		Cut-off value	**Sensitivity**	**Specificity**	**PPV %**	**NPV %**	**Overall accuracy %**
MMP-9	Acute attack	519.6	79.3	82.5	76.7	84.6	80.6
	Asymptomatic period	464	75	75	80.8	68.2	74.5
MMP-9/TIMP-1 Ratio	Acute attack	1.594	82.8	75	70.6	85.7	78
	Asymptomatic period	1.547	75	72.5	79.2	67.4	73

PPV: positive predictive value; NPV: negative predictive value; MMP-9: matrix metalloproteinase-9; TIMP-1: tissue inhibitor of metalloproteinase-1

Further, no statistically significant difference was detected between the two periods with respect to the duration of colchicine use.

The sensitivity, specificity, and positive/negative predictive values at specific cut-off points of MMP-9 and MMP-9/TIMP-1 in acute attack and asymptomatic periods derived from the ROC curve coordinates are shown in Table 2. ROC curves are shown in Figures 1 and 2. In the correlation analysis, a moderate negative correlation between patients' current age and TIMP-1 level of patients with acute episodes was detected (p=0.0008, r=−0.52) (Figure 3). Moreover, a moderate negative correlation between the duration of colchicine use and MMP-9 level of patients with acute episodes (p=0.04, r=−0.36) was also discerned. In addition, a moderate negative correlation between the duration of colchicine use and TIMP-1 level in patients with acute episodes (p=0.02, r=−0.39), a moderate negative correlation between erythrocyte sedimentation rate and TIMP-1 level in patients with acute episodes (p=0.01, r=−0.39) (Figure 3), a moderate negative correlation between fibrinogen level and TIMP-1 level in participants with acute episodes (p=0.04, r=−0.33), and a moderate negative correlation between the age of onset of illness and TIMP-1 level in patients with an asymptomatic period (p=0.0001, r=−0.56) were also detected.

MEFV gene mutations of patients are shown in Table 3.

Table 3. MEFV gene mutations of patients (42 patients available)

	Homozygous	**Heterozygous**	**Total**
M694V	15	19	34
E148Q	1	3	4
M680I	2	8	10
M694I	0	2	2
V726A	2	6	8

DISCUSSION

The most important finding of this study, which aimed to assess the importance of MMP-9 and TIMP-1 levels in patients with FMF, was that serum MMP-9 levels and MMP-9/TIMP-1 ratios were significantly higher in both asymptomatic periods and acute episodes of FMF than those in healthy controls (p=0.0001 and p=0.0001, respectively). However, there were no significant differences in TIMP-1 levels of patients with FMF when compared to healthy controls in both periods (p=0.88 and p=0.49, respectively) (Table 1). Based on these findings, the balance shifting towards MMP in both FMF periods may be a reflection of both acute inflammation and ongoing subclinical inflammation. If we just consider the 19 patients using data from both FMF periods, there was no significant difference in the levels of those mentioned markers between the two FMF periods, which means that MMP activation reaches a significant level even during the asymptomatic period.

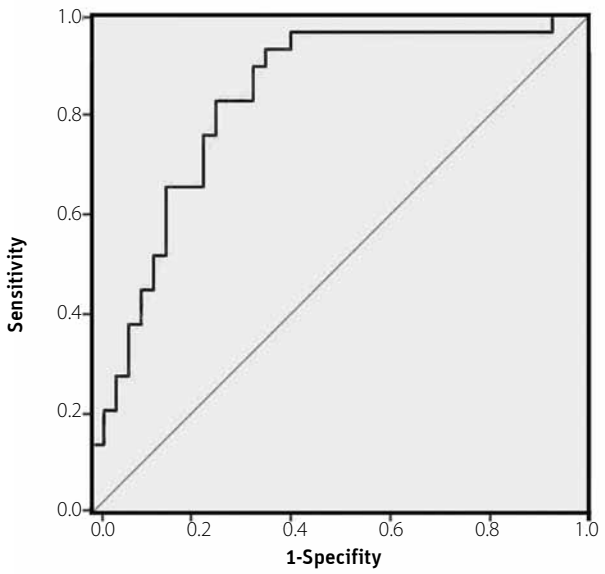

Figure 1. Receiver operating characteristic (ROC) curve of MMP-9/TIMP-1 ratio in predicting acute attack in familial Mediterranean fever patients.

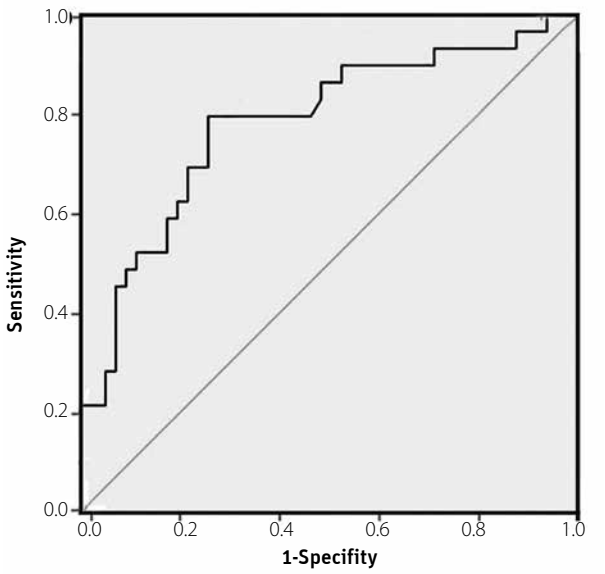

Figure 2. Receiver operating characteristic (ROC) curve of MMP-9 in predicting acute attack in familial Mediterranean fever patients.

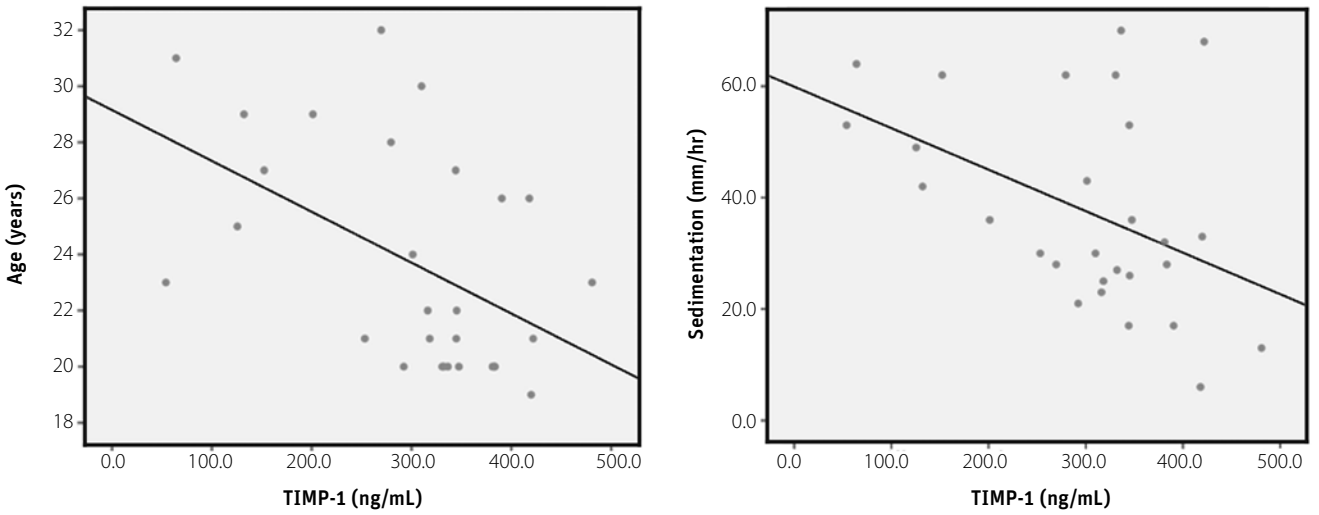

Figure 3. a,b. Spearman correlation between TIMP-1 and age (a) and erythrocyte sedimentation rate (b).

Besides, there was no statistically significant difference in the effects of colchicine use and colchicine dose on the levels of MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio in both FMF periods. This finding may be due to inappropriate colchicine use. The negative correlation between the duration of colchicine use and TIMP-1 levels in acute FMF episodes might indicate that a prolonged use of colchicine suppresses the inflammatory response, particularly in acute FMF episodes. If we accept that the MMP/TIMP system reflects the inflammatory response in FMF, it follows that the inhibitory effect of colchicine on FM-related chronic inflammation is inadequate.

In both FMF periods, TIMP-1 levels were at almost the same level as healthy controls, and TIMP-1 levels were not accompanied by high MMP-9 levels, i.e., a high TIMP-1 response had not occurred. This finding may be due to a disorder in TIMP-1 regulating mechanisms. Previously, it has been shown that in the case of myocardial infarction, the serum TIMP-1 level increases sharply because of inadequate blood supply (17,18). Also, in FMF, chronic peritonitis causes ischemic damage. However, it can be hypothesized that serosal damage may not be not as severe as myocardial ischemic damage, based on our unchanged TIMP-1 levels. Moreover, serosal membranes may be more resistant to ischemia, and this theory may explain the stable TIMP-1 levels in patients with FMF.

Patients of male gender and those with some FMF severity index parameters (early onset of the disease, frequency of acute episodes, and several risk factors for the development of amyloidosis) are more prone to chronic inflammation in FMF disease (7,19,20), and all of our participants were male. TIMP-1 levels were obviously higher in patients with FMF whose onset

of illness was less than 8 years of age than in those whose onset was after 8 years of age, particularly in asymptomatic periods (p=0.03). Compatible with this finding, a moderate negative correlation was found between age of onset and TIMP-1 levels of asymptomatic FMF patients (p=0.0001, r=–0.56). However, moderate negative correlations between sedimentation, fibrinogen level, and TIMP-1 level of participants were found (p=0.01, r=–0.39 and p=0.04, r=–0.33, respectively) during the acute FMF period. This means that TIMP-1 levels are reduced or not increased in acute FMF periods. These interesting findings suggest that the mechanisms that regulate the inflammatory response in various FMF phases are different. These findings may offer new insights to FMF researchers.

Increased inflammasome activation and hypersecretion of IL-1β play important roles in FMF pathogenesis (5,6). In a previous study, it was reported that IL-1β is a potent stimulator of MMP secretion, and IL-1β itself is destroyed by MMP-1,-2,-3, and -9. Moreover, this destruction is blocked by TIMP-1 (21). Hence, higher MMP-9 levels and MMP-9/TIMP-1 rates and stable TIMP-1 levels of FMF patients may be a preventive or adaptive response of the human body against IL-1β. The suppressor effect of MMPs in the IL-1β-mediated inflammatory response may explain the self-limited nature of FMF attacks. In addition, elevated MMP-9 levels during the asymptomatic FMF period may be a controlling response against ongoing subclinical inflammation. These theorical explanations should be explored with well-designed molecular and clinical studies.

Although we found some promising results, our study has some limitations. Firstly, our research was a case control study. Secondly, only 66 male patients with FMF were included in the current research. Because our hospital is a military hospital and generally cares for military personnel, we only performed the analysis for males. Finally, because of the short study period, we included a limited number of FMF patients to our research. Despite these limitations, our encouraging findings may positively contribute to the enlightening of the pathopyhsiology of FMF disease, which is not yet wholly understood. Moreover, our findings suggested that these markers are potential markers for FMF diagnosis. Therefore, MMP-9 and TIMP-1 levels may be not only indicators of disease activity but also a potential source of studies on the pathophysiology of FMF.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients' parents who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.D., A.T., Y.Y.; Design - M.D., A.T., Y.Y., Y.B.; Supervision - M.D., A.T., Y.Y., Y.B., L.D.; Resource - M.D., A.T., Y.Y., K.Ö., Y.Ö.; Materials - M.D., A.T., Y.Y., K.Ö., Y.Ö.; Data Collection and/or Processing - M.D., A.T., Y.Y., K.Ö., Y.Ö., S.H., M.K., İ.K., Y.S.S., L.D.; Analysis and/or Interpretation - M.D., A.T., Y.Y., Y.B.; Literature Search - M.D., A.T.,Y.Y., Y.B., K.Ö., Y.Ö., S.H.; Writing - M.D., A.T., Y.Y., Y.B., Y.S.S.; Critical Reviews - M.D., A.T., Y.Y., Y.B.

Conflict of Interest: No conflict of interest was declared by the authors

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