Matrix metalloproteinase-3 levels in relation to disease activity and radiological progression in rheumatoid arthritis

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Conflict of interest

None declared

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Abstract

Background. Rheumatoid arthritis (RA) is a chronic inflammatory and systemic disease of unknown etiology that primarily affects synovial joints and involves progressive destruction around the joints. Inflammation starting in the joint synovium causes the destruction of cartilage, bone and other adjacent tissues with pannus formation.

Objectives. The aim of this study was to evaluate serum matrix metalloproteinase-3 (MMP-3) levels and their clinical and radiological significance in patients with rheumatoid arthritis.

Material and methods. The study included 59 patients with RA and 30 healthy controls. Serum MMP-3 levels were measured using the enzyme-linked immunosorbent assay (ELISA) method. Patients with a Disease Activity Score 28 (DAS28) ≤ 3.2 were categorized as having lower disease activity, while a DAS28 score > 3.2 indicated patients with moderate/high disease activity. Additionally, the patients were divided into 2 groups in terms of disease duration: early RA (disease duration ≤ 2 years) and established RA (disease duration ≥ 2 years). Functional disability was evaluated using the Health Assessment Questionnaire (HAQ) and Nottingham Health Profile (NHP). Radiographs were scored using modified Larsen scoring.

Results. Serum MMP-3 levels in patients with RA were significantly higher than in controls ($p = 0.001$). Serum MMP-3 levels were correlated with laboratory and clinical parameters of disease activity, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), DAS28, and HAQ score; the exceptions were rheumatoid factor (RF) and cyclic citrullinated peptides (CCP). The serum MMP-3 levels of RA patients with moderate/high disease activity were found to be significantly higher than those of the patients with low disease activity ($p < 0.001$). However, MMP-3 levels were found to be similar in both established and early RA patients ($p = 0.927$). Additionally, the modified Larsen scores, which indicate structural damage, correlated significantly with serum MMP-3 levels ($p = 0.001$).

Conclusions. These results indicate that serum MMP-3 levels may be used as an indicator for structural damage such as erosions in the early stages of the disease, and to monitor disease activity.

Key words: rheumatoid arthritis, matrix metalloproteinase 3, disease activity, modified Larsen score

Rheumatoid arthritis (RA) is a chronic inflammatory and systemic disease of unknown etiology that primarily affects synovial joints and involves progressive destruction around the joints. Inflammation starting in the joint synovium causes the destruction of cartilage, bone and other adjacent tissues with pannus formation. Progressive joint deformations restrict mobility, and as a result, the patients' quality of life declines significantly.^{1–3} According to current guidelines, disease activity is the baseline in the treatment of RA; treatment is regulated on the basis of the "treat-to-target" (T2T) strategy, the aim of which is remission or low disease activity.⁴ However, this strategy has limitations in preventing structural damage.⁵ Radiological progression has been observed in some RA patients during the course of remission; this shows the limitations of the main disease activity indicators used for estimating disease progression.⁶ Since the common disease activity indicators were not specific for arthritis, inflammatory cytokines, destructive enzymes, collagen and non-collagen breakdown products of cartilage, as well as new multi-biomarker scores, including a few biomarkers, were quickly developed to assess structural damage in RA.^{7,8}

Matrix metalloproteinases (MMP) are important members of the extracellular proteinase family. Their most important task is to decompose extracellular matrix components (ECM). They are known to be involved in many physiological and pathological processes. Matrix metalloproteinase-3 (MMP-3) is a proteinase generated by synovial fibroblasts and chondrocytes in joints.⁹ Active MMP-3 can accelerate joint damage in RA by degrading aggregate nucleoprotein, cartilage-associated protein, fibronectin, and collagen types IV, VII, IX, and XI.¹⁰ Serum MMP-3 has been studied as an indicator of disease activity in RA. A few studies have shown that serum MMP-3 levels increase in patients with RA and that disease activity is positively correlated with synovial MMP-3 expression; in addition, it has been shown that serum MMP-3 is elevated in some RA patients in remission or with low disease activity.^{11,12}

The aim of this study was to investigate the association between serum MMP-3 levels and disease severity measured with disease activity, functional capacity and structural damage evaluated using conventional radiography.

Material and methods

Patient population

The study included 59 patients (43 females and 16 males) with a diagnosis of RA who had been monitored for at least 1 year. All of the patients met the 2010 American College of Rheumatology (ACR) criteria for an RA diagnosis.¹³ Any change (except dose changes) in treatment with disease-modifying antirheumatic drugs (DMARDs) in the previous 3 months was a reason for exclusion. Other exclusion criteria were diagnoses of autoimmune diseases other than RA; acute and chronic infections; malignancies; or serious

pulmonary, hepatic, kidney, or endocrinological diseases. Moreover, smokers, patients with hypertension (arterial blood pressure $\geq 140/90$ mm Hg or on anti-hypertensive drugs) and hypercholesterolemia, as well as those aged below 20 and above 70 years were excluded from the study. We also enrolled 30 healthy controls. The study was approved by the local ethics committee and written informed consent forms were obtained from all the participants.

Clinical evaluations

The severity of pain, fatigue and the patients' and physicians' global assessment of disease activity were assessed on a visual analogue scale (VAS). No change or addition is needed, because the assessment criteria are universal. The duration of morning stiffness was noted in minutes. Disease activity was evaluated using the Disease Activity Score 28 for Rheumatoid Arthritis (DAS28).¹⁴ Patients with DAS28 scores ≤ 3.2 were evaluated as having lower disease activity, while DAS28 scores >3.2 indicated patients with moderate/high disease activity.¹⁵ Additionally, patients were divided into 2 groups depending on disease duration: early RA (duration ≤ 2 years) and established RA (duration ≥ 2 years). Functional disability was evaluated using the Health Assessment Questionnaire (HAQ) and Nottingham Health Profile (NHP).¹⁶

Laboratory evaluations

The results of routine laboratory tests were recorded (erythrocyte sedimentation rate (ESR), blood chemistry, whole blood cell count, and urinalysis). Rheumatoid factor (RF) and C-reactive protein (CRP) levels were measured using nephelometric methods.

Matrix metalloproteinase-3 assay

Venous blood samples were drawn from the patients and healthy controls and centrifuged at 3,000 rpm for 10 min. The sera were kept at -20°C until analysis. Serum MMP-3 was studied with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Total MMP-3 ELISA Kit; Aviscera Bioscience Inc., Santa Clara, USA) in accordance with the manufacturer's instructions. The results were expressed in ng/mL.

Radiological evaluation

Standard plain hand radiographs of the patients taken within the previous 6 months were examined. Structural damage in the hand radiographs was evaluated by a radiologist who was blinded to the patients' clinical and laboratory data. The radiographs were scored using modified Larsen scoring. In this scoring system, 24 areas in both hands are scored (0 to 5 points for each area, with total scores of 0–120 points).¹⁷

Statistical analysis

The statistical analysis of the study was performed using SPSS for Windows, v. 20.0 IBM Corp., Armonk, USA). The results of the study were evaluated using parametric and nonparametric statistical methods for data with normal and non-normal distribution, respectively. Intergroup comparisons were performed using the independent samples t-test for parametric values and the Mann-Whitney U test for nonparametric values. Correlations between parameters were evaluated using Spearman's correlation coefficient. In statistical evaluations, $p < 0.05$ was accepted as the level of significance. The results were expressed as mean $\pm$ standard deviation (SD).

Results

The mean disease duration was 9.0 ± 7.5 years (range: 1–30 years) (Table 1). Rheumatoid factor was positive in 49 patients (83.0%) and anticyclic citrullinated peptide (anti-CCP) were positive in 45 patients (76.3%).

There were 49 patients (83.1%) using corticosteroids and the average prednisolone dose was 6.61 ± 4.42 mg/day (range: 2–16 mg/day). There were 10 patients (16.9%) using only methotrexate, 28 patients (47.5%) taking 1 or more DMARDs in addition to methotrexate, 6 patients (10.2%) using a biological agent in addition to methotrexate, and 15 patients (24.4%) taking only DMARDs other than methotrexate (such as leflunomide, hydroxychloroquine or sulfasalazine).

Serum MMP-3 levels were significantly higher in the patients with RA than in the controls ($p = 0.001$) (Table 1).

Similarly, the serum MMP-3 levels in the RA patients with moderate/high disease activity were found to be significantly higher than in the patients with low disease activity ($p < 0.001$) (Table 2). However, MMP-3 levels were found to be similar in both established and early RA patients ($p = 0.927$) (Table 3).

Serum MMP-3 levels were correlated with all of the clinical and laboratory parameters of disease activity except RF and anti-CCP. Furthermore, there was a statistically significant correlation between structural joint damage (modified Larsen scores) and serum MMP-3 levels (Table 4).

Serum MMP-3 levels were similar in the patients taking both methotrexate and a biological agent and the patients taking DMARDs other than methotrexate. No statistically significant difference was identified between these 2 treatment groups in terms of DAS28 score, ESR, CRP, RF, and anti-CCP levels.

In this study, the cut-off value of serum MMP-3 was determined as 18.73 ng/mL. The sensitivity and specificity of serum MMP-3 were found to be 93.2% and 82.8%, respectively.

Table 1. Comparisons of demographic, clinical and laboratory parameters among the RA patients and the healthy control group

Parameters	Rheumatoid arthritis group (n = 59)	Control group (n = 29)	p-value
Age [years]	45.3 $\pm$ 8.6 (22–70)	43.3 $\pm$ 12.6 (22–70)	0.371
Female/male	43/16	21/8	0.963
Duration of the disease [years]	9.0 $\pm$ 7.5	–	–
Morning stiffness [min]	54.58 $\pm$ 64.55	–	–
Pain [0–100 mm VAS]	40.68 $\pm$ 22.73	–	–
Fatigue [0–100 mm VAS]	45.08 $\pm$ 16.33	–	–
Patients global [0–100 mm VAS]	40.68 $\pm$ 19.92	–	–
Physicians global [0–100 mm VAS]	20.85 $\pm$ 16.19	–	–
Number of the swollen joints (0–28)	1.03 $\pm$ 2.30	–	–
Number of the tender joints (0–28)	4.36 $\pm$ 3.51	–	–
ESR [mm/h]	33.86 $\pm$ 25.15	11.90 $\pm$ 6.85	0.001
CRP [g/dL]	2.57 $\pm$ 2.72	0.33 $\pm$ 0.17	0.001
RF [IU/mL]	117.20 $\pm$ 162.46	7.99 $\pm$ 3.40	0.001
Anti-CCP [IU/mL]	407.3 $\pm$ 411.3	10.1 $\pm$ 2.9	0.001
Serum MMP-3 [ng/mL]	88.39 $\pm$ 63.11	14.24 $\pm$ 5.54	0.001
DAS28	3.94 $\pm$ 1.30	–	–
HAQ20 (0–3)	1.37 $\pm$ 0.71	–	–
Modified Larsen Score (0–120)	38.56 $\pm$ 30.60	–	–

VAS – Visual Analogue Scale; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; RF – rheumatoid factor; anti-CCP – anti-cyclic citrullinated peptide; DAS28 – Disease Activity Score 28; HAQ20 – Health Assessment Questionnaire 20.

Table 2. Comparisons of patients with moderate/high disease activity and those with low disease activity

Parameters	Moderate/high disease activity group (n = 32)	Low disease activity group (n = 27)	p-value
Age [years]	53.44 $\pm$ 11.88	50.37 $\pm$ 12.02	0.304
Female/male	19/13	24/3	0.011
Duration of the disease [years]	8.13 $\pm$ 7.64	10.04 $\pm$ 7.33	0.195
Prednisolone [mg/day]	7.59 $\pm$ 4.40	5.44 $\pm$ 4.23	0.035
Methotrexate [mg/week]	7.56 $\pm$ 5.19	6.53 $\pm$ 4.69	0.308
ESR [mm/h]	48.91 $\pm$ 24.63	16.04 $\pm$ 8.93	0.001
CRP [g/dL]	4.05 $\pm$ 2.89	0.81 $\pm$ 0.82	0.001
Serum MMP-3 [ng/mL]	122.07 $\pm$ 61.30	48.48 $\pm$ 36.80	0.001

ESR – erythrocyte sedimentation rate; CRP – C-reactive protein.

Table 3. The data for early and established rheumatoid arthritis

Parameters	Early RA group (<2 years) (n = 13)	Established RA group (>2 years) (n = 46)	p-value
Age [years]	46.3 ±13.3	53.6 ±11.1	0.087
Female/male	8/5	35/11	0.297
Serum MMP-3 [ng/mL]	111 ±66.3	81.97 ±61.38	0.927
Anti-CCP [IU/mL]	446.4 ±417.2	396.3 ±413.5	0.530
RF [IU/mL]	91.06 ±75.2	124.5 ±179.5	0.841
ESR [mm/h]	37.1 ±27.7	32.9 ±824.6	0.833
CRP [g/dL]	1.7 ±1.5	2.8 ±2.9	0.336
DAS28	4.37 ±1.86	2.75 ±0.42	0.426
HAQ20 (0–3)	1.66 ±0.85	1.20 ±0.65	0.179
Modified Larsen Score (0–120)	36.9 ±28.9	39.20 ±31.36	0.927

Anti-CCP – anti-cyclic citrullinated peptide; RF – rheumatoid factor; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; DAS28 – Disease Activity Score 28; HAQ20 – Health Assessment Questionnaire 20.

Table 4. Spearman's correlation coefficients (r) for serum MMP-3 levels and various clinical and laboratory parameters in patients with rheumatoid arthritis

Parameters	Serum MMP-3	
	p-value	r
Age	0.402	0.111
Period of disease	0.360	–0.121
Morning stiffness	<0.001*	0.696
Pain level	<0.001*	0.642
Number of swollen joints	<0.001*	0.706
HAQ score	<0.001*	0.688
ESR	<0.001*	0.462
CRP	<0.001*	0.464
RF	0.019*	0.304
Anti-CCP	0.229	0.159
DAS28	<0.001*	0.674
Modified Larsen score	<0.001*	0.706

HAQ – Health Assessment Questionnaire; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; RF – rheumatoid factor; anti-CCP – anti-cyclic citrullinated peptide; DAS28 – Disease Activity Score 28; numbers marked in bold indicate a statistically significant difference.

Discussion

Rheumatoid arthritis is one of the most common autoimmune diseases, affecting approx. 1% of the world population. Early diagnosis and treatment of RA is very important in order to prevent damage to joint tissues and disability. In patients with typical symptoms, a diagnosis can typically be made easily in the 1st year of the disease. Nevertheless, most of the time clinical symptoms are not explicit in the earliest stages of the disease. A long time

may pass from the onset of symptoms to the diagnosis in patients with atypical progression. Therefore, specific and sensitive serologic tests are necessary for a quick diagnosis. The most common autoantibodies in the ACR 2010 criteria for a diagnosis of rheumatoid arthritis are RF and anti-CCP. Rheumatoid factor is an autoantibody with high sensitivity but it is not sufficiently specific to RA; it can be found in healthy individuals and also accompanies various diseases, which means it is not an ideal autoantibody for a diagnosis.

As Porto et al. wrote: “The progression of RA brings an evolutionary potential to varying degrees of joint damage and functional disability. Thus, special attention should be [paid] to the identification of poor prognostic indicator parameters, because ideally the definition of therapeutic intensity level should be based on the reliable predictors of severity. It is already known that some features when present, are associated with a worse outcome of the disease, such as the presence of high titer rheumatoid factor, smoking and HLA-DRB1”.^{18,19}

Porto et al. continued: “Regarding the prognostic role of anti-CCP, its association with disease activity and functional capacity has still not been clarified, although many studies suggest that these antibodies are associated with more severe and erosive disease,^{20,23} especially in cases of initial RA.^{24,27} It is worth noting the methodological heterogeneity of the studies that analyzed the association of anti-CCP with structural damage. Although most studies have made use of conventional radiography as an evaluation tool, different radiographic score systems were used”.¹⁹

A study by Kumagai et al. showed that – in addition to RF – anti-CCP, anti-agalactosyl IgG antibodies and MMP-3 could be diagnostic indicators in RA.²⁸ With 81% sensitivity and 92.4% specificity, anti-CCP autoantibodies were identified as being more valuable than RF for the early diagnosis of RA. Joint damage progression was found to be faster in MMP-3-positive patients. Accordingly, MMP-3 that correlates strongly with CRP was determined to be very useful in assessing joint damage and RA disease activity.²⁸ In another study, serum MMP-3 and CRP levels showed a strong correlation. In this study, it was reported that the sensitivity of the MMP-3 and anti-CCP combination was 83.3% in early RA diagnosis.²⁹ In the present study, serum MMP-3 sensitivity was found to be 93.2% and its specificity was 82.8% in RA diagnosis. This result shows that MMP-3 is a useful marker in RA diagnosis despite the limited number of patients in the study. Furthermore, in parallel with the aforementioned studies, this study found a strong positive correlation between serum MMP-3 level and CRP values. Matrix metalloproteinase 3 is not commonly found in synovium and serum under normal conditions, but growth factors can be easily induced by cytokines such as interleukin 1 (IL-1) and tumor necrosis factor α (TNF- α).³⁰ The positive correlation between CRP and MMP-3 found in this study shows that pro-inflammatory

cytokines such as IL-1 and TNF- α , which are responsible for the production of acute phase reactants, also stimulate MMP-3 production.

One of the strongest indicators of long-term prognosis in rheumatoid arthritis is the progressive structural joint damage. The progressive cartilage and bone damage in rheumatoid arthritis are considered dependent on the intensive secretion of MMP enzymes.³¹ In their study, Taylor et al. determined that synovial liquid MMP-3 levels could be several hundred times higher than serum MMP-3 levels in RA patients, and that there was a strong correlation between serum and synovial liquid MMP-3 levels.³² Kobayashi et al. indicated that serum and synovial liquid in late RA patients had higher MMP-3 levels than in osteoarthritis (OA) patients when they compared the 2 groups functionally and radiographically.³³ Similarly, Klimiuk et al. compared the serum and synovial liquid samples of RA and OA patients and found that RA patients had higher MMP-3 levels than OA patients in all circumstances.³⁴ As in these studies, serum MMP-3 levels of the RA patients in our study were significantly higher than those of the healthy control individuals. This confirms that MMP-3 is released by inflammatory cells and clarifies why serum MMP-3 levels are high in patients with an elevated inflammatory cell load and intensive inflammation.

It has been reported in a few studies that high MMP-3 levels could be the most important independent indicator of radiological progression.^{35,36} Young-Min et al. reported that MMP-3 levels are superior to traditional indicators in foreseeing radiographical progression.³⁷ Our results concur with these studies, showing a positive correlation between MMP-3 levels and the modified Larsen score of the RA patients.

Identifying the number of sore joints (among a total of 28 joints) is one of the most important criteria used in the evaluation of disease activity in rheumatoid arthritis, and is considered one of the most reliable. Scores concerning sore joints generally comply with other clinical criteria. Radiological findings are indicators of joint damage in RA, and the progression rate can be considered a disease activity criterion.^{38,39}

Several studies in the available literature assert that serum MMP levels could reflect joint damage along with disease activity in RA and can be helpful in diagnosing the disease in the early stage.^{34,40–43} In this study, a statistically significant positive correlation was identified between MMP-3 and DAS28, ESR, CRP, and clinical disease parameters (swollen and sensitive joints, pain level, HAQ, NHP, the duration of morning stiffness, and asthenia). This shows that MMP-3 can reflect disease activity effectively.

Some studies have indicated that serum MMP-3 levels could be affected by the DMARDs, biological agents and corticosteroids often used in RA treatment. Serum MMP-3 levels decreased with the use of non-biological DMARDs and therefore, serum MMP-3 levels could reflect response to the treatment.^{44–46} It was stated in another study that

testing MMP-3 levels is a simple and non-invasive method for evaluating the efficiency of antirheumatic drug therapy, especially biological agents, and for monitoring synovial inflammation.⁴⁷

In this study, no significant difference was determined in terms of serum MMP-3 levels between the patient group taking methotrexate and a biological agent and the patients taking other DMARDs (sulfasalazine, leflunomide, hydroxychloroquine). This may result from the repression of MMP-3 levels by corticosteroids, biological agents and methotrexate in patients examined in this study.

The main limitation of this study is its cross-sectionality. Moreover, the limited number of patients, the absence of a patient control group and the examination of MMP-3 levels only in serum are other limiting factors of this study.

This study has shown that the serum MMP-3 levels of the RA patients increased and that disease activity was correlated with clinical and laboratory parameters. Accordingly, it can be concluded that increases in MMP-3 levels in RA patients may indicate progression of joint erosion as a result of synovial inflammation development. Serum MMP-3 levels can be used to assess joint erosion progression in the early stage of RA as an indicator of disease activation and to monitor patients' response to treatment.

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