Serum and synovial matrix metalloproteinase-3 as markers of disease activity in early rheumatoid arthritis

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Background
Matrix metalloprotein ase-3 (MMP-3) is one of the MMPs produced in rheumatoid arthritis (RA) joints.
Aim
The aim of this study was to evaluate serum and synovial fluid (SF) MMP-3 as markers of disease activity in early RA.
Patients and methods
Thirty early RA patients together with age-matched and sex-matched 12 primary knee osteoarthritis patients and 12 apparently healthy individuals as control groups were enrolled in this study. MMP-3 was measured in serum and SF samples using enzyme-linked immunosorbent assay. Assessment of disease activity in RA patients was carried out using disease activity score-28 (DAS-28), and radiographs of the hands, wrists, and forefeet were obtained and evaluated according to Larsen score.
Results
As regards mean serum levels of MMP-3, there was a statistically significant elevation in RA patients compared with the control groups ($P<0.001$). Moreover, the mean SF levels of MMP-3 in RA patients were statistically significantly higher than that in osteoarthritis patients ($P<0.001$). In RA patients, there was a statistically significant difference ($P<0.001$) between mean serum and SF levels, being higher in the SF. There was a statistically significant positive correlation ($P<0.05$) between serum MMP-3 with disease duration, DAS-28, and Larsen score. As regards mean SF MMP-3 levels, there was a high statistically significant positive correlation ($P<0.001$) with DAS-28 and a statistically significant positive correlation ($P<0.05$) with Larsen score.
Conclusion
Elevated serum and synovial MMP-3 levels reflect disease activity in RA patients; thus, it could be used as a useful marker for disease activity. The cross-sectional design of our study did not allow us to produce conclusions with respect to disease course and prognosis. Thus, we recommend further studies on large numbers of patients and serial measurements of MMP-3 to determine the rate of disease progression.

Keywords:
matrix metalloproteinase-3, rheumatoid arthritis, synovial fluid

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder that is more common among women and primarily involves joints in symmetrical pattern and may be remitting, but if uncontrolled it may lead to joint destruction and deformity [1]. There is a general consensus that RA is a multifactorial disease, resulting from the interaction of both genetic and environmental factors, which contribute to its occurrence and expression. The assessment of disease activity is useful for guiding the treatment and monitoring the patients to assess the treatment effectiveness [2]. Inflammatory biomarkers such as erythrocyte sedimentation rate (ESR) and high sensitivity C-reactive protein (CRP) may serve as helpful indicators for evaluation of disease activity. Patients positive for rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), and anti-mutated citrullinated vimentin antibodies are more likely to develop joint erosions compared with those negative for these antibodies, although they do not reliably change with disease activity [3]. Hence, it seems reasonable to determine more specific biomarkers in correlation with the RA activity, monitoring disease progression, and assessing the

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effectiveness of treatment [4]. Degradation of articular cartilage is one of the earliest features of the disease and is mediated by the increased activity of proteolytic systems. Among several enzymes involved in the process, matrix metalloproteinases (MMPs) have been shown to play an important role in the invasion of the synovial tissue, cartilage destruction, and bone erosion formation [5].

MMP-3 is one of the stromelysins expressed in several cell types, including human articular chondrocytes and synoviocytes, and is involved in the shedding of protein ectodomains from the cell surface and can degrade a broad spectrum of extracellular matrix substrates. Moreover, MMP-3 can proteolytically activate other MMPs such as pro-MMP-9, which has a role in inflammatory cell recruitment, and promotes the release of membrane-bound vascular endothelial growth factor; thus, it contributes to angiogenesis and disease progression [6].

Aim

The aim of this study was to evaluate serum and synovial levels of MMP-3 as markers of disease activity in early RA.

Patients and methods

Study approval

The study was approved by the Research Ethics Committee, Faculty of Medicine, Benha University, Egypt. The aim of the study was explained to all participants, and informed consent was obtained from all of them.

This study was carried out at Rheumatology and Rehabilitation Outpatients' Clinics and Inpatients' Department of Benha University Hospitals between October 2013 and March 2014.

Patients

This study included two groups. Group I included 30 patients with early RA (<12 months of duration). All of them met the new classification criteria of the American College of Rheumatology/European League against Rheumatism for RA classification (2010) [7].

Group II included 12 patients with primary knee osteoarthritis (OA) who fulfilled the criteria of American College of Rheumatology [8] and 12 apparently healthy volunteers. All were matched for age and sex with the RA patients.

Methods

All RA patients were subjected to the following: complete history taking, thorough clinical examination, assessment of disease activity using disease activity score-28 (DAS-28) [9], and laboratory investigations. Laboratory investigations included the following: complete blood count using a ESR determination using the Westergren method; CRP evaluation using the latex agglutination slide test; serum RF evaluation using the latex agglutination slide tests; anti-CCP antibodies and serum MMP-3 evaluation using enzyme-linked immunosorbent assay (ELISA) for RA patients and both control groups; and synovial MMP-3 evaluation using ELISA for both RA and OA patients [10,11].

Test principle

Preparation of samples

Five milliliters of blood was drawn from each patient (RA) and controls and 5 ml of synovial fluid (SF) was withdrawn from each RA and OA patient. The samples were separated by means of centrifugation and then labeled and stored at −20°C until use.

Matrix metalloproteinase-3 assay procedure

Human MMP-3 ELISA kit (USA) was based on standard sandwich ELISA technology. Human MMP-3-specific polyclonal antibodies were precoated onto 96-well plates. The human-specific detection polyclonal antibodies were biotinylated. Serum samples were diluted 1 : 20, whereas treated SF was diluted 1 : 2000. The test samples and biotinylated detection antibodies were added to the wells subsequently, followed by washing with PBS. Avidin–biotin–peroxidase complex was added and unbound conjugates were washed away with PBS. Horseradish peroxidase (HRP) substrate 3,3',5,5'-tetramethylbenzidine was used to visualize HRP enzymatic reaction. 3,3',5,5'-tetramethylbenzidine was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human MMP-3 amount of sample captured in plate. A standard curve was created by plotting absorbance of each standard concentration against MMP-3 concentration. For each sample, concentration was obtained from standard curve, and then multiplied by 10 (dilution factor).

Radiological evaluation

Plain radiography (posterior–anterior views) of both hands, wrists, and feet were obtained and scored according to Larsen score [12]. The Larsen index score 1995 was applied to proximal interphalangeal joints (PIPs) (2–5), metacarbophalangeal joints (MCPs) (2–5) in each hand, four quadrants in both

wrists, and metatarsophalangeal joints (MTPs) (2–5) in each feet. Thereafter, the score was summed up giving a maximum score of 160 when all joints of both hands are fully destroyed. Standard posterior–anterior views on both knees were obtained for RA and OA patients.

All RA and OA patients were presented with synovial effusion in one or both knee joints. Aspiration was obtained from the affected knee in unilateral cases, or from the worst knee in cases of bilateral effusion.

This study was not intended to analyze the putative effects of disease modifying anti rheumatic drugs (DMARD) therapy on the variables we studied. DMARDs were administrated according to conventional clinical judgment, and the time of sampling was unrelated to the start or discontinuation of DMARD therapy. Any influences are probably marginal and would not obscure the differences between groups. Intra-articular glucocorticoids had not been given within 3 months before knee joint fluid aspiration.

Statistical analysis

The data were recorded, tabulated, coded, and then analyzed using the computer program statistical package for the social science (SPSS; SPSS Inc., Chicago, Illinois, USA, version 16).

Descriptive data were presented in the form of mean ±SD and number.

Analytical statistics was carried out using Student's *t*-test, *F*-test (one-way analysis of variance) (a test of significance for comparison between more than two groups having quantitative variables with different variance) and Pearson's correlation coefficient (*r*) test. The accepted level of significance in this work was stated at *P*-value less than 0.05 was considered statistically significant (S). A *P*-value less than 0.0001 was considered highly significant (HS) in all analyses.

Results

We included 30 RA patients; there were 10 male and 20 female with a mean±SD age of 49.5±7.3 years and a mean disease duration of 7.56±3.36 months. In addition, there were 12 OA patients; there were three male and nine female patients with a mean ±SD age of 51.5±2.8 years and mean disease duration of 12±7.7 months. Moreover, 12 apparently healthy volunteers comprising four male and eight female patients with a mean±SD age of 47.53±5.11 were included in the study (Tables 1 and 2).

Table 1 Clinical and laboratory data of rheumatoid arthritis patients

Variables	
Clinical data (*n*)	
Arthritis	
Negative	0
Positive	30
Rheumatoid module	
Negative	22
Positive	8
GIT	
Negative	16
Positive	14
Neurologic	
Negative	25
Positive	5
Vasculitis	
Negative	28
Positive	2
Pulmonary	
Negative	26
Positive	4
Sicca symptoms	
Negative	24
Positive	6
Laboratory data (mean±SD)	
ESR (mm/h)	48.9±20.7
CRP (mg/dl)	22.4±10.4
HB% (g/dl)	11±1.3
WBCs (mm^3/ml)	6.5±1.8
Platelet (mm^3/ml)	229.7±52.1
RF [*n* (%)]	
Negative	5 (16.5%)
Positive	25 (83.3%)
Anti-CCP	
Negative	9 (30%)
Positive	21 (70%)

Anti-CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GIT, gasterointestinal tract; HB, hemoglobin; RF, rheumatoid factor; WBCs, white blood cells.

As regards mean serum levels of MMP-3, it was highly statistically significantly elevated in RA patients compared with the control groups ($P<0.001$). Moreover, the mean SF levels of MMP-3 in RA patients were highly statistically significantly elevated in RA than in OA patients ($P<0.001$). In RA patients, there was a statistically significant difference ($P<0.001$) between mean serum and SF levels, being higher in the SF (Table 3).

There was a highly statistically significant positive correlation ($P<0.001$) between serum MMP-3 and platelet ($r=0.55$) and a statistically significant positive correlation ($P<0.05$) with disease duration ($r=0.52$), tender joint count (TJC) ($r=0.37$), swollen joint count (SJC) ($r=0.41$), ESR ($r=0.52$), DAS-28 ($r=0.47$), RF titer ($r=0.44$), CRP ($r=0.59$), and Larsen

Table 2 Comparison between the mean serum and synovial fluid matrix metalloproteinase-3 levels among the studied groups

	Mean±SD	Test	*P*-value
Serum MMP-3 (ng/ml)			
RA	40.9±15.1	*F*=59.8	<0.001
OA	9.7±2.1		
HC	3.3±1.1		
Synovial fluid MMP-3 (ng/ml)			
RA	77.6±13.9	*t*=11.9	<0.001
OA	42.9±4.4		
RA			
Serum MMP-3	40.9±15.1	*t*=9.6	<0.001
Synovial fluid MMP-3	77.6±13.9		

HC, healthy control; MMP-3, matrix metalloproteinase-3; OA, osteoarthritis; RA, rheumatoid arthritis.

Table 3 Correlation between serum and synovial fluid matrix metalloproteinase-3 levels and the studied variables in rheumatoid arthritis patients

	Serum MMP-3		SF MMP-3	
	R	*P*-value	*R*	*P*-value
Serum MMP-3	–	–	0.38	<0.05*
SF MMP-3	0.38	<0.05*	–	–
Age	0.09	>0.05	0.12	>0.05
Disease duration	0.52	<0.05*	0.4	<0.05*
TJC	0.37	<0.05*	0.38	<0.05*
SJC	0.41	<0.05*	0.42	<0.05*
VAS	0.18	>0.05	0.004	>0.05
ESR	0.52	<0.01*	0.39	<0.05*
DAS-28	0.47	<0.01*	0.65	<0.001**
RF titer	0.44	<0.05*	0.5	<0.05*
ACPA	0.01	>0.05	0.03	>0.05
CRP	0.59	<0.01*	0.79	<0.001**
HB	–0.6	<0.001**	–0.51	<0.01*
WBCs	0.29	>0.05	–0.32	>0.05
Platelet	0.55	<0.001**	0.65	<0.001**
Larsen score	0.42	<0.05*	0.41	<0.05*

CRP, C-reactive protein; DAS-28, disease activity score-28; ESR, erythrocyte sedimentation rate; HB, hemoglobin; MMP-3, matrix metalloproteinase-3; RF, rheumatoid factor; SF, synovial fluid; SJC, swollen joint count; TJC, tender joint count; WBCs, white blood cells. *Statistically significant. **Statistically highly significant.

score (r=0.42). However, there were no statistically significant correlations (P>0.05) with age (r=0.09), visual analogue scale (VAS) (r=0.18), anti citrullinated peptide antibody (ACPA) (r=0.01), and white blood cells (r=0.29) and highly statistically significant negative correlation (P<0.001) with hemoglobin% (r=–0.6).

As regards mean SF MMP-3 levels, there were high statistically significant positive correlations (P<0.001) with DAS-28 (r=0.65), CRP (r=0.79), and platelet (r=0.65) and statistically significant positive correlations (P<0.05) with disease duration (r=0.4), TJC (r=0.38), SJC (r=0.42), ESR (r=0.39), RF titer (r=0.5), and Larsen score (r=0.41). However, there was a statistically significant negative correlation (P<0.05) with hemoglobin% (r=–0.51) and no statistically significant correlations (P>0.05) with age (r=0.12), VAS (r=0.004), ACPA (r=0.03), and white blood cells (r=–0.32) (Table 4).

Table 4 Radiological grades of rheumatoid arthritis patients

Larsen's grades	*N* (%) (*N*=30)
Grade I	12 (40.0)
Grade II	9 (30.0)
Grade III	5 (16.7)
Grade IV	4 (13.3)

According to Larsen score, about 40% [12] of RA patients were of grade I, 30% (9) were of grade II, 17% (5) were of grade III, and 13% (4) were of grade IV.

Discussion

In this study, the mean serum levels of MMP-3 were statistically significantly elevated in RA patients compared with the control groups (P<0.001). Moreover, the mean SF levels of MMP-3 in RA patients were statistically significantly higher than that in OA patients (P<0.001). In RA patients, there was a statistically significant difference (P<0.001) between mean serum and SF levels, being higher in the SF. Our results are in agreement with those Ma *et al.* [13] and Ally *et al.* [6], who found that the mean serum levels of MMP-3 were elevated in RA patients compared with healthy controls.

In line with our results, Kobayashi *et al.* [14] found that there were a statistically highly significant elevation in mean levels of serum and SF samples of MMP-3 in RA patients compared with OA patients (P<0.001).

Similar to our results, Galil *et al.* [15] reported that, in RA patients, serum MMP-3 levels correlate with levels produced by the synovium, and thus reflect the level of activity of rheumatoid synovitis and the baseline levels were significantly higher in those with high-progression, making it a strong prognostic marker of disease activity and an early predictor of progressive joint damage in recent-onset Egyptian RA patients.

In our study, there were no statistically significant correlations between serum or SF MMP-3 levels and age and a statistically significant correlation with disease duration in RA patients. This finding is in agreement with that of Faddaa *et al.* [16]. However, Mamehara *et al.* [17] found a nonsignificant

correlation with disease duration, wherein their patients had a longer disease duration (>5years).

In our study, there were statistically significant positive correlations ($P<0.05$) between mean serum levels of MMP-3 with TJC, SJC, ESR, DAS-28, RF titer, and CRP. In agreement with our results, Faddaa *et al.* [16] and Ma *et al.* [13] found that mean serum MMP-3 levels were significantly correlated with ESR, CRP, RF-positive, and anti-CCP-positive patients. In contrast, Mamehara *et al.* [17] found that there were no statistically significant correlations between mean serum levels of MMP-3 with ESR and CRP.

As regards mean SF MMP-3 levels, there were highly statistically significant positive correlations ($P<0.001$) with DAS-28, CRP, and platelet and statistically significant positive correlations ($P<0.05$) with disease duration, TJC, SJC, ESR, RF titer, and Larsen score. However, our results were different from those of Ma *et al.* [13], who reported that there were no significant correlations between SF MMP-3 level and TJC and SJC, and from those of Al-Sebaie *et al.* [18], who revealed no significant correlations with disease duration, nor RF positivity. As regards DAS-28, in our study, there was a statistically significant positive correlation and a highly statistically positive correlation with mean serum and SF MMP-3 levels, respectively. These results are in agreement those of with Faddaa *et al.* [16] and Tchetverikov *et al.* [19], who found that serum MMP-3 levels were significantly correlated with DAS-28. In contrast, these results are not in agreement with those of Ma *et al.* [13].

As regards Larsen radiographic score, in our study we found a statistically significant positive correlation ($P<0.05$) between serum and SF MMP-3 with Larsen score. This is in agreement with the findings of Houseman *et al.* [20] and Tchetverikov *et al.* [19], who found that elevated serum levels of MMP-3 were significantly associated with bone erosions and high radiographic scores. However, the study by Ally *et al.* [6] revealed a nonsignificant correlation between serum MMP-3 level and Larsen radiographic scores in early RA patients.

Conclusion

Elevated MMP-3 serum and synovial levels reflect disease activity in RA patients; thus, it could be used as a useful marker for disease activity. The cross-sectional design of our study did not allow us to produce conclusions with respect to disease course and prognosis. Thus, we recommend further studies on large numbers of patients and serial measurements of MMP-3 to determine the rate of disease progression.

Limitations of our study include small sample size and lack of serum MMP-3 measurements before and after treatment with corticosteroid and other DMARDs.

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Conflicts of interest
There are no conflicts of interest.

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