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

Rasha M. Fawzy¹, Yasser A. Abdel Hamid², Mona M. Albehesy³, Rasha F. Aboelmaged⁴

¹Lecturer of Rheumatology and Rehabilitation & Physical Medicine, Faculty of Medicine, Benha University, ²Professor of Rheumatology and Rehabilitation & Physical Medicine, Faculty of Medicine, Benha University, ³Lecturer of Chemical & Clinical Pathology, Faculty of Medicine, Banha University, ⁴President at Banha Teaching Hospital, Rheumatology and Rehabilitation & Physical Medicine Department, Benha, Egypt

Correspondence to Rasha Mohamed Fawzy, Lecturer of Rheumatology and Rehabilitation & physical Medicine, Faculty of Medicine Benha University, MD in Rheumatology and Rehabilitation Benha, Kayiobia, Egypt, Tel: 01003934274; fax: 0133226525; e-mail: Dr.rasha.fawzy@hotmail.co

Received 12 June 2016
Accepted 10 July 2016

Egyptian Rheumatology & Rehabilitation 2016, 43:178–183

Background
Matrix metalloproteinase-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

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), anticyclic 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

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work noncommercially, as long as the author is credited and the new creations are licensed under the identical terms.
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), metacarpophalangeal joints (MCPs) (2–5) in each hand, four quadrants in both
wrist, 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).

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).

| 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 (mm³/ml) 6.5±1.8                                         |
| Platelet (mm³/ml) 229.7±52.1                                  |
| RF [n (%)] 5 (16.5%)                                           |
| 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.

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

| Test   | Mean±SD  | 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 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 |
|----------|-------------|----------|
| Serum MMP-3 | –           | –        |
| SF MMP-3  | 0.38        | <0.05*   |
| Age       | 0.09        | >0.05    |
| Disease duration | 0.52 | <0.05*  |
| TJC       | 0.37        | <0.05*   |
| SJC       | 0.41        | <0.05*   |
| VAS       | 0.18        | >0.05    |
| ESR       | 0.52        | <0.01*   |
| DAS-28    | 0.47        | <0.01*   |
| RF titer  | 0.44        | <0.05*   |
| ACPA      | 0.01        | >0.05    |
| CRP       | 0.59        | <0.01*   |
| HB        | 0.59        | <0.001** |
| WBCs      | 0.29        | >0.05    |
| Platelet  | 0.55        | <0.001** |
| Larsen score | 0.42   | <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), SJ (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).

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.

Table 4 Radiological grades of rheumatoid arthritis patients

| Grade'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)   |

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 (>5 years).

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.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

---

**References**

1. Boers M, Buttgerell F, Saag K, Alten R, Grahn A, Storey D, et al. What is the relationship between morning symptoms and measures of disease activity in patients with rheumatoid arthritis? Arthritis Care Res (Hoboken) 2015; 67:1202–1209.
2. Farheen K, Agarwal SK. Assessment of disease activity and treatment outcomes in RA. J Manag Care Pharm 2011; 17:9–13.
3. Liu X, Jia R, Zhao J, Li Z. The role of anti-mutated citrullinated vimentin antibodies in the diagnosis of early rheumatoid arthritis. J Rheumatol 2009; 36:1136–1142.
4. Dessein PH, Joffe BI, Slamwix AE. High sensitivity C-reactive protein as a disease activity marker in rheumatoid arthritis. J Rheumatol 2004; 31:1095–1097.
5. Kaneko M, Tomita T, Nakase T, Ohswa Y, Seki H, Takeuchi E, et al. Expression of proteinases and inflammatory cytokines in subchondral bone regions in the destructive joint of rheumatoid arthritis. Rheumatology (Oxford) 2001; 40:247–255.
6. Ally MM, Hodkinson B, Meyer PW, Musenge E, Tikly M, Anderson R. Serum matrix metalloproteinase-3 in comparison with acute phase proteins as a marker of disease activity and radiographic damage in early rheumatoid arthritis. Mediators Inflamm 2013; 2013:183653.
7. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO III, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum 2010; 62:2569–2581.
8. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29:1039–1049.
9. Prevoo MLL, van ’t Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:44–48.
10. Saus J, Quinones S, Olani Y, Nagase H, Harris ED, Kukinien M. The complete primary structure of human MMP-3: identify with stromelysin. J Biol Chem 1988; 263:6742–6745.
11. Medley TL, Kingwell BA, Gatza KD, Pillay P, Cote TJ. Matrix metalloproteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. Circ Res 2003; 92:1254–1261.
12. Larsen A. How to apply Larsen score in evaluating radiographs of rheumatoid arthritis in long-term studies. J Rheumatol 1995; 22:1974–1975.
13. Ma JD, Zhou JJ, Zheng DH, Jian D, Jing J, Dong H, et al. Serum matrix metalloproteinase-3 as a noninvasive biomarker of histological synovitis for diagnosis of rheumatoid arthritis. Mediators Inflamm 2014; 2014:175284.
14. Kobayashi A, Naito S, Enomoto H, Shiomoi T, Kimura T, Ohsawa Y, et al. Serum levels of matrix metalloproteinase-3 (stromelysin 1 for monitoring synovitis in rheumatoid arthritis. Arch Pathol Lab Med 2007; 131:563–570.
15. Gall SM, El-Shafey AM, Hagrass HA, Fawzy F, Sannak AE. Baseline serum level of matrix metalloproteinase-3 as a biomarker of progressive joint damage in rheumatoid arthritis patients. Int J Rheum Dis 2016; 19:377–384.
16. Faddaa S, Abolkheir E, Alfie R, Gamal M. Serum matrix metalloproteinase-3 in rheumatoid arthritis patients: correlation with...
disease activity and joint destruction. Egypt Rheumatol 2016; 38:153–159.

17 Mamehara A, Sugimoto T, Sugiyama D, Morinobu S, Tsuji G, Kawano S, et al. Serum matrix metalloproteinase-3 as predictor of joint destruction in rheumatoid arthritis, treated with non-biological disease modifying anti-rheumatic drugs. Kobe J Med Sci 2010; 56:98–107.

18 Al-Sebaie MA, AL-Yasaky AZ, Assaf NY, Mohammad M. Serum and synovial fluid levels of MMP-3 and TIMP-1 in rheumatoid arthritis and osteoarthritis. Egypt Rheumatol Rehab 2003; 30:841–860.

19 Tchetverikov I, Lard LR, DeGroot J, Verzijl N, TeKoppele JM, Breedveld FC, et al. Matrix metalloproteinases-3, −8, −9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. Ann Rheum Dis 2003; 62:1094–1099.

20 Houseman M, Potter C, Marshall N, Lakey R, Cawston T, Griffiths I, et al. Baseline serum MMP-3 levels in patients with rheumatoid arthritis are still independently predictive of radiographic progression in a longitudinal observational cohort at 8 years follow up. Arthritis Res Ther 2012; 14:R30.