

Higher levels of matrix metalloproteinase-3 in patients with RA reflect disease activity and structural damage

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Aims. To evaluate the serum levels of matrix metalloproteinase-3 (MMP-3) as a potential marker of disease activity and joint damage in 92 patients with rheumatoid arthritis (RA), compared to 24 osteoarthritis (OA) patients and 26 healthy controls.

Methods. The concentrations of MMP-3 were measured by ELISA using the commercial kit AESKULISA DF MMP-3 (AESKU.Diagnostics, Germany) and compared with other laboratory parameters routinely used to assess the disease status, clinical score (DAS28) and radiographic stage in the group of RA patients.

Results. The mean serum concentrations of MMP-3 were 199.1 ± 160 ng/mL in RA patients, 113.9 ± 96.9 ng/mL in OA patients and 48.3 ± 19.2 in healthy controls. The differences were highly significant: RA patients and healthy controls ($P < 0.0001$), RA and OA patients ($P = 0.008$) as well as between OA patients and controls ($P = 0.009$). MMP-3 concentrations were further compared with other laboratory parameters and clinical and structural damage data. There were correlations between MMP-3 and CRP ($r = 0.304$, $P < 0.01$), DAS28 ($r = 0.301$, $P < 0.05$), levels of anti-cyclic citrullinated peptide antibodies ($r = 0.241$, $P < 0.05$), erythrocyte sedimentation rate ($r = 0.200$, $P = 0.059$) and radiographic disease stage ($r = 0.197$, $P = 0.063$).

Conclusion. These results demonstrated that measurement of MMP-3 could become a marker of disease activity in RA patients.

Key words: rheumatoid arthritis, disease activity, matrix metalloproteinase-3

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder of synovial tissue characterized by progressive, erosive and symmetrical polyarthritis associated with various extra-articular manifestations and with variable prognosis, significant morbidity, functional damage, disability and increased mortality. It affects approximately 0.5% of the adult population¹. The pathological process leads to destruction of articular cartilage and bone and may eventually result in ankylosis of the affected joints. It is now generally accepted that the activity of cells within synovium and, in particular, the cytokine and enzyme products which they generate are involved in the destruction of underlying matrix components^{2,3}. The destruction of connective tissue is also driven by the action of matrix metalloproteinase enzymes (MMPs) released by synovial fibroblasts, chondrocytes and monocytes in response to IL-1 β , TNF- α , interferon gamma, as well as by serum amyloid A (SAA) and epidermal growth factor and platelet-derived growth factor⁴⁻⁸. MMPs participate in the maintenance and remodeling of extracellular matrix (ECM) that is important for creating cellular environments. These enzymes have the ability to cleave several

constituents of ECM. They play a central role not only in many physiological processes but also in many diseases. Increased activity, caused by either up-regulation of their expression or down-regulation of their inhibitors, is implicated, for example, in arthritis, atherosclerosis and aneurysms, cancer metastases^{9,10}, nephritis, skeletal growth-plate disorders, tissue ulcerations and fibrosis¹¹. MMP-3 is involved in the pathogenesis of RA as it degrades a range of matrix proteins including proteoglycans, laminin, fibronectin and gelatin found in connective tissue in the synovial joint.

Recent advancement in the treatment of RA require reliable tools for the monitoring of disease activity, evaluation of disease prognosis and choice of the appropriate therapy.

Tracking new markers such as MMP-3 could help identify patients who have a higher risk of pathological changes earlier in the course of the disease and may help in effective intervention with therapy of the disease¹²⁻¹⁴. The present study investigated the association between MMP-3 disease activity and structural involvement with the final objective of assessing and evaluating patient prognosis based on MMP-3 serum levels more effectively.

OBJECTIVE

This study aimed to evaluate the serum levels of MMP-3 in patients with RA, those with osteoarthritis (OA) of hands and healthy controls to find the association with clinical disease activity, routine tests used in RA and the radiographic stage of disease.

MATERIALS AND METHODS

Patients

A total of 92 patients with RA (mean age 54.04 ± 12.22 years; mean disease duration 15.66 ± 9.04 years; 60 females and 32 males; mean DAS28 3.74 ± 1.47), 24 patients with OA (mean age 50.12 ± 15.95 years, mean disease duration 6.25 ± 3.43 years; 17 females and 7 males) and 26 healthy subjects (mean age 48.28 ± 13.38 years; 13 females and 13 males) were included in the study after signing informed consent. The study was approved by the local ethic committee of Faculty of Medicine and

Dentistry, Palacký University Olomouc. RA patients met the EULAR/ACR classification criteria for RA (ref.¹⁵) and OA patients met the ACR clinical classification criteria for OA of hand¹⁶. Twenty-six RA patients (28.2%) received biological therapy; the majority of RA patients (84 pts; 91.3%) were treated with synthetic disease-modifying antirheumatic drugs (DMARDs).

Laboratory analysis

MMP-3 was measured in serum by ELISA using the commercial kit AESKULISA DF MMP-3 (AESKU. Diagnostics, Germany). The microtiter plate was measured with the ELISA photometer Tecan Spectra (wavelength 450 nm). The standard range was 0–800 ng/mL.

The kit measures total MMP-3 (pro- and active MMP-3) and it is a tool for risk stratification of the development of joint destruction and for monitoring disease activity in RA patients. The instruction manual states that the normal ranges are 18–60 ng/mL and 24–120 ng/mL for females and males, respectively.

Anti-cyclic citrullinated peptide (CCP) antibodies

Table 1. Demographic data and results of MMP 3 measurement in RA, OA groups and in healthy controls and activity, laboratory and radiographic profile of RA patients.

	RA	OA	Healthy controls
N	92	24	26
Age (years)	54.04 ± 12.22	50.12 ± 15.95	48.28 ± 13.38
F/M ratio	60/32	17/7	13/13
Duration of disease	15.66 ± 9.04	6.25 ± 3.43	NA
MMP-3 (ng/mL)			
x±SD	199.1 ± 160	113.9 ± 96.9	48.3 ± 19.2
M	168	64	47.5
min-max	26-800	25-367	25-81
DAS 28			
x±SD	3.74 ± 1.47		
M	3.6	NA	NA
min - max	1.1 -6.84		
CRP (mg/mL)			
x±SD	12.2 ± 16.2		
M	4.5	ND	ND
min - max	0.3 -89.9		
ESR (mm/h)			
x±SD	18.9 ± 15.5		
M	13.9	ND	ND
min - max	1-62		
RF (IU/mL)			
x±SD	179.8 ± 242.7		
M	50.7	NA	NA
min - max	10.1 -724		
Anti CCP (IU/mL)			
x±SD	1479.3 ± 1288.6		
M	1420	NA	NA
min - max	25 - 3200		
x-ray stage			
x±SD	2.3 ± 0.9		
M	2	NA	NA
min - max	1-4		

x - mean, SD - deviation, M - median, min - minimum, max - maximum, NA-not applicable, ND- not done

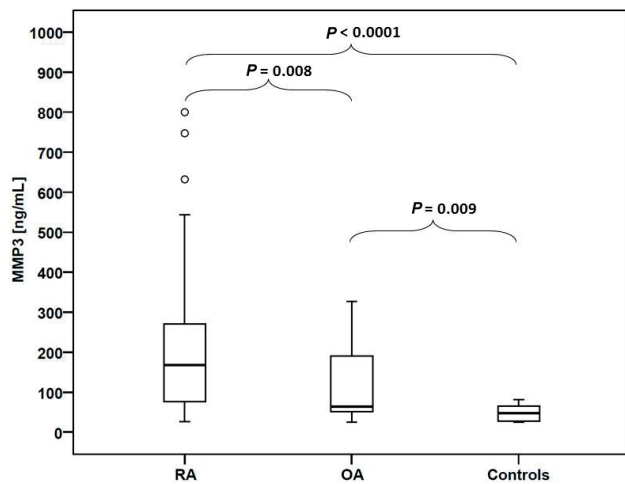


Fig. 1. Differences in serum concentration of MMP-3 between RA patients, OA patients and healthy controls. box shows the distance between the quartiles, with the median as a line, and the whiskers show minimum and maximum values of MMP3. Outliers are shown as separate points. The *P*-values show statistical significances of differences between groups.

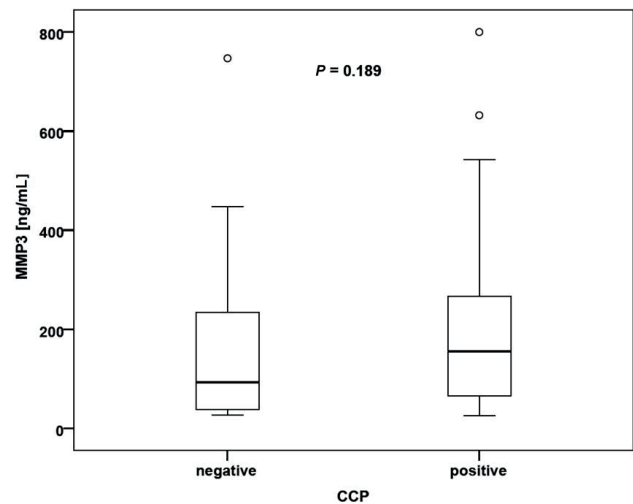


Fig. 2. MMP-3 serum levels in anti-CCP negative and anti-CCP positive RA patients. Box shows the distance between the quartiles, with the median as a line, and the whiskers show minimum and maximum values of MMP3. Outliers are shown as separate points. The *P*-value show statistical significance of differences between groups.

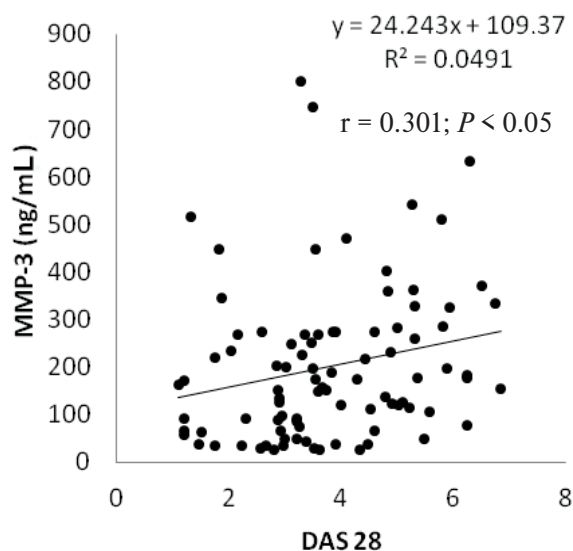


Fig. 3. Correlation between MMP-3 and DAS28.

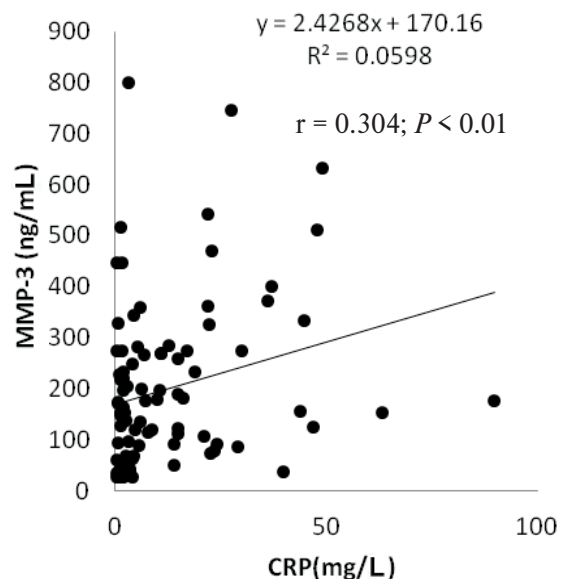


Fig. 4. Correlation between MMP-3 and CRP.

were measured by ELISA using the Immunoscan CCPlus kit (Euro Diagnostica, Sweden). The standard curve range is 25–3200 U/mL. These values were arbitrarily chosen by Euro Diagnostica since no generally recognized international standard exists for expressing the titer of anti-CCP antibodies¹⁷. Samples with results below 25 U/mL are defined as negative.

Rheumatoid factor (RF) was measured by nephelometry using the BN II system and N Latex RF Kit (Siemens, Germany). The result is evaluated by comparison with a standard of known concentration¹⁸. Samples with results below 15 IU/mL are defined as negative.

Erythrocyte sedimentation rate (ESR) was determined using the Westergren method. The rate of fall of red blood

cells is measured in millimeters after in 1 h. The normal rates are 0–15 mm (mean, 4 mm) for males and 0–20 mm (mean, 5 mm) for females¹⁹.

Clinical assessment

Disease activity score (DAS28) (ref.²⁰) was measured by a single assessor. Radiographic stage of disease was evaluated according to the Steinbrocker method^{21,22}.

Statistical analysis: The Shapiro-Wilk test of normality revealed non-normal distribution of the data. The data were expressed as mean, standard deviation, median, minimal and maximal value. Differences between independent groups were analyzed by the Kruskal-Wallis test and post hoc tests with Bonferroni correction. Differences between

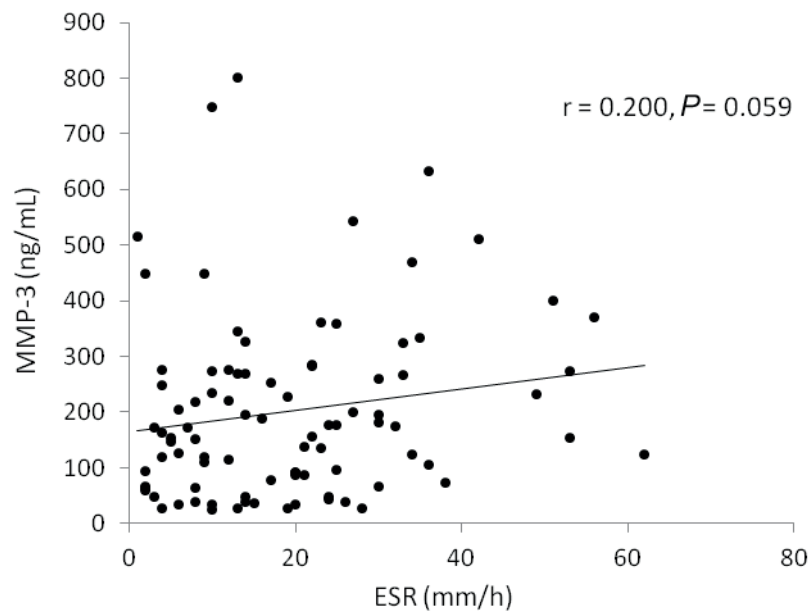


Fig. 5. Correlation between MMP-3 and ESR.

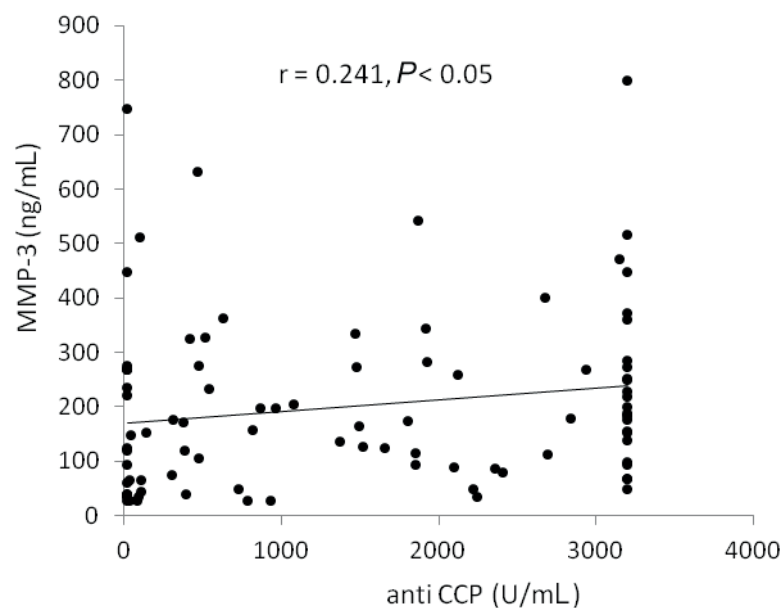


Fig. 6. Correlation between MMP-3 and anti CCP antibodies.

two groups were analysed using Mann-Whitney U-test. Correlations were sought using Spearman's correlation analysis. Values with P less than 0.05 were considered statistically significant. All statistical analyses were conducted with IBM SPSS Statistics 23 (IBM Corporation, 2015).

RESULTS

Table 1 summarizes the demographic data of study groups, results of MMP-3 serum levels measurement in RA and OA patients and in healthy controls and the laboratory profile of RA patients. The mean concentrations

of MMP-3 were 199.1 ± 160 ng/mL in RA patients, 113.9 ± 96.9 ng/mL in OA patients and 48.3 ± 19.2 ng/mL in healthy controls.

The differences in serum concentrations of MMP-3 were statistically significant between RA and controls ($P < 0.0001$), between RA and OA groups ($P = 0.008$) as well as between non-RA and controls ($P = 0.009$). The distribution of MMP-3 in groups and the achieved levels of statistical significance are plotted using a quartile box plot in Fig. 1.

The difference in MMP-3 serum levels between CCP-negative and CCP-positive RA patients was not statistically significant ($P = 0.189$). Fig. 2 shows the distribution of MMP-3 serum levels in both subgroups.

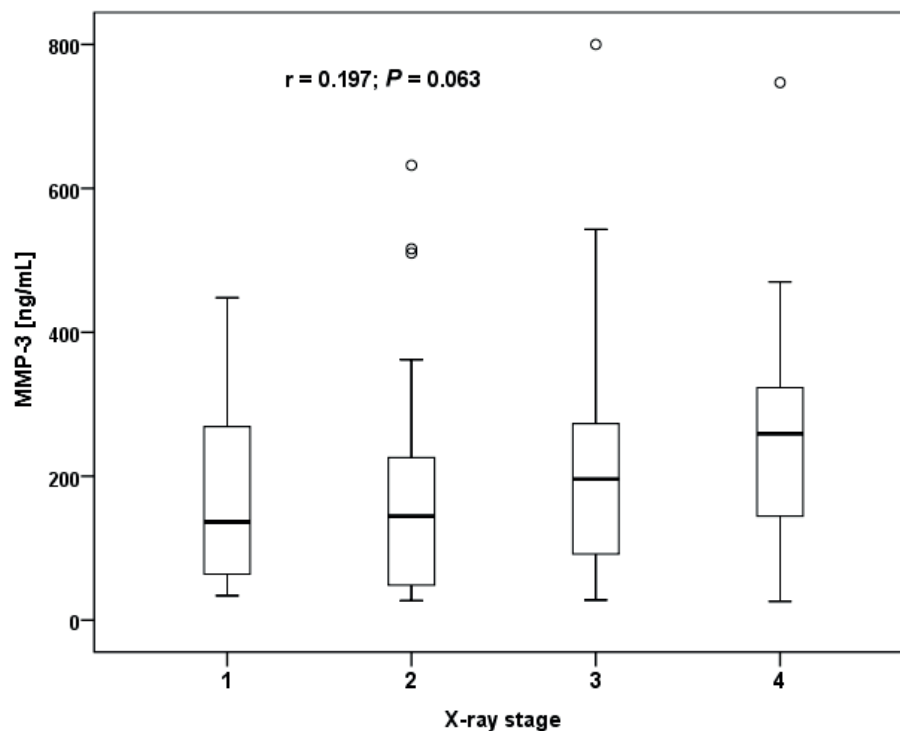


Fig. 7. Correlation between MMP-3 and radiographic stage. Box shows the distance between the quartiles, with the median as a line, and the whiskers show minimum and maximum values of MMP3. Outliers are shown as separate points.

MMP-3 concentrations were further compared with other laboratory parameters and clinical and radiographic data.

Spearman's correlation analysis showed correlations between MMP-3 and DAS28 ($r=0.301$, $P<0.05$; Fig. 3), CRP ($r=0.304$, $P<0.01$; Fig. 4), ESR ($r=0.200$, $P=0.059$; Fig. 5), CCP antibodies ($r=0.241$, $P<0.05$; Fig. 6), and radiographic stage ($r=0.197$, $P=0.063$; Fig. 7). No correlation was found between MMP-3 and RF.

DISCUSSION

The MMP family includes both classical enzymes secreted in a latent form and enzymes anchored in the membrane. Under physiological conditions, the activities of MMPs are regulated at the level of transcription, interaction with specific ECM components and inhibition by endogenous inhibitors. Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors that participate in controlling the local activities of MMPs in tissues. The increasing knowledge of the structures of MMPs and of the signal transduction pathways that control MMP gene expression may provide new opportunities for therapy and development of new medicaments^{23,24}. MMP-3 is one of the most important proteases involved in cartilage degradation; it is mainly secreted by synovial cells, fibroblasts and cartilage cells and may be activated by several cytokines such as IL-1 and TNF- α . MMP-3 in synovial fluid

may directly degrade the cartilage and bone and mediate the development of RA. The serum levels in patients with active RA are markedly elevated in comparison with those in remission. MMP-3 expression is regulated by a promoter gene and exhibits polymorphism with the 5A/6A alleles, but the effect of MMP-3 gene polymorphism on the development of RA is still not clear²⁵. Burrage et al. described the role of MMPs in the process of collagen degradation. The authors mention that the expression of other MMPs such as MMP-1, MMP-2, MMP-3, MMP-9 and MMP-13 is increased in arthritis and these enzymes degrade non-collagen matrix components of the joints. They suppose that influencing MMP gene expression might provide a new approach in the therapy of joint destruction²⁶.

In both RA and OA, inflammatory cytokines stimulate the production of several MMPs (e.g. MMP-1, MMP-2, MMP-3, MMP-9 or MMP-13). MMP-3 is thought to play a pivotal role in joint destruction in RA (ref.^{27,28}). Some studies have demonstrated significant serum expression increase in comparison to healthy controls and its correlation with concentration in synovial fluid. Therefore, serum MMP-3 could reflect a local inflammatory process in the affected joints. High levels of serum MMP-3 can be predictive of destructive processes in the joints even in early disease^{27,29}.

The presented study demonstrated that the serum concentration of MMP-3 is increased in RA patients, as compared with both controls and OA patients. Moreover,

it also correlates with disease activity assessed by the DAS28 score, ESR, CRP and serum levels of anti-CCP antibodies.

This conclusion is supported by Keyszer et al.³⁰ investigating how MMP-1, MMP-3, TIMP-1 and MMP1/TIMP-1 complex (MT complex) reflect the clinical activity compared to cytokines, CRP, ESR and RF. They demonstrated that the correlation of MT complex with clinical data was weaker than that of MMP-3 alone, which also reflected disease activity of RA better than cytokine levels or other markers of connective tissue turnover.

The correlation between MMP-3 and DAS28 in the present study is also concordant with results reported by Ribbens et al.³¹. They demonstrated that serum MMP-3 might serve as a consistent synovial derived marker of RA disease activity and that early changes of MMP-3 predict disease outcome. Serum MMP-3 as a predictor of joint destruction in RA was also dealt with by Mamehara et al. who concluded that MMP-3 could predict joint destruction for RA patients treated with non-biological DMARDs by reflecting response to the drugs²⁷. A recent study demonstrated that low levels of MMP-3 at the onset of the disease predict a patient subgroup that exhibits no radiographic progression over 3-year methotrexate monotherapy and does not require combination therapy³². Another study found that continuously elevated serum MMP-3 may predict radiographic evidence of progression over 1 year in patients treated with various DMARDs using a treat-to-target protocol³³. The above findings are also supported by those by Nawata et al. demonstrating that high levels of MMP-3 predict a poor effect of infliximab and radiographic progression, especially in joint space narrowing, with high CRP levels being a negative prognostic factor for joint erosions. If the results are confirmed in other RA populations, they could establish MMP-3 as a valuable predictor of disease outcome³⁴.

The role of MMPs, in particular MMP-3, is currently being examined in a wide range of other diseases. High levels of MMP-3 and gene polymorphism may play an important part in the pathogenesis of cardiovascular diseases. In a study by Abd El-Aziz and Mohamed, significantly higher levels of MMP-3 were displayed in patients with acute myocardial infarction (AMI) (ref.³⁵). The role of gene polymorphism for MMP-3 in ischemic heart disease was also confirmed by Teplakov et al.³⁶. This finding deserves special mention particularly considering RA itself as an independent risk factor for developing ischemic heart disease.

Furthermore, MMPs are increased in infections due to their role in controlling immune responses³⁷.

The authors of the present study are aware of its limitations. It was a cross-sectional study including patients with various stages and durations of the disease. The levels of MMP-3 could have been influenced by therapy. The authors intend to follow these patients to see the development of the disease based upon the baseline MMP-3 levels. The future study should also contain the investigation of MMP-3 polymorphism in RA and RNA as well as proteomic study.

CONCLUSION

The present study demonstrated increased serum levels of MMP-3 in RA patients compared to OA patients and healthy controls. The concentration of MMP-3 correlated with DAS28, CRP, anti-CCP, ESR and radiographic stage of the disease. These findings support the notion that MMP-3 plays an important role in the pathology of RA and that elevated serum levels may help identify patients at the highest risk for developing severe disease. By reflecting the current development in the field of MMP-3 measurement in RA as seen from other studies it becomes obvious that it represents a useful marker for prediction of joint destruction³⁸ and helps define the risk of progression of the disease.

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Author contributions: SM, HP, HZ: literature search; SM, HP, KA: clinical data collection; HZ: laboratory analysis; LK: data analysis; SM, HP, KA: manuscript writing, final approval.

Conflict of interest statement: None declared.

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