ORIGINAL ARTICLE

Serum Matrix Metalloproteinase-3 Predicts Radiographic Joint Damage and Functional Disability in Rheumatoid Arthritis

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ABSTRAK

Pencarian bio-penanda baru telah menjadi agenda utama aktiviti penyelidikan bagi penyakit Rheumatoid Arthritis (RA) pada dekad yang kebelakangan ini. Tujuan kajian ini adalah untuk menentukan korelasi serum matriks metalloproteinase-3 (MMP-3) dengan aktiviti penyakit, kerosakan sendi dan ketidakupayaan pada pesakit dengan RA. Ini adalah satu kajian kes-kawalan keratan rentas yang melibatkan pesakit RA yang berada di bawah rawatan susulan di Pusat Perubatan Universiti Kebangsaan Malaysia (PPUKM). Maklumat mengenai ciri-ciri penyakit RA telah diperolehi daripada rekod perubatan dan semua pesakit RA telah dinilai untuk DAS28 (skor aktiviti penyakit berdasarkan 28 sendi) dan Stanford Penilaian Kesihatan Questionnaire (HAQ) 8-item Indeks Upaya (HAQ-DI). Radiograf tangan pesakit RA dinilai dengan menggunakan Skor Modified Sharp (MSS). Serum MMP-3 dari pesakit RA dan kawalan yang sihat diukur dengan menggunakan kaedah ELISA. Seramai 77 pesakit RA dan 18 kawalan sihat telah mendaftar dalam kajian ini. Tahap serum MMP-3 adalah lebih tinggi di kalangan pesakit RA (p <0.05). Terdapat hubungan yang signifikan antara serum MMP-3 dan MSS (r = 0.327) dan HAQ-DI (r = 0.256), kedua-dua p <0.05. Tahap min serum MMP-3 pada pesakit RA dengan hakisan tulang radiografik adalah jauh lebih tinggi daripada pada pesakit tanpa hakisan (p <0.05). Tahap min MMP-3 di kalangan pesakit dengan ketidakupayaan yang signifikan iaitu HAQ-DI 1; jauh lebih tinggi berbanding pesakit RA tanpa kecacatan yang signifikan (p <0.05). Berdasarkan analisis multivariat, HAQ-DI kekal sebagai prediktor serum MMP-3 di kalangan pesakit RA. Serum MMP-3 adalah penanda bio yang berpotensi meramalkan kerosakan sendi radiografik dan ketidakupayaan di kalangan pesakit RA.

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ABSTRACT

The search for novel biomarkers has taken centre stage in the past decades of research in Rheumatoid Arthritis (RA). The purpose of the present study was to determine the correlation of serum matrix metalloproteinase-3 (MMP-3) with disease activity, joint damage and functional disability in patients with RA. We consecutively recruited RA patients who were under follow-up at the Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Information on the RA disease characteristics were obtained from the medical records and all RA patients were assessed for DAS28 (disease activity score based on 28 joints) and Stanford Health Assessment Questionnaire (HAQ) 8-item Disability Index (HAQ-DI). The hand radiographs of the RA patients were assessed for joint damage using the Modified Sharp Score (MSS). Serum MMP-3 levels from RA patients and healthy controls were measured using the ELISA method. We recruited a total of 77 RA patients and 18 healthy controls. The serum MMP-3 levels were significantly higher among the RA patients ($p<0.05$). There were significant correlations between the serum MMP-3 levels and MSS (r =0.327) and HAQ-DI (r=0.256), both $p<0.05$. The mean serum MMP levels in RA patients with radiographic joint erosions was significantly higher than in patients without erosions ($p<0.05$). Likewise, the subjects with significant functional impairment i.e HAQ-DI 1; had significantly higher mean MMP-3 levels compared to RA patients without significant disability ($p<0.05$). Using multivariate analysis, HAQ-DI remained the independent predictor of serum MMP-3 in RA patients. Serum MMP-3 is a potential biomarker and predictor of radiographic joint damage and functional disability in RA.

Keywords: acquired joint deformity, matrix metalloproteinases, rheumatoid arthritis

INTRODUCTION

Rheumatoid Arthritis (RA) is the most common autoimmune inflammatory polyarthritis (Rothschild et al. 1988). It is characterized by synovial inflammation that may lead to irreversible destruction of the cartilage, tendons and bones. RA is also associated with autoantibody production (rheumatoid factor and anti–citrullinated protein antibody (ACPA) with systemic features which may involve the cardiovascular, pulmonary and haematological systems (McInnes & Schett 2011). The consequent joint damage in RA often leads to deterioration in quality of life and severe functional impairment (Welsing et al. 2001).

Despite the major advances in the treatment of RA with novel biologic therapies, there are still several unmet needs. These include reliable biomarkers which are able to accurately reflect the disease activity, prognosis and severity of the disease. Serum C-reactive protein (CRP) and Eryhtrocyte Sedimentation Rate

(ESR) are the classical inflammatory biomarkers that are being used in the disease activity assessment of RA. Unfortunately, these biomarkers are non-specific and can be raised in many other inflammatory conditions eg infections and malignancies. A specific biomarker has a pivotal role in the management of RA to avoid undertreatment which leads to joint damage, or overtreatment which may pose unnecessary risks.

Matrix metalloproteinase-3 (MMP-3) is a proteolytic enzyme and it is produced mainly by the chondrocytes and synovial fibroblasts. It has been found to be elevated in the serum and synovial fluid in patients with RA (Kobayashi et al. 2007; Mahmoud et al. 2005; Ribbens et al. 2002). Its production is upregulated by the proinflammatory cytokines IL-1β, TNF, IFNγ, and IL-17A, as well as by serum amyloid A (SAA) in RA (Galliera, et al. 2010; Hueber et al. 2010). It also contributes to the progression of RA by promoting the recruitment of many other inflammatory cells ie neutrophils, monocytes and T cells (Jones et al. 2008). Since MMP-3 is produced in the inflamed joint, and subsequently circulated in the blood stream, it is a potential biomarker which may indicate the disease activity in RA (Zucker et al. 1994). In addition, elevated levels of MMP-3 may indicate ongoing destructive processes within the bones and synovium with resultant joint damage. The above however, is theoretical and has to be supported by clinical studies.

Therefore, the main aim of the present study was to explore the correlation of serum MMP-3 with disease activity, joint damage and functional disability in RA.

MATERIALS AND METHODS

STUDY DESIGN AND STUDY POPULATION

This was an observational, case-control study involving RA patients who were under follow-up at the Universiti Kebangsaan Malaysia Medical Centre. This study was approved by the institutional review board and ethics committee (DLP 2013-039). The following were the inclusion criteria of this study; i) Patients diagnosed with RA based on American College of Rheumatology 1987 (Arnett et al. 1988) or 2010 (Aletaha et al. 2010), ii) Patients aged 18 yrs and above.

The following patients were excluded: i) Patients who had previous hand and/or wrist surgeries which may obscure the assessment of the hand radiographs, ii) Patients with active infections, iii) Patients with malignances.

The subjects with RA were assessed for DAS28 (disease activity score based on 28 joints) and Stanford Health Assessment Questionnaire (HAQ)8-item Disability Index (HAQ-DI). A total of 77 RA patients and 18 healthy controls consented and were tested for serum matrix metalloproteinase-3 (MMP-3). The hand radiographs of the RA patients were scored using the Modified Sharp Score (MSS) by a single musculoskeletal radiologist who was blinded to the subjects.

DATA COLLECTION

The medical records of all the subjects with RA were reviewed to gather information on the disease duration and seropositivity. The sociodemographic

data, Patient Global Assessment (PGA) scores and Stanford HAQ-DI scores were determined by questioning the subjects. The Stanford HAQ –DI consists of eight questions regarding the functional limitations in performing their activities of daily living. Patients were required to answer on how difficult it was to perform an activity on a scale of 0 (without any difficulty) to 3 (unable to do). The scores were averaged to a single total score (Fries et al. 1980). The Patient Global Assessment (PGA) required patients to state how they felt generally using a scale of 0 (very well) to 10 (very poor) (Rohekar & Pope 2009).

In this study, the DAS 28 calculation was performed by certified rheumatologists. The four parameters used to calculate DAS 28 were; number of swollen joints, number of tender joints, C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), and the patient's global assessment (Fransen & van Riel 2005; Prevoo et al. 1995). A DAS 28 of 3.2 and above (moderate to high disease activity) is considered as active disease.

Using the modified Sharp scoring (MSS) system (Pincus et al. 1997), each joint was scored for joint space narrowing and erosion. Fifteen sites in each hand and wrist were examined for joint space narrowing on a scale of 0-4. Similarly, the erosions were scored individually at 16 sites in each hand and wrist.

SERUM MATRIX METALLOPROTEINASE-3(MMP-3)

Serum samples from all subjects were stored at 80°C until analysis. Serum levels of soluble MMP-3 were measured with a human MMP-3 sandwich enzyme immunoassay detection kit (Quantikine, R&D systems) according to the manufacturer's instructions. Measurements were done in duplicate. Serum samples were placed in designated microwells and both positive and negative calibrators were added to the designated microwells in order to determine a standard curve. The plates were then incubated for 30 mins at 26°C and washed with a buffer solution.

STATISTICAL ANALYSIS

The statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) package for Windows version 21. Continuous variables were expressed as mean SD. Differences between the RA patients and healthy controls were analysed using the Chi-square test (categorical variables) and independent t-test (continuous variables). Correlations between the serum MMP-3 levels and clinical indicators were analysed by the Spearman's rank order correlation test. A p value of <0.05 was considered statistically significant.

RESULTS

DEMOGRAPHIC DATA

A total of 77 RA patients and 18 healthy controls were enrolled in this study. The majority of the patients were females (89.6%). The subjects were predominantly Malays for both the RA patients (53.3%) and the controls (88.9%). The mean age of the subjects

with RA was 58.6 ± 10.3 yrs whereas for the controls was 56.4 ± 7.3 yrs. The demographic characteristics were matched between the RA patients and the controls (Table 1). The serum MMP-3 levels were significantly higher among the RA patients (p<0.05). The mean serum MMP-3 level in RA patients was higher than the normal range (18–60 ng/mL) whereas the healthy controls were within the range.

CORRELATIONS BETWEEN SERUM MMP-3 LEVELS AND CLINICAL MEASURES OF DISEASE ACTIVITY, JOINT DAMAGE AND FUNCTIONAL DISABILITY

Spearman's rank order correlation test showed significant correlations between the serum MMP-3 levels and MSS (r value of 0.327) and HAQ-DI (r value of 0.256) (Table 2). The strength of the relationship however was only moderate for MSS (r within 0.3-0.5) and weak for the HAQ-DI (r within 0.1-0.3). The mean serum MMP-3 levels in RA

patients with radiographic joint erosions was significantly higher (p=0.017) than in patients without erosions (Figure 1). Likewise, the subjects with significant functional impairment i.e HAQ-DI ≥1; had significantly higher mean MMP-3 levels (90.7 ng/ml) compared to RA patients without significant disability (80.3 ng/ml). There was no appreciable difference in this regard (p=0.268) between the active disease and inactive disease groups (Figure 1). Age and disease duration had an inverse relationship with the serum MMP-3 levels. There were no significant correlations with the disease activity and inflammatory markers such as ESR and CRP (Table 2).

A multiple regression analysis was performed to predict serum MMP-3 from HAQ-DI, MSS, DAS 28, ESR and CRP. These variables statistically significantly predicted serum MMP-3, $F_{(5,95)} = 7.368$, $p < 0.005$, $R^2 = 0.450$. However, only HAQ-DI added significantly to the prediction, p<0.05.

Table 1: Clinical data of the RA patients and the controls

	RA patients (n = 77)	Controls (n = 18)	p-value
Age (years)	58.6 ± 10.4	56.4 ± 7.3	0.398
Gender, n(%)			0.542
Male	8 (10.4%)	1 (5.5%)	
Female	69 (89.6%)	17 (94.4%)	
Ethnicity, n(%)			0.237
Malay	42 (54.5%)	16 (88.9%)	
Chinese	20 (25.9%)	1 (5.5%)	
Indian	14 (18.2%)	1 (5.5%)	
Others	1 (1.2%)	0 (0%)	
Serum MMP-3	82.73 ± 8.18	31.45 ± 3.29	<0.05

Data presented as either counts (percentages) or mean ± SD

Table 2: Correlation analysis between serum MMP-3 levels and clinical parameters in RA

Variable	Spearman's rho	p value
Age	-0.041	0.734
Disease Duration	-0.095	0.432
DAS 28	0.072	0.546
MSS	0.327	**0.005**
HAQ-DI	0.256	**0.030**
ESR	0.123	0.383
CRP	0.094	0.435

CRP; C reactive protein, ESR: erythrocyte sedimentation rate, HAQ-DI; Health Assessment Questionnaire Disability Index, MSS: Modified Sharp Score , DAS 28: 28 joint based Disease Activity Score
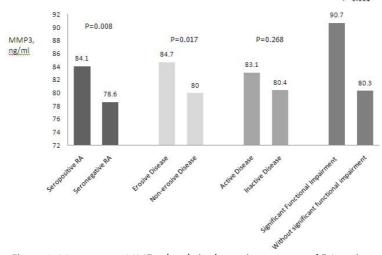

Figure 1: Mean serum MMP-3 levels in the various groups of RA patients

DISCUSSION

This study highlighted that serum MMP-3 levels had a significant association with radiographic joint damage and functional impairment in RA. There is paucity of data on the predictive role of serum MMP-3 with regard to functional disability. However, a Japanese study (Shinozaki et al. 2007) reported that elevated serum MMP-3 predicted progressive disability based on JHAQ (Japanese version of HAQ) especially in female patients without corticosteroid.

Galil et al. (2016), on the contrary, disclosed no positive correlations between serum MMP-3 levels and HAQ. Of note, the patients recruited by Galil et al. (2016) had joint symptoms for less than a year and hence, did not have significant functional disability. Our patients, on the other hand, had a mean disease duration of 10.5 yrs. We found that HAQ-DI was the only variable which significantly predicted serum MMP-3 using multiple regression analysis.

The finding of significant positive correlation between radiographic joint damage and serum MMP-3 echoes the results of the previous studies in this regard (Cheung et al. 2000; Cunnane et al. 2001; Galil et al. 2016). Some of these studies could demonstrate the above link despite using a different joint scoring system i.e the Larsen score (Yamanaka et al. 2000). MMP-3 acts directly on the matrix of the cartilage. In animal models, MMP-3 was inducible and detectable in chondrocytes even in the early phase of arthritis (Okada et al. 1989; Singer et al. 1995). Progressive degradation of the cartilage leads to joint space narrowing. Articular erosions are due to bone resorption which is facilitated by MMP-3-mediated removal of the outer osteoid layer (Cunnane et al. 2001) .

In contrast to many previous studies (Ally et al. 2013; Galil et al. 2016; Ichikawa et al. 1998), we found no relationship between serum MMP-3 and either disease activity or inflammatory markers (ESR,CRP). The difference in the study population may partially explain our discrepant findings. We recruited all RA patients regardless of disease duration. Many of the studies which demonstrated a link between disease activity and MMP-3 included patients with only early disease (Urata et al. 2012; Ally et al. 2013). The mean DAS 28 in this study was 2.19 (in remission). In general, patients with early disease tend to have higher disease activity due to unoptimised treatment and while in the interim before the onset of action of disease modifying anti-rheumatic drugs.

We do acknowledge the limitations of our study. As with most cytokines and biomarkers, MMP-3 levels may fluctuate in the blood circulation. Ideally, 2-3 samples of blood should have been obtained from each subject on separate occasions. The average measurement of MMP-3 could have been used in the analysis. Each subject only had a single blood sample of MMP-3 taken; for convenience. Besides, ultrasound has been proven to be more sensitive in detecting joint erosions in RA. Early or mild erosions can be easily overlooked with plain radiographs (Filippucci et al. 2006).

CONCLUSION

In conclusion, serum MMP-3 is a useful predictor of radiographic joint damage and functional disability in RA. The findings of this study have clinical implications. Novel therapies in RA should target MMP-3 to minimise joint destruction. Several models of MMP-3 inhibitors are currently under phase I and II of clinical trials.

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