Clinical significance of matrix metalloproteinase-3 in systemic lupus erythematosus patients: a potential biomarker for disease activity and damage

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ABSTRACT

Objectives: To assess the serum level of matrix metalloproteinase-3 (MMP-3) in systemic lupus erythematosus (SLE) patients and correlate it with clinical manifestations, laboratory findings, disease activity and damage.

Methods: Forty-two female SLE patients were included in the present study. Full history taking, thorough examination and investigations were performed. Disease activity was assessed using the SLE Disease Activity Index (SLEDAI). Furthermore, Systemic Lupus International Collaborating Clinics /American College of Rheumatology damage index (SLICC/ACR DI) was also assessed. Renal biopsy was done in those with lupus nephritis. Thirty age and sex matched subjects were included as control. Serum MMP-3 was measured by ELISA.

Results: The mean serum MMP-3 level in SLE patients was significantly higher (80.9±45.8 ng/ml) than in the control (10.01±2.6 ng/ml) (p <0.0001). The level in patients with arthritis, nephritis or hematologic disorders were significantly higher than in those without (p<0.0001, p=0.02 and p=0.04 respectively). The MMP-3 was significantly different among the subclasses of renal biopsy (p=0.01) being higher in those with class IV (137.5±45.6 ng/ml). It significantly correlated with the SLEDAI, SLICC, white blood cells and platelet counts (r=0.37, p=0.02; r=0.36, p=0.02; r=0.32, p 0.04 and r=0.38, p=0.01 respectively). On linear regression analysis with age, disease duration and body mass index as independent factors, the SLEDAI and SLICC were not significant predictors.

Conclusion: Serum MMP-3 was found to be high in SLE patients and associated with arthritis, nephritis and hematological manifestations. MMP-3 correlated with disease activity and damage making it a possible biomarker, and its measure of considerable interest, related to the potential therapeutic responses and disease outcome.

Keywords: MMP-3; Arthritis; Nephritis; SLE; SLEDAI; SLICC.

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INTRODUCTION

The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that degrade the components of the extracellular matrix and are essential for tissue remodeling and repair during development and inflammation[1]. It is known that MMPs are implicated in bone resorption, contribute to tissue destruction and are up regulated by ultraviolet radiation in skin[2].

Measurement of MMP-3 (stromelysin-1) is widely accepted as a clinically useful method for evaluating disease activity of rheumatoid arthritis (RA)[1] and showed a remarkable association with inflammation, cartilage destruction and bone erosions[3]. Elevated MMP-3 level is not specific for RA or for erosive joint diseases in general but reflects disease activity better than cytokine levels or markers of connective tissue turnover[4]. It was also elevated in patients with polymyalgia rheumatica, crystal arthritis, psoriatic arthritis[5] and ankylosing spondylitis[6].

MMP-3 levels were markedly elevated in systemic lupus erythematosus (SLE)[5] and were abundantly

expressed by keratinocytes in skin samples[2,7]. In chronic inflammatory diseases without synovitis, tissues other than the inflamed synovium may be sites of the increased production of MMP-3. Its production has been identified in glomerular, tubular epithelial and mesangial cells. Patients with mesangial proliferative glomerulonephritis such as IgA and lupus nephritis have increased levels[7].

Patients with active renal disease had a significantly higher serum MMP-3 level with a possible role in the pathogenesis of lupus nephritis[8]. A 3-5 fold increase in serum MMP-3 levels in SLE patients was found compared to healthy subjects. Contrary to expectations, serial measurements of MMP-3 in individual patients did not correlate with fluctuation in disease activity scores. Accordingly, it was suggested that MMP-3 may not be primarily involved in the initial tissue damage in SLE, but rather participates in a later aspect of inflammation involving tissue repair[7]. On the other hand, Ribbens *et al.*[5] found that serum MMP-3 did not change from control subjects in patients with cutaneoarticular or renal lupus, systemic sclerosis or vasculitides.

The aim of the present study was to assess and compare the serum level of MMP-3 in SLE patients and correlate it with clinical manifestations, laboratory findings, disease activity and damage.

METHODS

Forty-two SLE patients were included in the present study recruited from the Rheumatology department and outpatient clinic of Cairo University Hospitals, during the period from February till July 2013. All patients fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE[9]. Full history taking, thorough examination, laboratory and relevant radiological investigations were performed for all the patients. Comparison between those with current arthritis and those without any previous history was considered. Similarly, association with other clinical manifestations was taken into consideration. Disease activity was assessed for all the patients using the Systemic lupus erythematosus disease activity index (SLEDAI)[10]. Furthermore, SLICC/American College of Rheumatology damage index (SLICC/ACR DI) was also assessed[11]. Renal biopsies were done in those with lupus nephritis. The specimens were processed for light microscopy and classified according to the 1982 modified world health organization (WHO) morphologic classification of lupus nephritis[12]. Thirty age and sex matched subjects were considered as a control group and were recruited from the relatives of the patients and healthcare providers in the hospital. Serum MMP-3 was measured by enzyme linked immunosorbent assay (ELISA) in all subjects. The study was approved by the local university ethical committee and the study performed in accordance with the ethical standards of the 1964 Helsinki declaration. All patients gave their informed consent prior to their inclusion in the study.

STATISTICAL ANALYSIS

The data were collected, tabulated and analysed by SPSS package version 15 (SPSS corporation, USA). Data was summarized as mean ± SD. Mann–Whitney tests was used for comparative analysis of 2 quantitative data. Non-parametric analysis of variance (Kruskall–Wallis) was used for comparison of more than two groups; Spearman's correlation was used for detection of the relation between 2 variables. Linear multiple regression analysis was performed to detect predictors of MMP-3 level. Results were considered significant at $p < 0.05$.

RESULTS

Forty-two female patients suffering from SLE were studied. The demographic, clinical and laboratory feature of the patients are shown in Table I.

Joints were involved in the form of nonerosive oligoarthritis especially affecting the proximal interphalageal joint (PIPs), wrists and knees with arthralgias in other body joints. All the patients were treated with corticosteroids. In addition, 23 were receiving hydroxychloroquine (HCQ). Nine others treated with azathioprine and 8 gave history of cyclophosphamide administration. The anti-nuclear antibody (ANA) was positive in 90.5%, while the anti-double stranded deoxyribonucleic acid (anti-dsDNA) was positive in 57.1%. In the control, the mean age was 32.2±5.4 years.

The MMP-3 level was significantly higher in the patients (80.9±45.8 ng/ml) compared to the control (10.01±2.6 ng/ml) ($p < 0.0001$). The mean serum MMP-3 level in patients with arthritis (103.9±44.3 ng/ml) was significantly higher than in those without (55.8±32.9 ng/ml) ($p < 0.0001$). On considering those

TABLE I. DEMOGRAPHIC, CLINICAL AND LABORATORY FEATURES OF THE SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

Feature		SLE patients (42) mean±SD
Age (years)		33.2±11.6
Disease duration (years)		6.3±5.2
BMI		25.2±3.4
Steroid dose (mg/day)		24.5±16.7
SLEDAI		11.9±6.8
SLICC		2.6±1.9
Clinical Manifestations		N(%)
Mucocutaneous		32 (76.2)
Arthritis		23 (54.8)
Nephritis		21 (50)
Hematological		20 (47.6)
Neuropsychiatric		12 (28.6)
Serositis		17 (40.5)
FMS		6 (14.3)
APS		16 (38.1)
Laboratory features	**mean±SD**	**Reference range**
ESR (mm/1st hr)	47.7±27.9	M <20, F<30
Hemoglobin (g/dl)	10.3±2.7	M 13.8-17.2, F 12.1-15.1
WBCs (x103/mm3)	7.6±3.5	4-11
Platelets (x103/mm3)	308.7±165.2	150-400
Creatinine (mg/dl)	0.9±0.5	M 0.6-1.2, F 0.5-1.1
Urea (mg/dl)	36.4±21.8	7-20
Urine protein (g/24hr)	1.4±1.9	0-0.15
C3 (g/l)	0.6±0.3	0.9-1.8
C4 (g/l)	0.2±0.1	0.2-0.5

BMI: Body mass index; SLEDAI: Systemic lupus erythematosus disease activity index; SLICC: Systemic Lupus International Collaborating Clinics; FMS: Fibromyalgia syndrome; APS: Antiphospholipid syndrome; ESR: Erythrocyte sedimentation rate; C: Complement; WBC: white blood cells; M: males; F: females.

with lupus nephritis, the level was significantly higher (97.6±49.2 ng/ml) than in those without (64.3±36 ng/ml) (p=0.02). Twenty-one nephritis patients had a renal biopsy examined. The mean MMP-3 level, according to the predominant WHO renal classes, were as follow: one patient had class I (70 ng/ml), 4 had class II (125.8±39 ng/ml), 3 class III (105±35 ng/ml), 6 class IV (137.5±45.6 ng/ml), 5 class V (51±15.9 ng/ml) and 2 had class VI (45±21.2 ng/ml) nephritis. The MMP-3 was significantly different among the subclasses of renal biopsy (p=0.01). The level of MMP-3 was significantly higher in those with hematologic disorders (96.5±56.2 ng/ml) than in those without (66.8±28.4 ng/ml) (p=0.04) (Figure 1). While it was not different in the presence or absence of the other clinical manifestations, no significant difference in the MMP-3 level was present according to anti-dsDNA positivity, neither between those receiving HCQ, azathioprine or cyclophosphamide and those who didn't.

The serum MMP-3 significantly correlated with the white blood cells (WBC) and platelet counts (r=0.32, p 0.04 and r=0.38, p=0.01 respectively). The MMP-3 significantly correlated with the erythrocyte sedimentation rate (ESR), SLEDAI and SLICC (r=0.39, p=0.01; r=0.37, p=0.02 and r=0.36, p=0.02 respectively). Otherwise there were no significant correlations of the MMP-3 with the other studied parameters. On linear regression, analysis with age, disease duration and

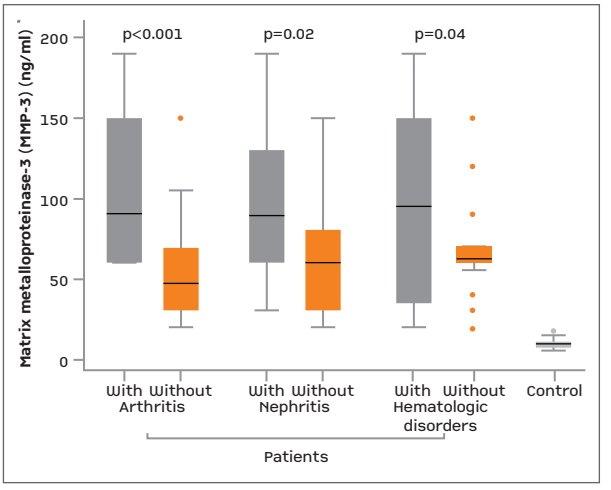

FIGURE 1. Serum MMP-3 levels in SLE patients in presence and absence of arthritis, nephritis and hematological disorders compared to the control

body mass index as independent factors, the SLEDAI and SLICC were not significant predictors. The presence of arthritis and nephritis were significant factors ($p < 0.0001$ and $p=0.001$ respectively).

DISCUSSION

Predicting the disease course of SLE may allow better targeting of aggressive treatment to improve the therapeutic outcome[13]. Matrix metalloproteinase-3, secreted by synovium cells, chondrocytes, and fibroblasts is strongly involved in joint tissue destruction[14]. Most MMPs occur naturally only in very low levels in normal tissues. Their expression is tightly regulated, induced only when needed, so as to avoid uncontrolled and excessive tissue destruction[15]. In the present study, the serum level of MMP-3 was significantly elevated. These findings are in agreement with the results of Zucker *et al.*[7] who stated that serum MMP-3 levels were significantly increased in SLE patients compared to healthy subjects. The frequency of SLE patients with high MMP-3 levels was comparable to that observed in RA[8].

In the present study, serum level of MMP-3 significantly correlated with the ESR, SLEDAI and SLICC/DI. Furthermore, patients with lupus nephritis showed significantly higher level of MMP-3. The increased level of serum MMP-3 is closely associated with clinical features relevant to lupus nephritis, suggesting its role in the pathogenesis. Serum MMP-3 in RA strongly correlated with the ESR and reflected disease activity[16]. *Yamanaka* et al.[16] stated that moderate elevation of MMP-3 in SLE patients is associated with disease activity especially nephritis. In another study[7], the elevated levels of MMP-3 did not correlate with disease activity. The potential use of MMP-3 levels as marker of disease outcome should thus be made with precaution.

The MMP-3 was significantly different among the subclasses of renal biopsy being highest among those with class IV. It may be true that MMPs represent the factor that disintegrates membranes in progressive lupus nephritis[17]. MMP-2 has been reported to be increased in patients with membranous nephropathy[18] while in another study its immunoreactivity was not detected in any type of glomerulonephritis (GN). MMP-9 was increased in mesangial proliferative GN[19]. Furthermore, no significant correlation appeared between the extent of MMP-11 positivity and histological severity of the types of GN[20]. More recently, the MMP-2 and MMP-9 mRNA levels were reported significantly up-regulated in class IV lupus nephritis[17].

The neuropsychiatric features were mild in the SLE patients included in the present study and the serum level of MMP-3 was not affected. So far there is no clear information about the relation of serum MMP-3 and the neuropsychiatric manifestations in SLE[15].

In our study, there was a significant positive correlation between serum MMP-3 level and both the WBC and platelet counts in the SLE patients. This was also observed in long term RA patients[16] while the serum and synovial fluid levels significantly correlated with the ESR and platelet count in juvenile idiopathic arthritis (JIA) patients[21]. Platelet–leukocyte aggregation is essential for leukocyte recruitment, an important step of inflammatory and immune reactions and is partly regulated by MMPs[22]. In the present study, no significant correlation was found between MMP-3 level and the corticosteroid dose received. This is in line with other studies that reported that MMP-3 serum levels were significantly increased in inflammatory rheumatic diseases patients, whether treated or not by corticosteroids and in those with synovitis, whether acute or chronic, erosive or not[5].

It could be concluded that serum level of MMP-3 was found to be high in SLE patients suggesting its role in the pathogenesis. The increased level was associated with important clinical features of the disease, namely, arthritis, nephritis and hematologic disorders.

Further longitudinal studies on a larger sample are needed to confirm our findings.

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