RAISED SERUM MATRIX METALLOPROTEINASES-3 AND -9 IN EARLY RHEUMATOID ARTHRITIS

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KEY WORDS: SERUM MATRIX METALLOPROTEINASE-3, SERUM MATRIX METALLOPROTEINASE-9, EARLY DIAGNOSTIC CRITERIA OF RA.

ABSTRACT

Aim of work: *To study whether serum levels of matrix metalloproteinases-3 and -9 (MMP-3 and MMP-9) are specifically increased in early rheumatoid arthritis patients (RA) as compared to osteoarthritis knee patients. Also, the relation of such rise to different disease activity parameters and to the development of joint damage as detected radiologically.*

Methodology: *Serum concentration of MMPs (3 and 9) were measured in 30 RA patients with disease duration less than one year and 30 OA knee patients with sandwich enzyme immunoassay (EIA). In RA patients, routine laboratory variables and plain x-rays of both hands & feet were assessed in parallel and were correlated with two variables.*

Results: *Serum concentrations of MMP-3 and MMP-9 were significantly higher in early RA patients as compared to OA patients ($p < 0.01$). There were significant correlations between MMP-3 and MMP-9 with swollen joint count (SJC), ESR and CRP. There were no significant correlations with disease activity score (DAS) or tender joint count (TJC) or Ritchie articular index (RAI). There were significant correlations between MMP-3 and MMP-9 and joint space narrowing (JSN) score. But there were no significant correlations with erosions and total sharp score (TSS).*

Conclusion: *Increased serum levels of MMP-3 and MMP-9 were not specific for RA. MMP-3 and MMP-9*

correlated with other markers of disease activity such as SJC, ESR and CRP. Of the radiological scores, as disease outcome measures, MMP-3 and MMP-9 correlated with joint space narrowing. Therefore, MMP-3 and MMP-9 can be used as synovial derived markers of RA activity.

INTRODUCTION

The matrix metalloproteinases *(MMPs)* are a family of enzymes that collectively degrade all components of the extracellular matrix. MMPs play crucial roles in normal physiologic processes such as development and wound healing, where levels of these enzymes are typically low. In contrast, aberrant MMP expression occurs in several disease states, including atherosclerosis, tumor invasion, and arthritis. MMPs mediate irreversible matrix degradation and subsequent joint destruction in rheumatoid arthritis *(RA)* and osteoarthritis *(OA)* Inflammatory cytokines, produced predominantly by macrophages in the synovial pannus, induce the expression of MMPs from the synovium and cartilage in RA. These MMPs then degrade the matrix components of bone, tendons and cartilage *(Mengshol et al., 2002).*

Osteoarthritis is characterized by destruction of joint cartilage and subchondral bone, with degradation of the extracellular matrix. Inflammatory cytokines, acting in an autocrine loop, induce MMPs production by chondrocytes, leading to localized cartilage degradation *(Martel-Pelletier et al., 1994).*

In RA, cytokines play a major role in local joint inflammation and destruction as well as in the systemic acute phase response. The relationship between acute phase proteins like C-reactive protein *(CRP)* and disease activity and radiological progression has been described *(Van Leeuwen et al., 1994 and Van Leeuwen et al., 1993).* Although CRP appears to be a good variable for prognostic purposes *(van der Heijde et al., 1992)* and for monitoring treatment effects *(Stegner et al., 1998),* it is an indicator of inflammation in general that may be influenced by other stimuli of the acute phase response *(Cheung et al., 1996).* MMPs that are locally produced and activated within the affected joint as a result of cytokine mediated stimulation, could be more specific markers of joint inflammation *(Taylor et al., 1994)* and especially joint destruction *(Sasaki et al., 1994).*

In the present study, we measured the serum levels of MMP 3 and 9 in early RA patients and knee osteoarthritis *(OA)* patients to detect whether these enzymes are specifically increased in RA or not. Furthermore, we

investigated the relationship between serum MMP-3 & -9 levels and different disease activity parameters as well as joint damage as detected radiologically.

MATERIALS AND METHODS

Thirty RA patients diagnosed according to the 1987 American College of Rheumatology criteria *(Arnett et al., 1988),* with joint symptoms of less than one year and who had not previously received disease modifying anti-rheumatic drugs *(DMARDs)* were included in this study. For comparison, 30 knee osteoarthritis (OA) OA patients knee according to *Altman et al. (1986)* were used as a control group. Two groups were matched for sex and age.

Clinical markers of disease activity: Fifty-two peripheral joints were examined for tenderness and soft tissue swelling. The following articular indices were determined: Ritchie articular index *(RAI) (Ritchie et al., 1968),* tender joint count *(TJC),* swollen joint count *(SJC),* and the disease activity score *(DAS)* according to *van der Heijde et al.,(1990),* with 3 variables; RAI, number of swollen joints and erythrocyte sedimentation rate *(ESR).*

Radiological analysis: Joints of both hands and feet were assessed with *Sharp's* method with some modifications as described by *van der Heijde et al., (1995).* By this method, joint space narrowing *(JSN)* and erosions were scored separately and combined to a total Sharp score *(TSS)* with a maximum TSS of 448 points. The radiographs were scored without knowledge of clinical and laboratory data.

Blood sampling: Blood samples were obtained between 9:00 am and 10:00 am, when the subjects had been awakened and physically active for more than 3 hours. Serum was isolated from the blood samples, frozen at -80° C, and assayed within 3 months.

Determination of MMP levels: Serum concentrations of MMP-3 and MPP-9 were measured with a single-step sandwich enzyme immunoassay *(EIA),* using a solid-phase monoclonal antibody and a horse radish peroxidase-labeled monoclonal antibody as previously described *(Fujimoto et al., 1994 and Obata et al., 1992).* The sensitivity *(limit of detection)* of the assay system was 12.5 ng/ml for MMP-3 and 3.1 ng/ml for MMP-9. The normal ranges of these protein concentrations in the serum are 15 - 72 ng/ml for MMP-3 and 12 - 71 ng/ml for MMP-9.

CRP was measured using nephelometer 100 (Dade Behring analyzer, USA) and ESR according to Westergren. IgM RF was measured with ELISA *(Van Leeuwen et al., 1995) (normal value < 10 IU/ml).*

Statistical analysis:

The significance of differences between the 2 study groups was determined by the Wilcoxon 2-sample test. p-values less than 0.05 were considered significant. For blood samples, the significance of differences between the two groups and the normal ranges *(as given above)* was also determined by Fisher's 2-tailed exact test because the data were not normally distributed. p-values less than 0.05 were considered significant. Logistic discriminate analysis was performed to determine cutoff levels of serum MMP, when any of these protein levels were significantly different between two groups. Spearman's rank correlation coefficient was used for the assessment of correlations between the different variables.

RESULTS

The characteristics of the 30 early RA patients are summarized in table (1).

Table (1): Characteristics of the 30 early RA patients.

Characteristic	No.	Range
Age in years	50	Range 17 – 72
Sex (female / male)	22 / 8	73% Females
Disease duration in months	6.5	Range 1.5 – 12
IgM RF positive	25	83%
Tender joint count*	19	1 – 45
Swollen joint count*	13	1-28
Ritchie articular index**	16	1 – 50
Disease activity score***	4.5	2.0 – 7.0
CRP, mg/l	25	0.6 – 172
ESR, mm/h	43	5 – 102
Sharp score****	3	0 – 37
No. of patients with normal radiographs (%)	9	27%

Values are the median *(range).* Maximal scores: * tender and swollen joint count 52, ** Ritchie articular index 78, *** disease activity score 10.0, ****Sharp score 448.

Serum concentrations of MMP-3 and MMP-9: The mean *(± SD)* serum levels of MMP 3 and 9 in the early rheumatoid arthritis patients and OA patients are summarized in table (2). Serum levels of MMP-3 were

higher than the normal range in 69% of RA patients in comparison to 20% of OA patients. Furthermore, 94% of RA patients showed high serum levels of MMP-9 in comparison to 5% of OA patients. Serum concentrations of MMP-3 and MMP-9 were significantly higher in early RA patients as compared to OA patients ($p < 0.01$).

Table (2): Serum Levels of MMP-3 & -9 in early RA patients and knee OA patients.

	RA		OA	
Patients Number	30		30	
	MMP-3	MMP-9	MMP-3	MMP-9
Mean ± SD	100.43 ± 63.38*	190.81 ± 187.83*	40.99 ± 28.44	41.23 ± 14.42
Median	86.5*	121.5*	31.25	38.9
Range	15.1 – 223	28.5 – 747	12.6 – 112	25 – 79.9
% above normal range	69	94	20	5
% below normal range	0	0	15	0

Values are in ng/ml. Normal ranges are 15 - 72 ng/ml for MMP-3 and 12 - 71 ng/ml for MMP-9
p < 0.01 versus osteoarthritis patients *(OA)*

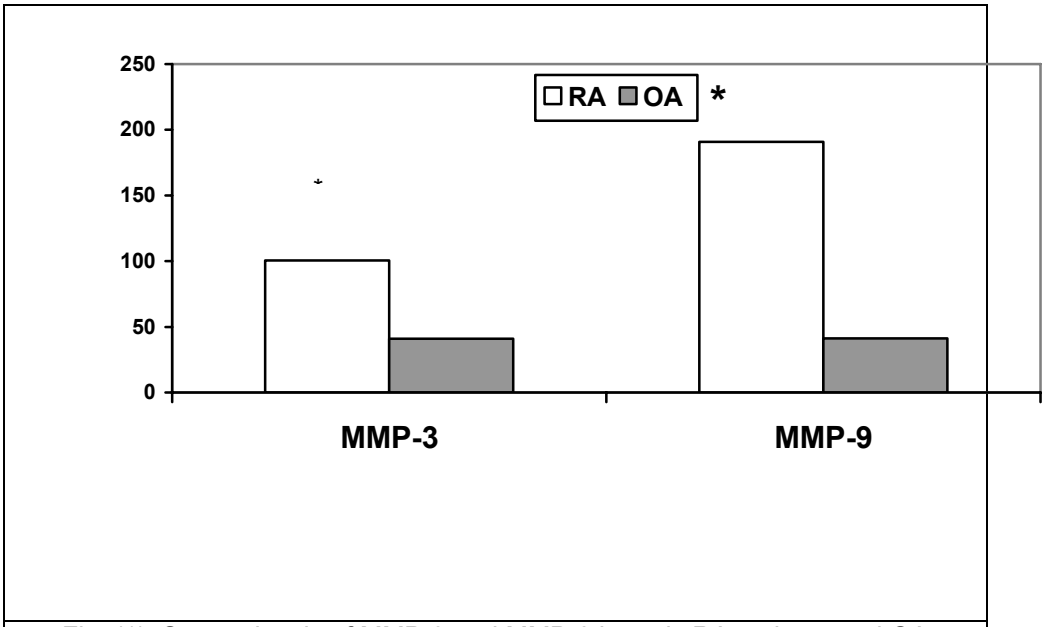

Fig. (1): Serum levels of MMP-3 and MMP-9 in early RA patients and OA patients knee.

Matrix metalloproteinase *(MMP)* Rheumatoid arthritis *(RA)* Osteoarthritis *(OA)* p < 0.01 RA versus OA.

MMP-3 and MMP-9 vs. markers of disease activity: Correlation coefficients between MMP-3 & MMP-9 and markers of disease activity, joint scores, DAS, CRP and ESR are shown in table (3). MMP-3 and MMP-9 were closely correlated with SJC, ESR and CRP. This correlation was

more marked with MMP-9. There were no correlations between MMP-3 & MMP-9 and TJC or RAI or DAS.

Table (3): Spearman correlations between MMP-3 and MMP-9 with swollen joint count (SJC), tender joint count *(TJC),* CRP, ESR, disease activity score *(DAS),* and Ritchie articular index *(RAI) (n = 30).*

	SJC	TJC	CRP	ESR	DAS	RAI
MMP-3	0.49*	0.12	0.72**	0.57**	0.23	0.07
MMP-9	0.54**	0.15	0.81**	0.62**	0.33	0.17

Values are Spearman, * $p < 0.01$, ** $p < 0.001$

Serum MMP-3 and MMP-9 vs. development of joint damage detected radiologically: Table *(4)* shows correlation coefficients between MMP-3 & MMP-9 and radiological scores: joint space narrowing *(JSN),* erosions and total sharp score *(TSS).* MMP-3 and MMP-9 were correlated only with JSN. There were no correlations between MMP-3 and MMP-9 and erosions or TSS.

Table (4): Spearman correlations between MMP-3 and MMP-9 with joint space narrowing, erosions and total Sharp score *(n = 30).*

	Joint space narrowing	Erosions	Total Sharp score
MMP-3	0.48*	0.12	0.33
MMP-9	0.51*	0.19	0.34

Values are Spearman r, * $p < 0.01$

DISCUSSION

In the present study, MMP-3 & MMP-9 were detected in both RA and OA patients. The mean serum levels of MMP-3 in RA patients were 2 - 3 times higher than that in OA knee patients. Serum levels of MMP-3 in early RA patients were significantly higher than those in OA patients knee. MMP-3 *(stromelysin-1)* has been thought to play an essential role in the degradation of the cartilage matrix. This protein could be produced by synovial fibroblasts as well as by chondrocytes in articular cartilage. Increased serum levels of MMP-3 have been observed in RA as well as OA *(Yoshihara et al., 1995 and Sasaki et al., 1994).*

The mean serum levels of MMP-9 in RA patients were 4 - 5 times higher than that in OA knee patients. Serum level of MMP-9 was significantly higher in early rheumatoid arthritis patients than in OA patients. It is possible that pro-MMP-9 could be activated by MMP-3 in

vitro *(Ogata et al., 1992),* and thus, an increased concentration of MMP-3 could contribute to the increase in levels of active MMP-9 in RA patients. MMP-9 has been implicated in cellular migration and invasion in patients with such conditions as inflammation, tumor invasion, and metastasis. In addition, studies of this proteinase in the skeleton suggested that MMP-9 could exist in matrix vesicles *(D'Angelo et al., 2001),* digest cartilage proteoglycans *(Ito et al., 1995 and Colnot & Helms, 2001),* play important roles in chondrocyte apoptosis *(Vu et al., 1998),* and osteoclastic bone resorption *(Rice et al., 1997; Kusano et al., 1998 and Hill et al., 1994).*

MMP-9 can also be produced by activated osteoclasts *(Tezuka et al., 1994).* A recent study also demonstrated that MMP-9 was strongly expressed in the subchondral region in RA patients. Enhanced production of MMP-9 by synovial cells in RA patients could partly account for an increase in the serum levels of this protease. A direct route into the blood stream via the subchondral microcirculatory system and an indirect route via the blood-synovium barrier could also be considered, as described for MMP-3 *(Kaneko et al., 2001).*

In the present study, serum MMP-3 & MMP-9 is correlated with other markers of disease activity such as swollen joint count, ESR and CRP. Of the radiological scores, joint space narrowing is closely correlated to MMP-3 and MMP-9. The highest correlation was between MMP-9 and CRP. Ribbens et al. *(2000)* concluded that in addition to CRP, a systemic marker of inflammation, serum MMP-3 may serve as a consistent synovial derived marker of RA disease activity, its early changes of which predict outcome.

In RA, a distinction must be made between process variables like joint tenderness, joint swelling, ESR, CRP, etc., and outcome measures like radiological progression. The course of the disease is generally monitored by serial measurements of one or more process variables. As radiological outcome is essentially the result of what has happened during the course of the disease. So, serial measurements or time integrated values of MMPs may reflect radiological outcome. In RA, MMP-3 and MMP-9 are mainly locally produced and activated in the affected joints and in that way, they are more direct reflection of joint inflammation. The acute phase protein CRP is produced indirectly by the liver after cytokine stimulation. Production can also be influenced by other stimuli of the acute phase response, such as bacterial infections. Nevertheless, there are no stronger associations between serum MMPs and markers of disease activity in comparison with CRP, in this study or other studies *(Ribbens et al., 2000*

and Keyszer et al., 1999). This confirms the value of CRP level as a good marker for disease activity of RA in clinical practice.

Conclusion:

In conclusion, our results suggest that there are significant differences between early RA patients and OA knee patients as regard MMP-3 and MMP-9. MMP-3 and MMP-9 showed a close correlation with other markers of disease activity such as swollen joint count, ESR and CRP in early RA patients. Of the radiological scores as outcome measures, the item joint space narrowing was closely correlated with MMP-3 and MMP-9. We proposed that serum MMP-3 and MMP-9 may be an additional serological marker indicative of joint inflammation and cartilage degrading activity in RA.

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زيادة كمية الإنزيم الميتالوبروتينيز البينخلوي الثالث و التاسع في مصل مرضى الرثيان المفصلي الحديث

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قسم الروماتيزم والتأهيل- كلية الطب جامعة عين شمس و قسمي الكائنات الدقيقة والمناعة* و الأشعة التشخيصية** – كلية الطب جامعة القاهرة

هدف البحث: دراسة مستوي الأنزيم الميتالوبروتينيز البينخلوي الثالث والتاسع في مرضي الرثيان المفصلي وتحديد هل هذه الأنزيمات علي علاقة محددة وخاصة بالمرضي ومدى علاقتة بدلالات نشاط المرض والتغيرات التي تحدث في المفصل المشخصة إشعاعياً.

طريقة البحث: تم دراسة نسبة الأنزيم الميتالوبروتينيز البينخلوي الثالث والتاسع بطريقة الإليزا في مصل ثلاثين من مرضي الرثيان المفصلي حديثي التشخيص وتمت مقارنته بثلاثين من مرضي خشونة غضاريف الركبة كذلك تم عمل الفحوصات المعملية الروتينية الأخرى وأشعة للقدمين و اليدين.

نتيجة البحث: كان هناك زيادة ذات دلالة إحصائية في كمية الأنزيم الميتالوبروتينيز البينخلوي الثالث والتاسع معامل الرثياني، في مرضي الرثيان المفصلي مقارنة بمرضي الفصال العظمي الركبة كما كانت هناك علاقة طر دية بين نسبة الأنزيمات وعدد المفاصل الملتهبة، و المعامل الرثياني، سرعة الترسيب والبروتين (سي) المتفاعل ولم تكن هناك علاقة بين الأنزيمات وعدد المفاصل المؤلمة أو مؤشر نشاط المرض(داس) أو مؤشر ريتشي. أما بالنسبة للتغيرات المفصلية المشخصة إشعاعيا كانت هناك علاقة طر دية بين الأنزيمات و ضيق المسافة المفصلية.

خلاصة البحث: تدل هذه الدراسة علي زيادة نسبة الأنزيم الميتالوبروتينيز البينخلوي الثالث والتاسع في مرضي الرثيان المفصلي وأنه من الممكن استخدامه كمؤشر لنشاط المرض إضافة إلى الفحوصات المعملية الأولية مثل سرعة الترسيب وبروتين (سي) المتفاعل حيث أن هذه الإنزيمات الميتالوبروتينيز البينخلوي الثالث والتاسع ينتج مباشرة من المفصل الملتهب ولهذا هو مؤشر مباشر لانحلال الغضاريف.