SERUM AND SYNOVIAL FLUID LEVELS OF MMP-3 AND TIMP-1 IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS


Rheumatology & Rehabilitation and Internal Medicine* Departments Ain Shams University Faculty of Medicine and Clinical Pathology Department**, Tanta University Faculty of Medicine

KEY WORDS: MMP-3, TIMP-1, RA, OA.

ABSTRACT

Objective: Matrix metalloproteinases (MMPs) are cytokine-modulated enzymes that play an important role in the pathogenesis of RA by inducing bone resorption and cartilage destruction. Tissue inhibitors of metalloproteinases (TIMPs) are naturally occurring MMPs inhibitors. In rheumatoid arthritis (RA), there is a disturbed balance between MMPs and TIMPs favoring proteolysis. This study was performed to evaluate the significance of measuring the serum and synovial fluid (SF) levels of MMP-3 and TIMPs in RA and osteoarthritis (OA) patients in an attempt to provide more insight in their role in the pathogenesis of those two diseases.

Materials & Methods: Serum levels of MMP-3 and TIMP-1 were measured from 30 RA, 20 OA patients and 20 healthy controls using double-antibody ELISA. Also, their levels were measured in the SF of 14 RA and 9 OA patients. RA disease activity was assessed using the Multivariate Analysis of Mallya and Mace and joint erosions were assessed using Larsen score.

Results: Serum and SF levels of MMP-3 and TIMP-1 were significantly higher in RA than OA patients and in OA patients than controls. Their levels were significantly higher in the SF than in the serum. Serum and SF TIMP-1: MMP-3 ratio was significantly lower in RA as compared to OA patients and normal controls and this ratio was significantly lower in the SF than in the serum of RA patients.
Serum levels of MMP-3 and TIMP-1 correlated strongly with clinical and laboratory parameters of rheumatoid disease activity, and the serum levels of MMP-3 showed a significant positive correlation with the number of joint erosions but TIMP-1 levels did not show this positive correlation.

**Conclusion:** Serum and SF MMP-3 and TIMP-1 levels were significantly higher in RA than OA patients and normal controls. They appear to play a critical role in joint inflammation and destruction, especially MMP-3, which may serve as an additional marker for assessment of RA disease activity and severity.

**INTRODUCTION**

Destruction of bone and cartilage is a characteristic feature of many inflammatory arthropathies, particularly rheumatoid arthritis (RA). This destruction is mediated largely by a variety of proteolytic enzymes. The matrix metalloproteinases (MMPs) are thought to play a critical role in this process (Murphy et al., 1991). MMPs are a family of zinc-dependant endopeptidases that are capable of degrading all components of extracellular matrix (Woessner, 1991). They are fundamentally responsible for many diverse biological functions of the cell including cell migration, proliferation and wound healing. They contribute to joint destruction in RA by directly degrading the cartilage and bone and indirectly promoting angiogenesis (Jackson et al., 2001).

MMPs are classified into four major subclasses: collagenases, gelatinases, stromelysins and membrane type MMP. All MMPs share several common properties and are inactivated by tissue inhibitors of metalloproteinases (TIMPs), EDTA and zinc chelating agents (Birkedal et al., 1993). Gene expression of MMPs has been observed in the synovial lining layer (McCachren, 1991), in scattered cells in the sublining area (Gravallese et al., 1991), and in activated synovial endothelial cells (Shingu et al., 1993). However, it is thought that non-articular sources may also contribute to elevated serum level of MMP-3 (Zucker et al., 1999).

Higher levels of these enzymes have been detected in the serum (So et al., 1999), synovial fluid (SF) (Ishiguro et al., 1999), and synovial membrane (Konttinen et al., 1999) of arthritis patients. They have also been observed at sites of cartilage erosions (Tetlow & Woolley, 1995). The distribution of MMP-3 in the synovial membrane has been reported to be wider than other MMPs, it is the predominant metalloproteinase synthesized in the human articular cartilage (Yoshihara et al., 2000). It can degrade many components of extracellular matrix (Birkedal et al., 1993); but it is
not the dominant enzyme in proteoglycan breakdown. It plays a vital role in the initiation of joint damage, possibly through the activation of MMP-1 (Van Meurs et al., 1999). It also induces aggrecan disruption, which may be a necessary first step in allowing MMP-1 access to collagen fibrils (Hasty et al., 1990).

The enhanced expression of MMPs by synovial fibroblast, monocytic phagocytes in synovial lining cells and infiltrating inflammatory cells are responsible for connective tissue degradation in synovial joints of RA patients (Mengshol et al., 2002). Also it is thought that MMPs production by chondrocytes leads to localized cartilage degradation in OA (Martel-Pelletier et al., 1994).

TIMPs are naturally occurring MMP inhibitor, specific MMP inhibitors form non-covalent, tight-binding complexes with active MMPs (Cawston, 1996). TIMP-1 is a small glycoprotein that is produced locally by the same cells that secrete MMPs (McCachren, 1991).

MMP production is controlled during different stages: synthesis, secretion, activation and inhibition (Cawston, 1996). Interleukin-1 (IL-1), tumor necrosis factor and fibroblast growth factor promote MMP production, while transforming growth factor β, IL-6, IL-11 and leukemia inhibitory factor increase TIMP levels (Ishiguro et al., 1996). These effects suggest that MMP and TIMP are regulated differently, and a disproportionately low level of TIMP secretion may be part of the acquired functional abnormalities of RA synovial cells (Jackson et al., 1998). In RA an excess of MMPs over TIMPs results in an imbalance favoring proteolysis (Seitz and Dayer, 2000). Elevated serum and SF levels of MMP-3 and TIMP-1 have been previously reported in RA patients, but TIMP: MMP ratios were significantly lower (Cunnane et al., 2001).

Corticosteroids decrease MMP production and increase TIMP levels (McGuire et al., 1981), while methotrexate and interferon-β reduce MMP levels without affecting TIMP levels (Smeets et al., 2000). So suppression of MMP production may be an effective therapeutic approach in RA patients. Also intra-articular administration of corticosteroids can reduce serum level of MMP.

Keyszer et al. (1999) reported that the disturbed proteinase and proteinase inhibitor balance caused by inflammatory cytokines increases MMPs expression but not TIMP-1. He stated that MMP secretion by microvascular endothelial cells is an essential step in angiogenesis in RA patients. So new approaches for RA treatment include inhibition of specific MMPs, inhibition of signaling molecules involved in MMP gene expression and induction of natural inhibitors of MMP, drugs that can specifically
block the active MMPs proved to prevent the destruction of cartilage in animal models (Heather & Rowan, 2001).

**Aim Of The Study:**

To evaluate the significance of measuring the serum and SF levels of MMP-3 and TIMP-1 in RA and OA patients and to correlate these levels with the parameters of disease activity in an attempt to provide more insight regarding their role in the pathogenesis of those two diseases.

**MATERIALS AND METHODS**

This study was carried out on 50 adult patients, 30 of them were diagnosed to have RA according to the revised criteria of the American College of Rheumatology (ACR) (Arnett et al., 1988), and 20 were diagnosed to have primary OA of the knees according to the criteria of Altman for classification and reporting of OA (Altman et al., 1986). An additional 20 apparently healthy individuals, age and sex matched, served as a control group.

**All Patients And Controls Were Subjected To The Following:**

* Careful history taking and thorough clinical examination.
* Complete blood count (CBC) using the Coulter Counter (T660).
* Erythrocyte sedimentation rate (ESR) using Westergren method.
* Rheumatoid factor (RF) detection using latex agglutination test.
* C-reactive protein (CRP) detection using standard enzyme-linked immunosorbent assay (ELISA) kit.

The disease activity in RA cases was assessed clinically using the Multivariate Analysis (MVA) according to Mallya & Mace (1981). Each patient was assessed with the following six parameters: the duration of morning stiffness, the severity of pain, the grip strength, joint tenderness, and ESR and hemoglobin percentage (HB %). Each parameter was given a score of 4. The scores obtained from these parameters were summed-up together, and the result was divided by six to obtain the index of disease activity (IDA) which again was graded into four grades called the mean disease grading activity (MDGA).

Joint damage and erosions were assessed for RA severity, with the standardized Larsen score at the time of assessment. The Larsen score was established using standard reference films by taking hand X-rays (Larsen et al., 1977). Wrists, metacarpophalangeals 2-4 and proximal interphalangeals 2-4 were scored on a fine-point scale: 0=no abnormalities, 1=slight abnormalities (joint space narrowing, or band like osteoporosis), 2=small, but definite erosions, 3=medium erosions, 4=severe destructive
abnormalities, 5= mutilating abnormalities. The score for the wrist was then multiplied by 2, so that the total score ranged from 0 to 100.

MMP-3 and TIMP-1 levels (in serum and in SF) were measured using double-antibody enzyme-linked immunosorbent assay (ELISA technique) kits provided by oncogen research product, Cambridge, USA. Venous blood samples were collected in pyrogen free tubes, allowed to clot for one hour and centrifuged at 1000 xg for 10 minutes. The serum obtained was divided into two aliquots and stored at -20° C until assessment. SF was obtained from arthrocentesis performed on 23 patients, who presented with knee effusion (14 RA and 9 OA cases). SF was treated with 3.000 units/ml of hyaluronidase (type IV from bovine tests; Sigma, St. Louis, MO) for 1 hour to reduce viscosity. All fluids were then centrifuged at 1000 xg for 5 minutes prior to assay.

Measurement of MMP-3:

Serum samples were diluted 1: 20, while treated SF was diluted 1:2,000. Microtiter 96-well plates were coated overnight at 4°C with 200 µl of rabbit anti-proMMP-3 IgG in phosphate buffered saline (PBS). Then the wells were washed in PBS Tween. 100 µl of diluted standard or patient samples were placed in the wells for 1 hour at room temperature. After washing step, 100 µl of biotinylated anti-proMMP-3 IgG in normal horse serum was added for 1 hour. A further washing step was followed by 100 µl/well of HRP-conjugated steptavidin in PBS for 30 minutes. After washing, tetramethylbenzidine (Sigma) in potassium citrate was added at 100 µl/well and incubated for 10 minutes. The reaction was stopped with 50 µL/well of M2SO4 and the absorbance was measured at 450 nm.

Measurement of TIMP-1:

Serum samples were diluted 1: 20, while treated SF was diluted at 1: 40 – 1: 80. The TIMP-1 ELISA was performed in a manner similar to that of the MMP-3 assay.

Statistical Analysis:

Was performed using the SPSS program, V-10 under windows. Descriptive statistics: for continuous variables by mean, standard deviation (+SD), standard error (+SEM), and range and for qualitative data by number and percent. As the data were nonparametric, analysis for quantitative data by Mann-Whitney U-test and Spearman rank order correlation. Graphic representation was conducted using HGW program.
RESULTS

The thirty RA patients included in this study comprised 24 females and 6 males with a mean age of 43.8±7.85 years (range 29-60 years). As for OA cases, their ages ranged from 30 to 69 with a mean age of 45.6±10.76. They were 16 females and 4 males. The healthy controls age ranged from 25-65 with a mean age of 47.3±9.84. They were 15 females and 5 males. Statistical comparison showed that the three groups were matched in age and sex.

Regarding RA cases, the mean duration of their illness was 9.2±6.07 ears (range 2-26 years). Out of the 30 cases, there were 21 sero-positive and 9 seronegative cases. CRP was 23.4±17.33 (SEM=3.16) and ranged from 0-52. The mean of ESR was 57.5±21.8 (SEM=3.99) and ranged from 26 – 110 mm/hr. The mean of Hb% was 10.13±1.16 (SEM=0.21) and ranged from 8.1-13 gm/dl. The mean of erosions of small joints of both hands (Larsen score) was 1.4±2.8 (SEM=0.51) and ranged from 0-12. The assessment of disease activity showed that no patient was in grade I, 4 patients were in grade II, 20 patients were in grade III and 6 patients were in grade IV. Descriptive data of MMP-3, TIMP-1 and TIMP-1: MMP-3 ratio in both serum and SF in the three groups are shown in (Table 1).

Table (1): Descriptive data of MMP-3 & TIMP-1 (in serum and SF).
Fig. (1): Mean level of MMP-3 and TIMP-1 in RA, OA and Control groups

Fig. (2): Mean level of TIMP-1:MMP-3 in RA, OA and Control groups
Table (2): Comparison between the groups regarding MMP-3 & TIMP-1 (in serum and SF).

<table>
<thead>
<tr>
<th></th>
<th>MMP-3 (ng/ml)</th>
<th>TIMP-1 (ng/ml)</th>
<th>TIMP-1: MMP-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mann-Whitney p &amp; significance</td>
<td>Mann-Whitney p &amp; significance</td>
<td>Mann-Whitney p &amp; significance</td>
</tr>
<tr>
<td>RA x OA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>19.0</td>
<td>28.0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.004 (S)</td>
<td>0.02 (S)</td>
<td>0.03 (S)</td>
</tr>
<tr>
<td>Synovial</td>
<td>19.5</td>
<td>27.5</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>0.004 (S)</td>
<td>0.02 (S)</td>
<td>0.03 (S)</td>
</tr>
<tr>
<td>RA x Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>10.0</td>
<td>72.0</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td>0.000 (HS)</td>
<td>0.000 (HS)</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>Synovial</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>OA x Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>46.0</td>
<td>122.5</td>
<td>78.5</td>
</tr>
<tr>
<td></td>
<td>0.000 (HS)</td>
<td>0.03 (S)</td>
<td>0.001 (HS)</td>
</tr>
<tr>
<td>Synovial</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

From tables (1 & 2) we can find that:

* The synovial levels of both MMP-3 and TIMP-1 showed a highly significant increase than their serum levels in the studied groups (RA & OA). In the control group, we could not compare serum and synovial levels as we did not take SF sample.

* The mean level of TIMP-1 (in both serum and SF) were significantly higher than levels of MMP-3 in all the studied groups, except in the few individual cases of the RA group, who had active joint inflammation at the time of SF sampling where we found their MMP-3 level bypass the TIMP-1 level.

* The TIMP-1: MMP-3 ratio in the serum of RA was $4.33 \pm 3.58$ (range= 0.91-15), while its synovial level was $2.74 \pm 1.73$ (range 0.9-5.7). This shows a significant increase of its serum level than its synovial level.

I- Comparison between RA & OA groups:

a) Regarding MMP-3:

* The mean(SEM) of serum MMP-3 in the RA group was $180.6 \pm 33.54$, while in the OA group was $70.5 \pm 11.93$. Statistical comparison showed that there was a significant difference being higher in the RA group.

* The mean(SEM) of synovial MMP-3 in the RA group was $1769.49 \pm 394.11$, while in the OA group it was $420.22 \pm 124.04$. Statistical comparison showed that there was a significant difference being higher in the RA group.
b) Regarding TIMP-1:

* The mean±SEM of serum TIMP-1 in the RA group was 397.33±28.13, while in the OA group it was 272.0±30.48. Statistical comparison showed that there was a significant difference being higher in RA group.

* The mean±SEM of synovial TIMP-1 in the RA group was 2844.0±278.48, while in the OA group it was 1880±410.34. Statistical comparison showed that there was a significant difference being higher in the RA group.

c) Regarding TIMP-1: MMP3 ratio:

* The mean±SEM of serum TIMP-1:MMP3 in the RA group was 4.33±0.65, while in the OA group it was 6.34±1.31. Statistical comparison showed that there was a significant difference being higher in the OA group.

* The mean±SEM of synovial TIMP-1:MMP3 in the RA group was 2.74±0.46, while in the OA group it was 8.05±2.51. Statistical comparison showed that there was a significant difference being higher in the OA group.

II- Comparison between RA & Control groups:

a) Regarding MMP-3:

* The mean±SEM of serum MMP-3 in the RA group was 180.6±33.54, while in the control group it was 15.1±1.72. Statistical comparison showed that there was a highly significant difference being higher in the RA group.

b) Regarding TIMP-1:

* The mean±SEM of serum TIMP-1 in the RA group was 397.33±28.13, while in the control group it was 188.0±21.27. Statistical comparison showed that there was a highly significant difference being higher in the RA group.

c) Regarding TIMP-1: MMP3:

* The mean±SEM of serum TIMP-1:MMP3 in the RA group was 4.33±0.65, while in the control group it was 14.89±2.13. Statistical comparison showed that there was a highly significant difference being higher in the control group.

III- Comparison between OA & Control groups:

a) Regarding MMP-3:

* The mean±SEM of serum MMP-3 in the OA group was 70.5±11.93, while in the control group it was 15.1±1.72. Statistical comparison showed that there was a highly significant difference being higher in the OA group.
b) Regarding TIMP-1:
* The mean±SEM of serum TIMP-1 in the OA group was 272.0±30.48, while in the control group it was 188.0±21.27. Statistical comparison showed that there was a significant difference being higher in the OA group.

c) Regarding TIMP-1: MMP3:
* The mean±SEM of serum TIMP-1:MMP3 in the OA group was 6.34±1.31, while in the control group it was 14.89±2.13. Statistical comparison showed that there was a highly significant difference being higher in the control group.

Correlation Study:
In the RA group, correlation study was done between MMP-3, TIMP-1 and TIMP-1: MMP-3 ratio in both serum and SF. This is shown in table (3).

Table (3): Correlation matrix for MMP-3, TIMP-1 & TIMP-1: MMP-3 ratio in serum and synovial fluid.

<table>
<thead>
<tr>
<th></th>
<th>MMP-3 (SF)</th>
<th>TIMP-1 (serum)</th>
<th>TIMP-1 (SF)</th>
<th>TIMP-1: MMP-3 ratio (serum)</th>
<th>TIMP-1: MMP-3 ratio (SF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-3 (serum):</td>
<td>r = +0.99</td>
<td>p &lt;0.001</td>
<td>r = +0.75</td>
<td>p &lt;0.001</td>
<td>r = -0.92</td>
</tr>
<tr>
<td>Sign.</td>
<td>(HS)</td>
<td>(S)</td>
<td>(HS)</td>
<td>(HS)</td>
<td></td>
</tr>
<tr>
<td>MMP-3 (SF)</td>
<td>r = +0.38</td>
<td>p = 0.17</td>
<td>r = +0.73</td>
<td>p = 0.003</td>
<td>r &lt;0.001</td>
</tr>
<tr>
<td>Sign.</td>
<td>(NS)</td>
<td>(S)</td>
<td>(HS)</td>
<td>(HS)</td>
<td></td>
</tr>
<tr>
<td>TIMP-1 (serum)</td>
<td>r = +0.33</td>
<td>p = -0.31</td>
<td>r = -0.31</td>
<td>p = +0.05</td>
<td></td>
</tr>
<tr>
<td>TIMP-1 (SF)</td>
<td>Sign.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP-1:MMP-3 (serum)</td>
<td>r = +0.92</td>
<td>p = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sign.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

r = Spearman rank order correlation  p = Level of significance.
S = significant,   HS= highly significant,   NS = not significant.
From table (3) we can find that:

* MMP-3 (serum level) showed a highly significant positive correlation with its synovial level. Also it showed a significant positive correlation with TIMP-1 (in both serum and SF). It showed a highly significant negative correlation with TIMP-1: MMP-3 ratio (in both serum and SF).

* TIMP-1 (serum level) did not show any significant correlation with TIMP-1 (synovial level), nor TIMP-1: MMP-3 ratio (in both serum and SF).

In the RA group, correlation study was done regarding serum levels of MMP-3, TIMP-1 and TIMP-1: MMP-3 ratio versus clinical and laboratory parameters of disease duration, disease activity and severity. This is shown in table (4).

Table (4): Correlation study between serum MMP-3, TIMP-1 and TIMP-1:MMP3 ratio and other parameters.

<table>
<thead>
<tr>
<th></th>
<th>MMP-3 (serum)</th>
<th>TIMP-1 (serum)</th>
<th>TIMP-1:MMP-3 (serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease Duration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(years)</td>
<td>r 0.26</td>
<td>+0.15</td>
<td>-0.23</td>
</tr>
<tr>
<td></td>
<td>p 0.16</td>
<td>0.42</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td><strong>RF positivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Latex)</td>
<td>r +0.003</td>
<td>0.00</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>p 0.84</td>
<td>0.98</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td><strong>CRP (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r +0.52</td>
<td>+0.47</td>
<td>-0.44</td>
</tr>
<tr>
<td></td>
<td>p 0.05</td>
<td>0.007</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(S)</td>
<td>(S)</td>
</tr>
<tr>
<td><strong>ESR (mm/hr)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r +0.66</td>
<td>+0.45</td>
<td>-0.57</td>
</tr>
<tr>
<td></td>
<td>p &lt;0.001</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(HS)</td>
<td>(S)</td>
</tr>
<tr>
<td><strong>HB (gm/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r -0.43</td>
<td>-0.42</td>
<td>+0.18</td>
</tr>
<tr>
<td></td>
<td>p 0.02</td>
<td>0.02</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(S)</td>
<td>(S)</td>
</tr>
<tr>
<td><strong>Larsen score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r +0.57</td>
<td>-0.12</td>
<td>-0.17</td>
</tr>
<tr>
<td></td>
<td>p 0.001</td>
<td>0.52</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(HS)</td>
<td>(NS)</td>
</tr>
<tr>
<td><strong>MDGA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r +0.42</td>
<td>+0.40</td>
<td>-0.43</td>
</tr>
<tr>
<td></td>
<td>p 0.02</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(S)</td>
<td>(NS)</td>
</tr>
</tbody>
</table>
From table (4) we can find that:

**a) Regarding disease activity:**

* Serum levels of MMP-3 shows significant positive correlation with clinical and laboratory parameters of disease activity as ESR (r= +0.66), CRP (r= +0.52), MDGA (r= +0.42) and significant negative correlation with Hb% (r= -0.43).

* Serum levels of TIMP-1 shows significant positive correlation with clinical and laboratory parameters of disease activity as ESR (r= +0.45), CRP (r= +0.47), MDGA (r= +0.40) and significant negative correlation with Hb% (r= -0.42).

* Serum levels of TIMP-1: MMP-3 ratio shows significant negative correlation with clinical and laboratory parameters of disease activity as ESR (r= -0.57), CRP (r= -0.44) and MDGA (r= -0.43).

**b) Regarding disease severity:**

* Serum levels of MMP-3 showed a significant positive correlation with the number of joint erosions (r= +0.57), but no significant correlation with disease duration (r= +0.26), nor RF positivity (r= +0.003).

* Serum levels of TIMP-1 showed no significant correlation with the number of joint erosions (r= -0.12), disease duration (r= +0.15), nor RF positivity (r= 0.00).

* Serum levels of TIMP-1: MMP-3 ratio showed no significant correlation with the number of joint erosions (r=-0.17), disease duration (r= -0.23), nor RF positivity (r= -0.03).

At last, correlation study between synovial level of MMP-3, TIMP-1 or TIMP: MMP-3 ratio, versus the number of joint erosions showed no significant correlation.

**DISCUSSION**

MMPs are thought to play an important role in the degradation of matrix components of bone, tendon and cartilage with subsequent destruction in chronic inflammatory arthritis (Jain et al., 2001). The overall MMPs activity is a balance between MMP and TIMP and the low level of TIMP or increased expression of MMP may shift the balance in favor of MMP with subsequent joint inflammation and destruction (Jackson et al., 1998 and Keyszer, 1999).

In our study, the serum levels of MMP-3 were significantly higher in RA patients (mean±SEM= 180.6±33.5), as compared to OA cases (mean±SEM= 70.5±11.9) and normal controls (mean±SEM= 15.1±1.7). Also, the levels were significantly higher in OA cases as compared to
controls. These observations were reported by other investigators who found a higher serum level of MMP-3 in RA and OA patients (Sasaki et al., 1994 and Yoshiharaa et al., 2000). Ribbens et al. (2000) reported that the serum level of MMP-3 increased in RA patients, and later on, in year (2002) they proved that the serum level of MMP-3 was higher in RA patients than OA patients and the difference was statistically highly significant. All these results agreed with ours. But in their study in year (2002), they found that the serum level of MMP-3 in OA patients was similar to that of controls. This may be explained by the mild to moderate cases of OA they have included in their study, while our OA samples had more joint inflammation which could explain why the increased MMP-3 levels were significantly higher than the control samples.

Yamanaka et al. (2001) found that serum MMP-3 levels were elevated in their RA patients as compared to healthy controls. They stated that MMP-3 is expressed in rheumatoid synovial tissue and shows potential activity in degrading the proteoglycan of cartilage. They added that it plays a pivotal role in joint destruction in RA, and that the serum concentration of MMP-3 is a useful marker for predicting bone damage in the early stage of RA. Furthermore, the suppression of MMP-3 production may be an effective therapeutic approach in RA patients.

Regarding the synovial level of MMP-3, it showed a significantly higher value in RA patients (mean±SEM = 1769.4±394.1) as compared to OA cases (mean±SEM=420.22±124.04). These results agree with Kageyama et al. (2000) who stated that the levels of MMP-3 in SF from RA patients were significantly higher than those in OA and they concluded that MMP-3 in SF may contribute in distinguishing RA from other arthritic diseases. As a whole, the evaluation of MMP-3 status, and in support of our results, Cunnane et al. (2001) stated that the serum and SF levels of MMP-3 were significantly higher among RA than in non RA patients.

Regarding TIMP-1, our results showed that the serum level of TIMP-1 was significantly higher in RA patients (mean±SEM=397.3±28.13) as compared to OA patients (mean±SEM=272.0±30.48); and its serum level showed a significant increase than serum sample in normal controls (mean±SEM=188±21.27). These results go with Manicourt et al. (1995) and Keyszer et al. (1999). Also Kageyama et al. (2000) had found that TIMP-1 levels were higher in RA patients than in controls.

Regarding TIMP-1 synovial level, we found that the mean±SEM of TIMP-1 was 2844±278.4 in RA cases, while in OA cases it was only 1880±410.3. This significant difference being higher in the RA group agrees with Cunnane et al. (1999) who stated in their study that synovial and serum
levels of TIMP-1 were significantly elevated in RA patients than in OA group.

When we compared RA serum MMP-3 (mean±SEM = 180.6±33.5), with its synovial level (mean±SEM = 1769.4±394.1); also RA serum TIMP-1 (mean±SEM = 397.3±28.1), with its synovial level (mean±SEM=2844.0±278.5), they showed a highly significant difference being more in SF. This result agrees with Cunnane et al. (2001) who found out that the SF levels of MMP-3 and TIMP-1 were significantly higher than their serum levels. They suggested that the enzymes in the serum are derived from the joint. This coincides with our results as we found highly significant positive correlation (r= +0.9) between serum and synovial level of MMP-3 (i.e., the higher its synovial level, the higher will be its serum level), but there was no significant association between serum and SF levels of TIMP-1 (r= +0.33). The same concept was also discussed in a previous study by Taylor et al. (1994) who stated that the levels of MMPs in SF were significantly higher than those measured in the serum due to the increased production by an inflamed joint. Also, Maeda et al. (1995) proved that the levels of MMP-3 in SF correlated with the degree of synovial membrane inflammation in the same joint.

From our results, serum and SF levels of MMP-3 were significantly lower than levels of TIMP-1 in all the studied cases. This higher value of TIMP than MMP-3 was also described in the study done by Cunnane et al. (2001), as they found the mean of MMP-3 in RA sera was 148.2 (median=61.1), while TIMP-1 mean was 336.7 (median=306.6). In some selected cases of RA, we encountered SF level of MMP-3 to be higher than their SF level of TIMP-1. These cases were found clinically to have active uncontrolled inflammation. These results also agree with Cunnane et al. (2001) who mentioned that the highest levels of MMP-3 in the SF of their RA patients were higher than the levels of TIMP-1 in some of their uncontrolled cases.

The mean±SEM of serum TIMP-1: MMP-3 ratio in the RA group was 4.33±0.65, in the OA group it was 6.34±1.31 and in the control group it was 14.89±2.13. Statistical comparison showed that there was a highly significant difference being higher in the control group than the patients’ group and in the OA than in the RA group. Also, this ratio was significantly lower in the SF (2.74±1.73, range 0.9-5.7) than in the serum (4.33±3.58, range= 0.91-15) in RA patients. All these results agree with Cunnane et al. (2001) who found that the ratio TIMP-1: MMP-3 in SF was significantly lower than that in the serum especially in the less active cases of RA. This explains their ongoing process of joint inflammation and destruction. Also they added that at a local level, an inadequate amount of inhibitor is
available to control proteolysis and the beneficial effects of different RA therapeutic modalities may in part result from modulation of the protease: inhibitor balance.

Regarding the correlation between either MMP-3 or TIMP-1 and disease activity, in the present study, we found out that the serum levels of MMP-3 showed a significant positive correlation with clinical and laboratory parameters of disease activity such as ESR (r= +0.66), CRP (r= +0.52), MDGA (r= +0.42) and significant negative correlation with HH% (r= -0.43). Approximately the same findings were encountered in TIMP-1. This concept goes with Posthumus et al. (2002) final conclusion as they reported that MMP-3 levels correlated with joint swelling, ESR, CRP, disease activity score (DAS) and joint space narrowing (i.e., markers of disease activity clinically, laboratory and radiologically). Also in a previous study done by Ribbens et al. (2000), they found that MMP-3 correlated significantly with CRP, DAS and they added that MMP-3 can serve as a consistent synovial derived marker of RA disease activity and its early changes predict the outcome. This was also proved more recently in 2002 by Klimiuk and his coworkers who studied serum and synovial samples from RA and OA patients to explore whether the serum level of MMP-3 and TIMP-1 correlated with the histological appearance of the disease, and they found that MMP-3 and TIMP-1 dominated in the serum of RA patients with follicular synovitis as compared with those with diffuse synovitis and it correlated as well with the clinical activity of the disease.

When we studied MMP-3 to clarify its relation to disease severity, we found that the serum levels of MMP-3 showed a significant positive correlation with the number of joint erosions (r=0.57). This result agrees with Cheung et al. (2000) who reported that there was a significant relationship between radiological damage and serum levels of MMP-3. Yamanaka et al. later on in (2001) stated that serum level of MMP-3 had a strong correlation with the Larsen score. Also Cunnane and his colleagues in 2001 found that serum level of MMP-3 and TIMP-1 correlated with the number of joint erosions. This was also confirmed more recently by Green and his colleagues (2003) who demonstrated that serum levels of MMP-3 and MMP-1 were greater in RA patients as compared to normal controls and these levels correlated with disease activity and predict the functional and radiographic outcome in early untreated RA patients. They may even have a particular value in predicting the progression of erosive disease in patients who are not erosive at presentation and in the group of patients with normal CRP at presentation.

This is consistent with the current understanding of the evolution of RA whereby damage begins early and usually in the peripheral joints, often
with normal CRP, and then progresses to involve large joints, at which it generates an elevated CRP and systemic features of RA. It is therefore understandable that markers of local damage such as MMP-3 would be particularly useful at this early stage of disease prior to the onset of an elevated acute-phase response.

Conclusion:
MMP-3 and TIMP-1 appear to play an important role in joint inflammation with subsequent joint destruction in RA patients. Moreover these markers especially MMP-3 may serve as an additional marker for assessment of RA disease activity and severity. Thus, we can select the RA patient who will start with early aggressive therapy especially with medications that affect the level of proteinases, and so prevent further destruction and provide good prognosis.

REFERENCES
Cheung NT, Dawes PT, Poulton KV, Ollier WE, Taylor DJ and Mattey (2000): High serum levels of pro-matrix metalloproteinases-3 are associated with greater radiographic damage and the presence the shared epitope in patients with rheumatoid arthritis.


دراسة مستوى إنزيم الميتالوبروتينيزي-3 والعامل النسيجي المثبط للميتالوبروتينيزي-1 في المصل والسائل الزاللي في مرضى الرثيان المفصلي والفصال العظمي

منى عبد الله السباعي، أحمد زكي البسقي، نجلاء يوسف عساف، ممدوح محمد مهدي* و ناهد علوان**

قسم الروماتزم والتهابات ونواة’’كلية الطب جامعة عين شمس، ومدينة البالونجية الإكلينيكية’’، كلية الطب جامعة طنطا

تطلب إنزيمات الميتالوبروتينيز دورا هاما في التهاب المفصل وما قد ينتج عنه من تأكل في الغضاريف، وهناك إنزيمات متصلة بالميتالوبروتينيز تتحكم في درجة إصابة المفصل.

الطريقة: أجريت هذه الدراسة على 30 مريضاً بحثياً من مرضى الرثيان المفصلي و20 مريضاً بحثياً من الفصالأ العظمي وعشرون من الأصحاء كعينة ضابطة.

وقد قياس مستوى إنزيم الميتالوبروتينيزي-3 والعامل النسيجي المثبط للميتالوبروتينيزي-1 في المصل والسائل الزاللي للمرضى والجموعة الضابطة بطريقة الإلزاما وتم دراسة نشاط مرضي الرثيان المفصلي إكلينيكياً وعملياً وتقييم نسبة تأكل المفصل بأشعة أكس على البدين ومن ثم تم مقارنة النتائج بعضها ببعض محاولاً التعرف على دور هذه الإنزيمات في هذا المرض.

النتائج: وأظهرت النتائج وجود زيادة ذات دلالة إحصائية في مستوى إنزيم الميتالوبروتينيزي-3 والعامل النسيجي المثبط للميتالوبروتينيزي-1 في المصل والسائل الزاللي في مرضى الرثيان المفصلي، ونسبة مرضى الرثيان المفصلي الذين يعانون من الفصالأ العظمي وعنه في العينة الضابطة، وأن مستواه في السائل الزاللي أكثر من مستواهم في المصل.

وأظهرت النتائج أيضاً نقص ذو دلالة إحصائية في نسبة (العامل المثبط: ر.الإلزيم) في مرضى الرثيان المفصلي عندها في مرضى الفصالأ العظمي وعنه في العينة الضابطة وأن هذه النسبة أقل في السائل الزاللي في مرضى الرثيان المفصلي عنها في المصل. وكذلك مستوي إنزيم الميتالوبروتينيزي-3 والعامل النسيجي المثبط للميتالوبروتينيزي-1 في المصل ينتاسب تناهي إيجابياً مع معدلات قياس نشاط المرض الإكلينيكية والمادية وأن مستوي إنزيم الميتالوبروتينيزي-3 في المصل يتسمب تناهي إيجابيا مع عدد التأكل في المفصل المصلي.

الاستخلاص: ومن ذلك نستخلص أن ارتفاع نسبة هذه الإنزيمات يلعب دوراً هاماً في انتهاش المفصل وما قد ينتج عنه من إصابة وتشوهات في صحة الرثيان المفصلي وخصوصاً إنزيم الميتالوبروتينيزي-3 والذي يرتبط ارتباطاً إيجابياً ببطء نشاط المرض وشدته. وذالك يمكن اختيار مرضى الرثيان المفصلي الذي يجب أن نبدأ مع العلاج المكمل مبدأ وخصوصاً العقائير التي تؤدي إلى انخفاض مستوى هذه الإنزيمات وذالك تمنع حدوث تشوهات في المفصل.