CLINICAL SIGNIFICANCE OF SERUM THROMBOMODULIN LEVEL IN RHEUMATOID ARTHRITIS PATIENTS

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KEY WORDS: THROMBOMODULIN, RHEUMATOID ARTHRITIS

ABSTRACT

Objective: To determine the soluble thrombomodulin (TM) level in sera of rheumatoid arthritis (RA) patients and to evaluate its relationship to disease activity in those patients.

Subjects & Methods: Twenty rheumatoid arthritis patients were included in this study. They were 18 females and 2 males. Their age ranged from 25 to 62 years. The Disease Activity Score (DAS) was used for the assessment of disease activity in the patients. The DAS was divided into 3 categories: ≤ 2.4 (low disease activity), > 2.4 and ≤ 3.7 (moderate disease activity), and >3.7 (high disease activity). Ten age and sex-matched healthy subjects were included as a control group.

The soluble thrombomodulin level was measured with a solid phase sandwich-Enzyme-Linked-Immuno-Sorbent Assay (ELISA) in the sera of the twenty RA patients and ten controls. The relationship between serum TM levels and disease activity in the patients was assessed.

Results: The frequency of patients with high serum TM levels (> 5.33 ng/ml) was 7/20 (35%). Serum TM levels in the patients were significantly higher than those in the control group (p < 0.05) and there was a significant positive correlation between serum TM levels and the DAS (p < 0.05). Comparison between patients with different categories of the DAS and controls regarding serum TM level showed that patients with high disease activity (DAS > 3.7) had a highly significant higher
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serum TM levels than the control group \( p < 0.001 \). Patients with moderate disease activity \((\text{DAS} > 2.4 \text{ and } \leq 3.7)\) showed significantly higher serum TM levels than the control group \( p < 0.05 \) while serum TM levels in patients with low disease activity \((\text{DAS} \leq 2.4)\) were not significantly different than those in the control group \( p > 0.05 \).

**Conclusion:** Serum TM levels were elevated in RA patients and correlated with the disease activity score. These results may indicate that serum TM measurement may be valuable in the evaluation of the RA disease activity.

**INTRODUCTION**

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune, inflammatory disorder in which an erosive, symmetric joint disorder maintains the center stage accompanied by a variable, but at times prominent, degree of extraarticular involvement (Mevorach & Paget, 2000). Several lines of evidence indicate that the homeostatic mechanism is closely linked to the inflammatory process in RA (Conway & Nowakowski, 1993).

Thrombomodulin (TM) is a transmembrane glycoprotein that interacts with thrombin, thereby serving as a cofactor in the activation of protein C (PC), a major physiologically relevant natural anticoagulant (Conway & Nowakowski, 1993). In baboon studies, activated protein C (APC) and its cofactor, protein S, have been demonstrated to modulate the manifestations of Escherichia coli-induced sepsis, thereby providing a definitive link between the coagulation system and inflammation (Esmon et al., 1991 and Taylor et al., 1991).

Elevated TM levels have been reported in patients with vascular damage and/or functional impairment of the kidney, such as systemic lupus erythematosus (SLE), disseminated intravascular coagulation, advanced diabetes mellitus or chronic renal failure, adult respiratory distress syndrome, acute hepatic failure (Takano et al., 1990), and thrombotic thrombocytopenic purpura (Takahashi et al., 1991).

In a study done by Kotajima et al. (1997), the frequency of patients with high TM levels was 21% in systemic lupus erythematosus (SLE), 16% in rheumatoid arthritis (RA), 12% in Sjogren’s syndrome, and 4% in systemic sclerosis (SSc).
Aim of work:

The aim of this work was to determine the soluble thrombomodulin TM level in the sera of RA patients and to evaluate its relationship to disease activity in these patients.

PATIENTS AND METHODS

Twenty rheumatoid arthritis patients diagnosed according to the 1987 revised criteria of the American College for Rheumatology (Arnett et al., 1988) were included in this study. They used to attend the Outpatient Clinic of the Rheumatology & Rehabilitation Department of Ain Shams University Hospitals. They were 18 females and 2 males. Their age ranged from 25 to 62 years (mean ± SD = 42.65 ± 10.06). Ten age and sex-matched healthy subjects were included as a control group.

All patients were subjected to the following:

- Full history taking.
- Thorough clinical examination.
- Disease activity assessment according to the Disease Activity Score (DAS) (Van der Heijde et al., 1990). It combines the Ritchie articular index (RAI) (Ritchie et al., 1968), a count of swollen joints, the erythrocyte sedimentation rate (ESR), and an assessment of the patient’s general health (GH). The DAS was divided into 3 categories: ≤ 2.4 (low disease activity), > 2.4 and ≤ 3.7 (moderate disease activity), and >3.7 (high disease activity) (Van Gestel et al., 1996)
- Rheumatoid factor using latex agglutination technique.
- ESR with Westergren method.
- Measurement of TM: The TM kit is a solid phase sandwich-Enzyme-Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for TM has been coated onto the wells of the microtiter strips provided. Samples, including standards of known TM concentrations, and unknowns are added into these wells. During the first incubation, the TM antigen and a biotinylated antibody specific for TM are simultaneously incubated. After washing, the enzyme (Streptavidin-peroxidase) is added, after incubation and washing to remove all the
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unbound enzyme, a substrate solution which acts on the bound enzyme is added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of TM present in the samples.

Statistical analysis:

Analysis was done using the SPSS base 7.5 statistical package. Results were expressed as mean ± SD. Comparisons were made by unpaired "t" test. Correlations between variables were analysed using Spearman’s rank correlation. p value > 0.05 was considered statistically nonsignificant (NS), p value < 0.05 was considered statistically significant (S) and p value < 0.001 was considered highly significant (HS).

RESULTS

This study included twenty RA patients. They were 18 females and 2 males. Their age ranged from 25 to 62 years (mean ± SD = 42.65 ± 10.06). Ten age and sex-matched healthy subjects were included as a control group.

The clinical and laboratory data of the patients are shown in Table (1). Assessment of disease activity revealed that seven patients had high disease activity (DAS > 3.7), 5 patients had moderate disease activity (DAS > 2.4 and ≤ 3.7) and 8 patients had low disease activity (DAS ≤ 2.4).

Table (1): The clinical and laboratory data of the patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>42.65 ± 10.06</td>
<td>25 - 62</td>
</tr>
<tr>
<td>Disease duration (in months)</td>
<td>47.1 ± 53.91</td>
<td>9 – 216</td>
</tr>
<tr>
<td>Morning stiffness (in minutes)</td>
<td>46.75 ± 32.82</td>
<td>10 – 120</td>
</tr>
<tr>
<td>Ritchie articular index (RAI)</td>
<td>6.7 ± 5.99</td>
<td>0 – 22</td>
</tr>
<tr>
<td>Number of swollen joints</td>
<td>5.5 ± 5.87</td>
<td>0 – 20</td>
</tr>
<tr>
<td>general health (GH)*</td>
<td>40.75 ± 18.59</td>
<td>10 - 80</td>
</tr>
<tr>
<td>ESR in (mm/hour)</td>
<td>43.5 ± 26.24</td>
<td>10 – 100</td>
</tr>
<tr>
<td>Disease Activity Score (DAS)</td>
<td>3.07 ± 1.23</td>
<td>1.03 – 5.52</td>
</tr>
</tbody>
</table>

* General health was quantified on a 100-mm visual analogue scale.

The frequency of patients with high serum TM levels (> 5.33 ng/ml) was 7/20 (35%). Six out of the 7 patients with high serum TM levels had high disease activity (DAS > 3.7) while the remaining one had moderate disease activity (DAS > 2.4 and ≤ 3.7). Serum TM levels in the patients
were statistically significantly higher than those in the control group \((p < 0.05)\) (Table 2 & Fig 1).

Patients with high disease activity \((\text{DAS} > 3.7)\) showed statistically highly significant higher serum TM levels than the control group \((p < 0.001)\). Patients with moderate disease activity \((\text{DAS} > 2.4 \text{ and } \leq 3.7)\) showed statistically significant higher serum TM levels than the control group \((p < 0.05)\) while serum TM levels in patients with low disease activity \((\text{DAS} \leq 2.4)\) were not statistically significantly different than those in the control group \((p > 0.05)\) (Table 2 & Fig 1).

Table (2): serum TM levels in patients with different DAS compared to controls.

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Mean ± SD</th>
<th>t</th>
<th>p</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>20</td>
<td>5.87 ± 2.21</td>
<td>2.01</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>4.43 ± 0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with DAS &lt; 2.4</td>
<td>8</td>
<td>4.46 ± 0.21</td>
<td>0.19</td>
<td>&gt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>4.43 ± 0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with DAS &gt; 2.4 and &lt; 3.7</td>
<td>5</td>
<td>4.9 ± 0.52</td>
<td>1.82</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>4.43 ± 0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with DAS &gt; 3.7</td>
<td>7</td>
<td>8.16 ± 2.4</td>
<td>4.86</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>4.43 ± 0.45</td>
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</tbody>
</table>

![Fig. (1): Comparison between controls and patients groups classified according to their DAS regarding mean values of TM.](image)

Correlation matrix was done with Spearman’s rank correlation test between serum TM levels and different measured patients’ parameters. There was a significant positive correlation \((p < 0.05)\) between serum TM
level and each of the DAS (r = 0.87) (Fig. 2), morning stiffness (r = 0.78), RAI (r = 0.94), number of swollen joints (r = 0.75), GH (r = 0.61) and ESR (r = 0.75) (Table 3).

Table (3): Correlations between serum TM levels and different measured patients' parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r</th>
<th>p</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning stiffness</td>
<td>0.78</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
<tr>
<td>RAI</td>
<td>0.94</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
<tr>
<td>number of swollen joints</td>
<td>0.75</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
<tr>
<td>GH</td>
<td>0.61</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
<tr>
<td>ESR</td>
<td>0.75</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
<tr>
<td>DAS</td>
<td>0.87</td>
<td>&lt; 0.05</td>
<td>S</td>
</tr>
</tbody>
</table>

**DISCUSSION**

TM is synthesized by several cells including vascular endothelial cells, platelets, megakaryocytes, mesothelial cells, neutrophils and the syncytiotrophoblast of placenta (Conway & Nowakowski, 1993). Ohdama et al. (1994) found that the mean plasma TM concentrations in patients with juvenile RA, systemic sclerosis (SSc), polymyositis and/or dermatomyositis, Wegener's granulomatosis, and active states of SLE, RA, and Behcet’s disease were significantly higher than those in the control group. In addition, they found that elevated plasma TM values were decreased along
with amelioration of the diseases by treatment. They stated that these results may indicate that plasma TM measurement may be helpful for evaluating vascular injury in patients with collagen diseases. *Boehme et al. (2000)* stated that soluble TM seems to be a promising, valuable serological disease activity marker in vasculitides.

In this study, there were increased serum TM levels in 35% of the patients. Also, the present study showed a significant positive correlation ($p < 0.05$) between serum TM levels and the DAS of the patients. In addition, patients with high disease activity (DAS > 3.7) showed statistically highly significant higher serum TM levels than the control group ($p < 0.001$). Patients with moderate disease activity (DAS > 2.4 and ≤ 3.7) showed statistically significant higher serum TM levels than the control group ($p < 0.05$) while serum TM levels in patients with low disease activity (DAS ≤ 2.4) were not statistically significantly different than those in the control group ($p > 0.05$) (Table 2 & Fig 1).

These results were in agreement with the results of *Ohdama et al. (1994)* who found that the mean plasma TM concentration in patients with active state of RA (12 patients) was significantly higher than that in the control group. Only one of the 12 patients in the active RA group showed a TM value within the normal limits. On the other hand, their results showed that the mean value for inactive RA (30 patients) was not statistically different from that of the control group.

*Kotajima et al. (1997)* found increased serum TM level in 16% of the RA patients while the frequency of patients with high serum TM levels in the present study was 7/20 (35%). This different frequency may be due to different percentage of patients with active disease in *Kotajima et al. (1997)* study than the present study.

*Conway & Nowakowski (1993)* stated that several lines of evidence indicate that the hemostatic mechanism is closely linked to the inflammatory process in RA. A mechanism to explain the elevated serum TM levels in disease associated with systemic or locally increased levels of inflammatory cytokines was proposed by *Boehme et al. (1996)*. They indicated that neither TNF-α nor neutrophils alone but the interaction of neutrophils with TNF-α-activated endothelial cells bearing increased adherent molecules might lead to the release of TM into the culture supernatant, concomitant with morphologically evident endothelial cell damage. By indirect immunofluorescence using monoclonal antibodies against TM, intense positive staining for TM was observed along the
capillary walls of glomeruli from patients with lupus glomerulonephritis by Tomura et al. (1994). These researchers also showed that the staining for TM was weak or negative in patients with non-lupus glomerulonephritis i.e. membranous glomerulonephritis, IgA glomerulonephritis, minimal-change nephrotic syndrome and hemolytic uremic syndrome. The enhanced presence of TM in the glomeruli of patients with lupus glomerulonephritis could lead to the increased release of soluble TM through the above-mentioned concerted action of cytokine-stimulated endothelial cells and neutrophils.

Harris (2001) stated that, in one sense, it is redundant to think of vasculitis as a complication of RA, because the initial pathologic change in RA is believed to occur in small blood vessels. However, it is useful to use the term vasculitis to group those extra-articular complications related not to proliferative granulomas but rather to inflammatory vascular disease.

Studies on rabbit and human articular tissue have indicated that synovial lining cells express TM on the cell surface (Boffa et al., 1987 and McCachren et al., 1991). The precise role of TM in the inflamed joint is currently largely a matter of speculation; however, the fact that the protein is known to have multiple distinct structural domains suggests that it probably has several yet to be defined functions (Conway and Nowakowski, 1993).

Although the process of inflammation in RA is likely driven by a variety of interactions between cells, cell-surface receptors, growth factors, cytokines, and lymphokines (Alvaro-Gracia et al., 1990), available evidence supports the critical role of proteases in the destructive process (Epstein, 1990 and Firestein and Zvaifler, 1987). Among others, the serine protease plasmin appears to play a major part (Martel-Pelletier et al., 1991). The synovial fluid of patients with RA and osteoarthritis (OA) contains plasminogen activator (PA), which may be produced by synovial lining cells, chondrocytes, or microvascular endothelial cells, or could also be derived from the circulation (Inman and Harpel, 1986). Plasmin, once formed from its precursor plasminogen, may directly degrade extracellular matrix of the joint, or alternatively activate otherwise inactive collagenase that then leads to rapid destruction of collagen-containing tissues (Conway and Nowakowski, 1993).

Recent data indicate that the PC-TM anticoagulant mechanism may play an important role in regulation of the fibrinolytic system and plasmin generation. TM, when complexed with thrombin, supports the conversion of PC to its activated form, whereupon the newly formed serine protease not
only suppresses further thrombin formation, but also is reported to enhance the fibrinolytic system by neutralizing PA inhibitor-1 (PAI-1) (Conway and Nowakowski, 1993). This inhibitor, which is present in synovial fluid, provides a major regulatory mechanism for the transformation of plasminogen to plasmin, and presumably therefore serves to protect the joint tissues from destruction. This would suggest that excess TM in the presence of adequate thrombin and PC might lead to further destructive processes within the acutely inflamed arthritic joint. However, in addition to several direct anticoagulant properties, recent in vitro and in vivo studies suggest that TM may also suppress fibrinolytic activity by accelerating thrombin’s inactivation of single-chain urokinase-type PA (scu-PA) (Molinari et al., 1992 and DeMunk et al., 1991). Therefore, the alternative scenario would be that TM directly interferes with plasmin generation, thereby protecting the joint from further deterioration.

Conway & Nowakowski (1993) stated that the known antiinflammatory role of APC in the baboon model (Esmon et al., 1991 and Taylor et al., 1991) supports the hypothesis that TM may also be critical in regulating the inflammatory response in RA. By enhancing the activation of PC, TM may therefore act indirectly to limit damage due to inflammation. Also, Conway & Nowakowski (1993) stated that little is known as to the role of fibrin deposition in the highly vascular synovial tissue as the proliferating lesion develops into a destructive pannus; however, areas of thrombosis are commonly seen. Lack of TM in the microvasculature caused by either PMN-derived elastase proteolysis or cytokine-induced downregulation of the surface-bound receptor could lead to further fibrin clot formation (Conway and Rosenberg, 1988 and Moore et al., 1989). Mechanisms to enhance TM expression may therefore provide a means to attempt to constrain the overwhelming forces to form intravascular clots in the expanding pannus.

Conclusion:

Serum TM levels were significantly elevated in the RA patients. There was a significant positive correlation between serum TM levels and the disease activity score in the patients. These results may indicate that serum TM measurement may be valuable in the evaluation of the RA disease activity.
REFERENCES


القيمة الإكلينيكية لمستوى الثرمبوموديولين في المصل في مرضى الرئة المنفصلي

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كان الهدف من هذه الدراسة هو تحديد مستوي الثرمبوموديولين في مصل مرضى الرئة المنفصلي مع تقييم علاقة النشاط المرضي في هؤلاء المرضى. وشملت هذه الدراسة على عشرين من مرضى الرئة المنفصلي الذين تراوحت أعمارهم بين 25 و 62 عامًا. وقد تم قياس نشاط المرض باستخدام عدد نقاط نشاط المرض ويشمل ثلاث فئات هي: أقل من أو يساوي 2.4 (نشاط مرضي منخفض) و أكبر من أو يساوي 3.7 (نشاط مرضي عالي). كما اشتملت الدراسة على عشرة من الأصحاء مماثلين في السن والجنس كجامعة ضابطة.

وتتم قياس مستوي الثرمبوموديولين بواسطة طريقة الإليزا في مصل المرضى وال مجموعة الضابطة وتم تقييم العلاقة بين مستوي الثرمبوموديولين في المصل ونشاط المرض في المرضى.

وقد أظهرت نتائج هذه الدراسة أن مستوي الثرمبوموديولين في المصل كان مرتفعًا في نسبة من المرضى (35%). وكان مستوي الثرمبوموديولين في المصل مرتفعاً ارتفاعًا في قيمة إحصائية في النعتين المريضاً عنة في المجموعة الضابطة. كما أظهرت النتائج وجود علاقة إيجابية ذات قيمة إحصائية بين مستوى الثرمبوموديولين في المصل وعدد نقاط نشاط المرض في المرضى. وبالمقارنة بين الفئات الثلاث للمرضي طبقاً لعدد نقاط نشاط المرض والمجموعة الضابطة أظهرت النتائج أن مستوي الثرمبوموديولين في المصل كان مرتفعاً ارتفاعًا في قيمة إحصائية عالية في المرضى ذو النشاط المرضي العلاجي عند في المجموعة الضابطة، كما كان مرتفعاً ارتفاعًا في قيمة إحصائية في المرضى ذو النشاط المرضي المتوسط عند في المجموعة الضابطة بينما لم يوجد فرق ذو قيمة إحصائية في مستوي الثرمبوموديولين في المصل بين المرضى ذو النشاط المرضي المنخفض والمجموعة الضابطة.

ويحلا هذه الدراسة أن مستوي الثرمبوموديولين في المصل كان مرتفعاً في مصل مرضى الرئة المنفصلي كما أنه ذو علاقة بنشاط المرض في هؤلاء المرضى. هذه النتائج قد تكون إلى أن قياس مستوي الثرمبوموديولين في المصل قد يكون ذو قيمة في تقييم نشاط المرض في مرضى الرئة المنفصلي.

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