POSSIBLE ROLE OF SERUM THROMBOMODULIN AS A MEDIATOR OF ENDOTHELIAL CELL DAMAGE IN SYSTEMIC SCLEROSIS

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ETIOPATHOGENESIS OF SYSTEMIC SCLEROSIS.

ABSTRACT

Objective: The aim of this study was to find out the role of thrombomodulin as a mediator of damage to endothelial cells in systemic sclerosis patients.

Subjects and Methods: This study was carried out on 20 systemic sclerosis patients who used to attend the Outpatient Clinic of the Rheumatology and Rehabilitation Department of Ain Shams University Hospitals. All patients fulfilled the American College of Rheumatology criteria for diagnosis of systemic sclerosis. Ten age and sex matched healthy controls were also included. Determination of serum thrombomodulin (TM) level was done for both patients and controls using sCD141 thrombomodulin kit, which is a solid phase sandwich enzyme linked immunosorbant assay (ELISA).

Results: Serum TM was higher in the patients group than in the control group with a highly significant difference ($t=6.75$, $p<0.001$). There was also, a significant difference between the level of serum TM in patients with Raynaud’s phenomenon, digital ulcers and interstitial pulmonary fibrosis as compared to those without these clinical manifestations ($t=0.034$, 0.013, 0.25 respectively, $p<0.05$). There was a significant positive correlation between serum TM level and the duration of illness, Raynaud’s phenomenon, digital ulcers and interstitial pulmonary fibrosis ($r=0.69$, 0.48, 0.58, 0.49 respectively, $p<0.05$). Also, there was a significant positive
correlation between serum TM level and ESR and serum urea 
\((r=0.72, 0.61\text{ respectively, } p<0.05)\).

**Conclusion:** We can conclude that the serum level of TM 
was higher in our patients and this may reflect its pathogenetic 
role (through endothelial cell damage) in systemic sclerosis.

**INTRODUCTION**

Systemic sclerosis (SSc) is a multisystem and multistage disorder 
characterized by proliferation of vascular tissue, obliterative microvascular 
lesions, and residual atrophy with fibrosis of multiple organs. These features 
occur to differing extents in various organ systems and lead to the clinical 
and pathological changes of the disease (Le Roy et al., 1988 and Blann et 
al., 1993). 

A wide spectrum of clinical presentations can occur in systemic 
sclerosis ranging from relatively benign and limited (ISSc) to more severe 
and diffuse forms (dSSc) of the same disorder. The former group includes 
the CREST variant, i.e. a combination of subcutaneous calcinosis, 
Raynaud’s phenomenon (RP), esophageal dysmotility, sclerodactyly, and 
telangiectasia. Raynaud’s phenomenon can occur as part of systemic 
disease, or with no sign of connective tissue disease. Once complete 
assessment fails to show any associated well-defined disease, it is classified 
as primary RP (PRP), a provisional diagnosis since such patient may later 
develop SSc (Medsger & Steen, 1996 and Salojin et al., 1997). 

In systemic sclerosis, endothelial damage and platelet activation may 
occur and both these processes may contribute to the peripheral ischemia so 
characteristic of this disease. The pathogenesis of SSc is, however, unclear. 
One school of thought is that the ‘primary fault’ in SSc is in the vasculature 
(Herrick et al., 1996).

Trifiletti et al. (2000) stated that systemic sclerosis is a 
 multifaceted disease characterized by proliferation and swelling of 
endothelial cells and other disorders. Raynaud’s phenomenon is a 
disturbance, with unknown pathogenesis, that may be precursor to SSc. 

Thrombomodulin (TM) is a vascular endothelial cell receptor for 
thrombin (membrane bound glycoprotein) which rapidly converts protein C 
to the active anticoagulant serine protease, activated protein C. Activated 
protein C in complex with protein S functions as anticoagulant by 
inactivating factors Va and VIIIa (Ohlin & Mailar, 1995). 

In addition to endothelial cells, thrombomodulin can be detected on 
human syncytiotrophoblasts and under circumstances, on vascular smooth
muscle cells in culture (Boehme et al., 1994). Thrombomodulin has also been reported to be synthesized by several cells, including megakaryocytes, platelets, monocytes, neutrophils (PMN), mesothelial cells, placental syncytiotrophoblasts and synovial lining cells (Conway & Nowakowski, 1993). A soluble form of thrombomodulin (sTM) is found in the serum, plasma and urine. It is an established marker of endothelial cell damage and vasculitis (Boehme et al., 2000).

Recently, Carson et al. (2000) stated that thrombomodulin is found predominantly on the surface of endothelial cells and is a receptor for thrombin. Thrombin bound to TM loses its procoagulant ability to convert fibrinogen to fibrin and rapidly converts the inactive zymogen protein C, into the anticoagulant, activated protein C. Anti-TM antibodies could inhibit protein C activation and tip the scales in favor of coagulation to predispose patients to thrombosis. Protein C also plays a role in inflammation, and in a similar way, anti TM antibodies could contribute to the inflammatory response. Deficiency of protein C anticoagulant function has been reported in many inflammatory disease states. In some cases this has been shown to be due to elevated C4 b BP as part of the acute phase inflammatory responses. In others, it has been suggested to be due to TM inhibitions.

Merci et al. (1997) detected circulating plasma thrombomodulin (TMp) with enzyme immunoassay and they considered it as a marker of endothelial damage. They found that TMp was elevated in collagen vascular diseases, where vascular endothelial damage is suspected. It was particualrly elevated in systemic lupus erythematosus and systemic sclerosis.

**Aim of Work:**

The aim of this study was to find out the role of thrombomodulin as a mediator of damage to endothelial cells in systemic sclerosis patients.

**PATIENTS AND METHODS**

This study was carried out on twenty systemic sclerosis patients who used to attend the Outpatient Clinic of the Rheumatology and Rehabilitation Department of Ain Shams University Hospitals. All patients fulfilled the American College of Rheumatology criteria for the diagnosis of systemic sclerosis (Masi et al., 1980).

The study was controlled with ten age and sex matched asymptomatic healthy individuals drawn from the hospital staff.

All patients were subjected to the following:

- Full medical history taking.
• Thorough physical examination (both general and local examination) with special attention to the manifestations of clinical vasculitis in the form of Raynaud’s phenomenon, digital ulcers, infarcts or gangrene, palpable purpura, cutaneous ulceration, trophic changes of nails, splinter hemorrhage (Valuente et al., 1997).

• Routine laboratory tests as follows:
  - Complete blood count (CBC) including hemoglobin level, red blood cell count, white blood cell count (total and differential) and platelet counts by coulter T540.
  - Erythrocyte sedimentation rate (ESR) with Westergren method.
  - Kidney function tests as serum urea and serum creatinine with Sychron CX5 (Beckman, US).

• Determination of serum thrombomodulin (TM) level was done for both patients and control groups.

**Principle of the method:**

The sCD141 thrombomodulin kit is a solid phase sandwich-enzyme linked immuno sorbent assay (ELISA). A monoclonal antibody specific for (sCD141) thrombomodulin has been coated onto the wells of the microtiter strips provided. Samples including standards of known TM concentrations and unknown are pipetted into these wells.

During the first incubation, the TM antigen and biotinylated antibody specific for TM are simultaneously incubated. After washing, the enzyme (streptavidin-peroxidase) is added, after incubation and washing to remove all unbound enzyme, a substrate solution which is acting on the bound enzyme is added to induce a color reaction product. The intensity of this colored product is directly proportional to the concentration of TM present in the samples.

**Statistical analysis:**

Statistical analysis was performed using the statistical package IBM-PC. Student’s “t” test of significance was applied for comparison of groups together with Pearson’s correlation coefficient for detection of different correlations. Values of p<0.05 were considered significant while p<0.001 were highly significant. Some data were graphically represented using Harvard graphics.
RESULTS

Our results showed that systemic sclerosis patients were 18 females (90%) and 2 males (10%). Their ages ranged from 25-56 years with a mean of 39.2±11.01. The disease duration ranged from 1 to 10 years with a mean of 5.8±2.5. These demographic data are represented in table (1). The clinical data of our patients are summarized in table (2). The results of routine laboratory data for systemic sclerosis patients are shown in table (3). The mean ± standard deviation (SD) of serum level of thrombomodulin in patient group was 12.21±3.4 while in the control group it was 4.7±0.59. These results are summarized in table (4). Statistical comparison between patients and controls as regards the mean serum thrombomodulin level using the student ‘t’ test showed a highly significant statistical difference (t=6.75, p<0.001) as shown in table (5).

Table (1): Demographic data of systemic sclerosis patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>39.20</td>
<td>25</td>
<td>56</td>
</tr>
<tr>
<td>Disease duration/year</td>
<td>5.8</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

Table (2): Frequencies of clinical data in SSc patients.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raynaud’s phenomenon</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>Digital ulcers</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Esophageal dysmotility</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Sclerodactly</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>Calcinosis</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Interstitial pulmonary fibrosis</td>
<td>8</td>
<td>40</td>
</tr>
</tbody>
</table>

Table (3): Routine laboratory data of SSc patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (gm/dl)</td>
<td>10.36±1.2</td>
<td>7.10</td>
<td>12</td>
</tr>
<tr>
<td>RBCs (X10⁶/mm³)</td>
<td>3.9±0.35</td>
<td>3.30</td>
<td>4.5</td>
</tr>
<tr>
<td>TLC (X10³/mm³)</td>
<td>8.03±1.87</td>
<td>4.20</td>
<td>11.4</td>
</tr>
<tr>
<td>Platelets (X10⁹/mm³)</td>
<td>222.95±38.45</td>
<td>162</td>
<td>293</td>
</tr>
<tr>
<td>ESR (mm/1st hr)</td>
<td>61.6±35.45</td>
<td>10</td>
<td>127</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.06±0.31</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Serum urea (mg/dl)</td>
<td>27.25±7.87</td>
<td>16</td>
<td>36</td>
</tr>
</tbody>
</table>
Table (4): Thrombomodulin level in both patient and control group.

<table>
<thead>
<tr>
<th>Thrombomodulin level in serum (ng/ml)</th>
<th>Mean ±SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td>12.21±3.44</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Controls group</td>
<td>4.75±0.59</td>
<td>4</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Table (5): Comparison between systemic sclerosis patients and controls as regards serum thrombomodulin level.

<table>
<thead>
<tr>
<th></th>
<th>Patients (mean±SD)</th>
<th>Controls (mean±SD)</th>
<th>t</th>
<th>p</th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum thrombomodulin</td>
<td>12.21±3.43</td>
<td>4.75±0.58</td>
<td>6.75</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>level (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The level of thrombomodulin was measured in patients with or without some clinical manifestations and comparison of its level was done as shown in table (6).

Table (6): Statistical comparison of serum thrombomodulin level in patients with different clinical manifestations.

<table>
<thead>
<tr>
<th>Clinical Manifestation</th>
<th>Serum thrombomodulin level (mean±SD)</th>
<th>t</th>
<th>p</th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raynaud’s phenomenon</td>
<td>-Present (15/20) 13.12±3.22</td>
<td>0.034</td>
<td>&lt;0.05,S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Absent (5/20) 9.44±2.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digital ulcers</td>
<td>-Present (6/20) 15±2.6</td>
<td>0.013</td>
<td>&lt;0.05,S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Absent (14/20) 11±3.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial pulmonary</td>
<td>-Present (8/20) 14.25±2.49</td>
<td>0.025</td>
<td>&lt;0.05,S</td>
<td></td>
</tr>
<tr>
<td>fibrosis</td>
<td>-Absent (12/20) 10.84±3.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was a significant difference between the level of serum thrombomodulin in patients who had Raynaud’s phenomenon, digital ulcers and interstitial pulmonary fibrosis as compared to those without these clinical manifestations (t=0.034, 0.013, 0.025 respectively, p<0.05).

Correlations between serum thrombomodulin level and duration of illness and some clinical manifestations of systemic sclerosis showed that there was a significant positive correlation between its level and duration of illness, Raynaud’s phenomenon, digital ulcers and interstitial pulmonary fibrosis (r=0.69, 0.48, 0.58, 0.49 respectively, p<0.05).
As regards correlation of serum thrombomodulin level and routine laboratory investigations, there was a significant positive correlation
between serum thrombomodulin level and ESR and serum urea (r=0.72, 0.61 respectively, p<0.05).

Table (8): Correlation of serum thrombomodulin level and routine laboratory investigations.

<table>
<thead>
<tr>
<th>Item</th>
<th>r value</th>
<th>p value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>-0.42</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>RBCs</td>
<td>-0.22</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>TLC</td>
<td>0.078</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.13</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>ESR</td>
<td>0.72</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>Serum Urea</td>
<td>0.61</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>0.13</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

DISCUSSION

Systemic sclerosis (SSc) encompasses a wide spectrum of clinical presentations. It is characterized by vascular changes leading to injury of endothelial cells. However, assessment of severity of endothelial damage is so notoriously difficult that the search for circulating markers has gone on for over a decade (Salojín et al., 1997).

Pearson (1991) concluded that pathogenetic mechanism of scleroderma might be due to two main theories. Firstly, failure of endothelial cell control may play a significant part in the abnormal regulation of vascular tone and permeability in scleroderma. Secondly, the ability of endothelial cells to take part in the initiation and development of
immune cell mediated in small vessels (which may additionally lead to endothelial cell damage or destruction), coupled with evidence that there is increased production of a variety of cytokines in scleroderma.

Thrombomodulin is a vascular endothelial surface glycoprotein receptor for protein C, protein S and α-thrombin. Assembly of this complex on thrombomodulin leads to activation of protein C. Vascular endothelial damage has been shown to result in the release into the circulation of cleavage fragments of thrombomodulin varying in molecular weight from 28 to 112 KDa (soluble thrombomodulin). Increased concentrations of soluble thrombomodulin have been demonstrated in patients with vasculitis, and are thought to be a specific indicator of vascular endothelial injury (Herrick et al., 1996).

The aim of this study was to find out the role of thrombomodulin as a mediator of damage to endothelial cells in systemic sclerosis patient.

We found that serum thrombomodulin level was elevated in our patient and statistical comparison of its level in both the patient group and the control group showed a highly significant difference (t=6.75, p<0.001). Moreover, when we compared its serum level in patients with or without some clinical manifestations as Raynaud’s phenomenon, digital ulcers and interstitial pulmonary fibrosis, we found increase in its level in those patients with the previously mentioned clinical manifestations than in patients without manifestations and there was accompanied statistical significant difference (t=0.034, 0.013, 0.25 respectively, p<0.05).

Soma et al. (1993) measured plasma thrombomodulin level in systemic sclerosis patient. They found that thrombomodulin levels were elevated in a subset of patients with systemic sclerosis. They suggested that the measurement of plasma thrombomodulin might be used to assess the state of endothelial cell injury in systemic sclerosis.

The plasma thrombomodulin concentration in different collagen vascular diseases was measured by (Ohdama et al., 1994). They found that the mean plasma thrombomodulin concentrations in patients with juvenile rheumatoid arthritis, systemic sclerosis, polymyositis/dermatomyositis, Wegener’s granulomatosis, active states of SLE, rheumatoid arthritis and Behcet’s disease were significantly higher than those in the control group. Patients with active interstitial pneumonitis or extensive pulmonary lesions showed higher values of plasma thrombomodulin than those without overt pulmonary involvement. They explained the increased level of plasma thrombomodulin might be caused by an accelerated release of it from injured endothelial cells by proteolytic activity generated on the surface of
endothelium. They concluded that vascular endothelial cell injuries occurring with the progression of the collagen vascular diseases could be assessed by the measurement of plasma thrombomodulin.

In 1997 Salojin et al., measured the plasma thrombomodulin concentration in patients with limited systemic sclerosis (ISSc), primary Raynaud’s phenomenon (PRP) and diffuse systemic sclerosis (dSSc). They found that plasma thrombomodulin concentration were higher in patients with dSSc compared with those with ISSc (p=0.037). They concluded that thrombomodulin is a specific glycoprotein liberated by damage of endothelial cells in patients with systemic sclerosis.

The level of soluble thrombomodulin was measured by (Kotajiama et al., 1997) in patients with systemic rheumatic diseases. They found that it was highest in patients with SLE, less high in patients with rheumatoid arthritis, systemic sclerosis and Sjogran’s syndrome. They explained the elevated serum level of thrombomodulin in diseases associated with systemic or locally increased levels of inflammatory cytokines. They stated that neither TNF-$\alpha$ nor neutrophils alone but, the interaction of neutrophils with TNF-$\alpha$ activated endothelial cells bearing increased adhesion molecules might lead to release of thrombomodulin into culture supernatant, concomitant with morphologically evident endothelial cell damage.

Boehme et al. (2000) compare in vivo soluble thrombomodulin as a marker of activity in SLE patients with established and recent serological parameters. They found that soluble thrombomodulin is the most important serological parameters of disease activity in SLE currently available, as shown by the in vivo studies. They concluded that soluble thrombomodulin is an excellent and promising marker of endothelial cell damage in vasculitides.

Recently, Trifiletti et al. (2000) investigated possible alterations in haemostatic system and examined whether there was a circadian variation in hemostatic variable in the initial stage of systemic sclerosis by measuring level of thrombomodulin and other parameters in plasma of patients with Raynaud’s phenomenon (secondary to systemic sclerosis). They found that only thrombomodulin levels were found to be higher in patients than controls. They concluded that in patients with Raynaud’s phenomenon, there is endothelial damage reflected by increased plasma level of thrombomodulin.

In 2000, Stratton et al. investigated the expression of thrombomodulin in scleroderma associated pulmonary hypertension. They found that soluble thrombomodulin was raised in scleroderma associated
pulmonary hypertension when compared with scleroderma controls. They explained this finding by number of reasons. Thrombosis of pulmonary arteries was an important factor in the development of severe pulmonary hypertension. One possible explanation for this observation was that in pulmonary hypertension, the pulmonary vascular endothelium was deficient in anticoagulant protein like thrombomodulin. Also injury to endothelial cells might contribute in the pathogenesis of scleroderma associated pulmonary hypertension and plasma soluble thrombomodulin was increased in conditions associated with endothelial cell damage. They concluded that the pathogenesis of scleroderma-associated hypertension may be distinct from the pathogenesis of other forms of pulmonary vascular diseases.

Only one study done by Herrick et al. (1996) in the contrary to our results, were they measured the level of soluble thrombomodulin in systemic sclerosis patients and they were not able to demonstrate increased its concentration in patients with systemic sclerosis.

In the present study, we found also a positive significant correlation between the level of serum thrombomodulin and duration of illness, Raynaud’s phenomenon, digital ulcers and interstitial pulmonary fibrosis. Moreover, we found a positive significant correlation with its level in ESR and serum urea.

After the previous discussion, we can clarify that our results were in agreement with most of the studies done as regards higher level of serum thrombomodulin in systemic sclerosis patients when compared to controls. Moreover, we found that it was higher in SSc patients with some clinical manifestations (related to vasculitis) when compared to those without clinical manifestations.

**Conclusion:**

Elevated soluble serum thrombomodulin in our patients may reflect its role in the pathogenesis of systemic sclerosis as a result of endothelial damage. Finally, we suggest that soluble thrombomodulin may also represent a promising serological parameter for therapeutical considerations and decisions.
REFERENCES


