

Assessment of Serum Thrombomodulin in Patients with Systemic Lupus Erythematosus in Rheumatology Research Center

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Received: 2 Sep. 2006; Received in revised form: 26 Oct. 2007; Accepted: 11 Nov. 2007

Abstract- At present, some clinical presentations and serological parameters such as products of complement activation and elevation of autoantibodies (e.g. dsDNA antibodies), erythrocyte sedimentation rate (ESR), levels of C-reactive protein (CRP), or cytokines such as IL-2/IL-2 receptor, IL-6 and IL-10 are used as indirect serological markers with variable degrees of significance. To date, no specific serological parameter is available to assess disease activity in SLE. Soluble serum thrombomodulin is a new marker of endothelial cell injury and vasculitis. The objective of this study was to determine in vivo soluble thrombomodulin as a marker of disease activity in SLE patients and compare serum thrombomodulin in SEL patients with and without renal involvement and inactive SLE patients. Sixty-four patients fulfilling ACR criteria with proven SLE with different disease activities were tested for serum levels of Thrombomodulin and dsDNA by ELISA. C3, C4 and FANA were also measured by standard laboratory tests. The clinical disease activity was evaluated by the Systemic Lupus Erythematosus Activity Index (SLEDAI). Elevated soluble thrombomodulin had significant correlations with an increased ANA level ($P=0.037$), decrease level of C3 ($P=0.017$), increase of SLEDAI ($P=0.003$) and strongly associated with renal involvement in SLE. Thrombomodulin level and Anti dsDNA in active patients with and without renal disease were higher than inactive patients. In SLE, serum thrombomodulin seems to be important marker for evaluation of disease activity.

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Acta Medica Iranica 2009; 47(2): 97-102.

Key words: Systemic lupus erythematosus, thrombomodulin, SLE disease activity

Introduction

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease of unknown etiology, which involves different organ systems to a variable degree (1). Vasculitis plays an essential role in the SLE pathogenesis. Many manifestations of systemic lupus erythematosus, and among them lupus nephritis, CNS involvement, cardio-pulmonary and severe hematologic involvement are important and some of them are closely related to vasculitis damage.

At present, the erythrocyte sedimentation rate (ESR) (2,3), products of complement activation (3-5), and levels of C-reactive protein (CRP) (2,3,6), autoantibodies (e.g. dsDNA antibodies) (2,3,5,7,8) or cytokines such as IL-2/IL-2 receptor (8-10), IL-6 (7,11) and IL-10 (12) are

used as indirect serological markers with variable degrees of significance.

In general, these factors do not correlate closely enough with disease activity. In contrast, soluble serum thrombomodulin (sTM) was recently established as a valuable new serological marker with good correlation to disease activity in SLE (12). Soluble TM is the endothelial cell transmembrane receptor for thrombin (13,14). Soluble TM is an established marker of endothelial cell damage (12). However, a direct comparison of sTM and other serological markers of disease activity in SLE is still lacking. In this study, we determined sTM in SLE patients with renal involvement and active SLE patients without renal involvement. We also determined sTM in inactive SLE patients. Finally, sTM was compared in these three groups.

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Table 1. Characteristics of SLE patients

| | Number (percentage) | Sex | | Mean Age | Mean of dis. duration |
|-------------------------------|------------------------|-----------|----------|------------|--------------------------|
| | | Female | Male | | |
| Inactive SLE | 16(25 %) | 15(93.8%) | 1(6.3%) | 31.75 year | 98.19 month |
| Active SLE without renal dis. | 27(42.2%) | 25(92.6%) | 2(7.4%) | 30.33 year | 71 month |
| Active SLE with renal dis. | 21(32.8%) | 17(81%) | 4(19%) | 30 year | 51.29 month |
| Total | 64(100%) | 57(80.9%) | 7(19.1%) | 30.58 year | 71.33 month |

Patients and Methods

Patients

Sixty-four Iranian SLE patients were recruited at Rheumatology Research Center, Tehran University for Medical Sciences. At the time of diagnosis all patients fulfilled the 1982 revised American College of Rheumatology (ACR) for the diagnosis of SLE and disease activity was evaluated with the SLE Disease Activity Index (SLEDAI). The patients were divided into three groups: 16 inactive SLE patients, 27 SLE patients with active lupus nephritis and 21 active SLE patients without renal disease.

Laboratory tests

Standard laboratory tests were used to measure the serum levels of CBC, diff, BUN and creatinine. Serum levels of soluble thrombomodulin were measured by ELISA method (Randox kits). The titer of anti-nuclear antibodies (ANA) and Anti-dsDNA antibodies were

detected also by ELISA. Levels of C3 and C4 components of complement were measured by nephelometry

Statistics

Pearson correlation test and ANOVA test were used for statistical evaluation of results. A probability (*P*) value less than 0.05 was considered as indicating a significant difference.

Results

The age, sex, SLEDAI score, duration of diagnosis, are summarized in Table 1. Sixteen SLE patients with inactive disease (15 females and one male) aged 31.75±2.78 yr (range: 19–56). 21 active SLE patients with renal disease (17 females and 4 males) aged 30±2.15 yr (range: 18-52) and 27 active SLE patients without renal disease (25 females and 2 males) were 30.33±2.14 yr (range: 17-60) (ANOVA, P=0.876).

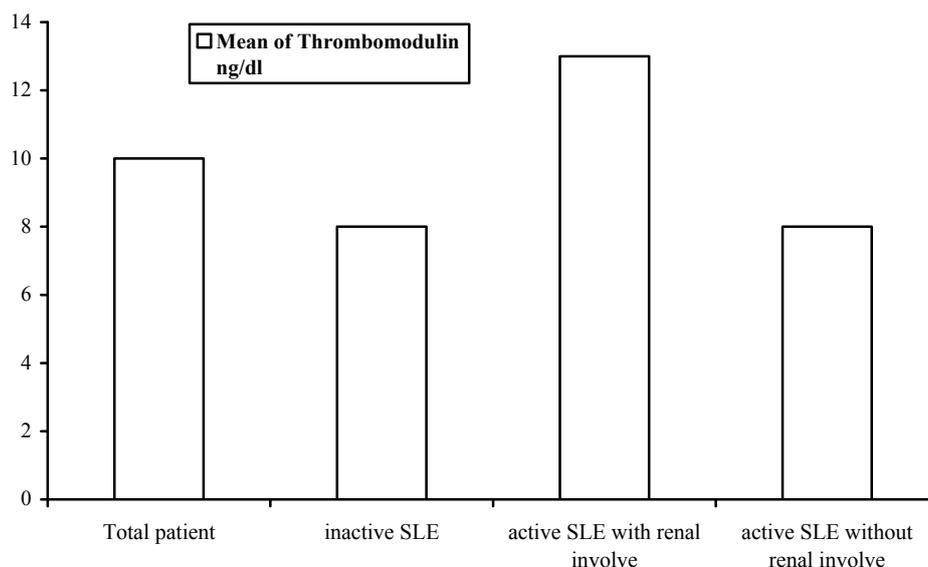


Figure 1. Mean of serum Thrombomodulin in patients

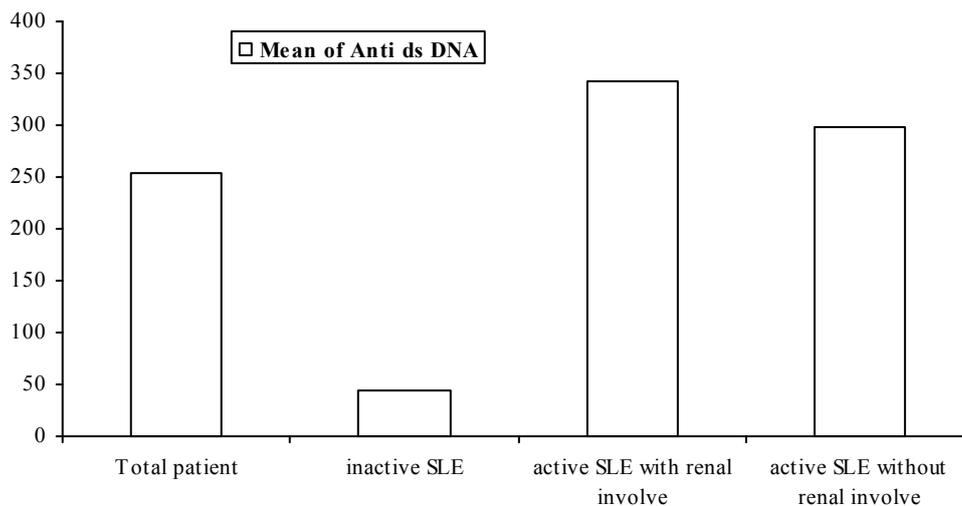


Figure 2. Mean of Anti dsDNA level in patients

The mean duration of SLE was 98.19 ± 21.57 months (range 12-360) for SLE patients with inactive disease, 51.29 ± 10.14 months (1-144) for SLE patients with renal disease and 71 ± 11.66 month (2-240 mo) for active SLE patients without renal disease. (ANOVA, Post Hoc Test, $P=0.031$)

As shown in figure 1, serum sTM was significantly higher in active SLE patients with renal disease than in other groups, Using ANOVA analysis of TM levels,

SLE patients with renal involvement was statistically different from the inactive SLE patients (13.12 and 8.4, $P=0.003$) and from active SLE without renal involvement (13.12 and 8.41, $P=0.001$)

Anti dsDNA was also higher in active SLE patients with and without renal disease than inactive SLE patients (Figure 2). (45.14 & 298.65, $PV=0.026$) and (45.14 & 342.05, $P=0.014$).

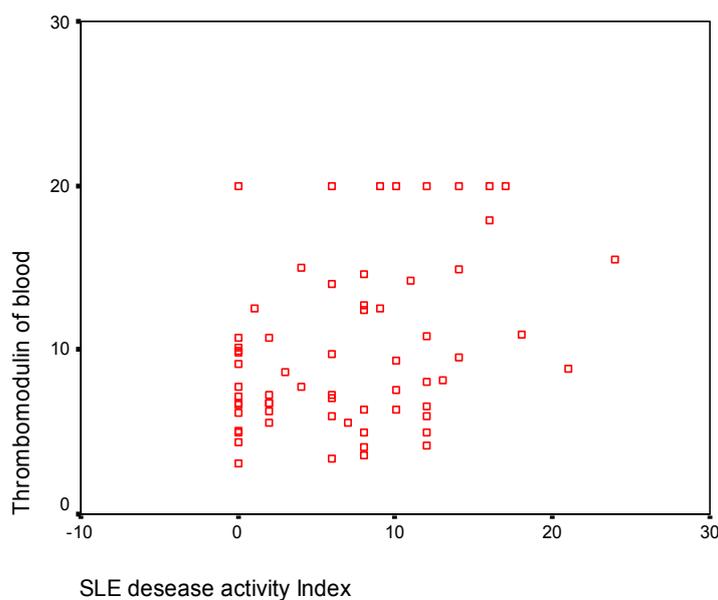


Figure 3. Scatter graph Thrombomodulin over SLEDAI

Serum thrombomodulin in SLE

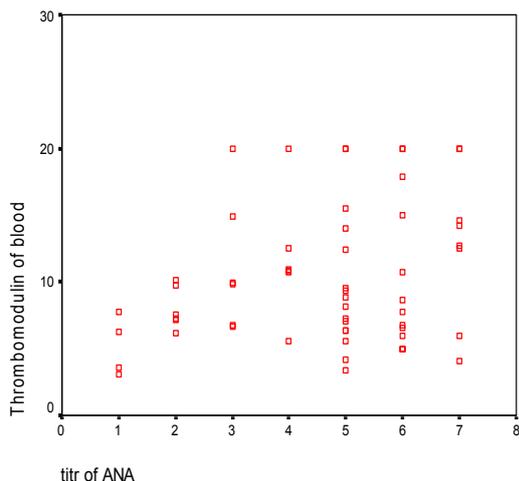


Figure 4. Scatter graph Thrombomodulin over ANA

Pearson's correlation between Thrombomodulin and ANA, Anti dsDNA, C3, C4, CH50 and SLEDAI was 0.273, 0.210, -0.306, -0.144, 0.139, 0.332.

As show in figure 3, 4 and 5 elevated soluble thrombomodulin had significant correlations with increase ANA level ($P= 0.037$), decrease level of C3 ($P =0.017$) and increase of SLEDAI ($P=0.003$) and strongly associated with renal involvement in SLE.

Discussion

Our data provide *in vivo* evidence for sTM as a good serological parameter of disease activity in patients with SLE. Serum sTM had correlation with SLE disease activity, positive ANA, elevated anti dsDNA and low complement components.

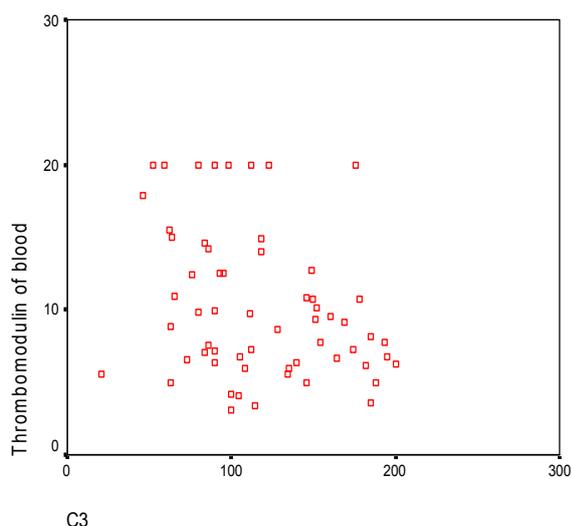


Figure 5. Scatter graph Thrombomodulin over low C3

Initially, only general, unselective parameters of disease activity were available such as ESR, CRP, or levels of immunoglobulins. Overall, these parameters show a weak correlation to disease activity and are influenced by a multiplicity of factors (2-6). Furthermore, the CRP response of patients with SLE was characterized in most cases by a weak increase (6). Elevated anti ds-DNA and low level of complement components have a good correlation with SLE disease activity. The levels of several complement components have proven to be of special value in the subgroup of patients with nephritis (4,5). A characteristic feature of SLE is the polyclonal B cell activation and the occurrence of a variety of autoantibodies. Particularly, autoantibodies to dsDNA have proven to be of high value for the diagnosis of SLE (7,8). In contrast, the titer of dsDNA antibodies is of limited value as a serological parameter of disease activity (5,7,8,15). Continuously elevated titers are found in a substantial subgroup of SLE patients independent of the clinical disease activity or relapse (5,7,16).

Pathophysiologically, SLE is characterized by an immune complex vasculitis and increased cytotoxicity of polymorphonuclear neutrophils (PMN) to endothelial cells (1,21). Recently, sTM was established as marker of endothelial cell damage (12). Thrombomodulin is the transmembranous glycoprotein receptor for thrombin, mainly expressed on endothelial cells and syncytiotrophoblasts. The sTM-thrombin complex acts as important anticoagulant resulting in accelerated activation of protein C (13,14). After physiological activation and reaction the complex is internalized and degraded. In vitro studies including Cr-release assays could confirm that soluble sTM is a reliable marker of endothelial cell damage independent of physiological activation (12,15). In addition, cytokine stimulation of endothelial cells results in decreased sTM expression on the cell surface due to sTM internalization with subsequent degradation and suppression of sTM transcription and translation. (17,18). Nevertheless, previously we showed in endothelial cell cultures in vitro and in patients receiving recombinant human tumor necrosis factor-alpha (rhTNF-[alpha]) for therapy, that soluble sTM is a reliable marker of endothelial cell damage due to endothelial-leucocyte adhesion and interaction after cytokine activation (19). In summary, sTM is a good and promising marker of endothelial cell damage in vasculitides. The clinical importance of sTM has further been documented recently in studies correlating sTM with disease activity in patients with various diseases resulting in vasculitides or vascular injury. These included patients with SLE (13,14), ulcerative colitis (18), Wegener's granulomatosis

sis (19,20), Takayasu's arteritis (19), Behcet's disease (19), giant cell arteritis (19), panarteritis nodosa (21), microscopic polyangiitis (20), diabetic microangiopathy (24), sepsis (22,23), malaria (24,25), and disseminated intravascular coagulation (22,24). However, a marked variation of sTM concentration (up to 10-fold) is found comparing the different reports. This might be due to the different standards used in the individual test kits, based on either recombinant or purified sTM from human placenta. Therefore, an international standardization is required. In conclusion, our in vivo data highlight sTM as the best serological activity marker in SLE with renal involvement available at present. Serum sTM reflects closely endothelial cell damage due to vasculitis and therefore, is closely related to the immunopathophysiological alterations in SLE. Our comparative study suggests that sTM may also represent a promising serological parameter for decisions.

Acknowledgments

The work is supported by the Research Center of Tehran University for Medicine.

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