

Urinary soluble VCAM-1 in systemic lupus erythematosus: A clinical marker for monitoring disease activity and damage

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ABSTRACT

Objective. To determine the urinary levels of soluble vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in patients with systemic lupus erythematosus (SLE) and to assess their relationship with clinical and laboratory features and the degree of activity and damage associated with the disease.

Methods. The study sample included 24 consecutive patients with SLE. 24-hour urine samples were collected for the determination of soluble VCAM-1 and ICAM-1 levels by ELISA. Disease activity was defined by the SLE Disease Activity Index (SLEDAI) and disease outcome by the Systemic Lupus International Collaborating Clinics/ American College of Rheumatology (SLICC/ACR) damage index.

Results. The urinary soluble VCAM-1 level was significantly higher in patients with SLE compared to normal controls (32.35 ± 34.27 vs. 4.66 ± 3.8 ng/mg creatinine, $p = 0.0005$) and statistically significantly correlated with disease activity (SLEDAI), a low serum C_3 level, decreased creatinine clearance and albuminuria, as well as with disease damage (SLICC/ACR damage index). In contrast, the urinary soluble ICAM-1 level was not significantly higher in the patients' group compared with the controls (4.5 ± 5.19 vs. 2.72 ± 2.31 ng/mg creatinine, $p = 0.2$), but was statistically significantly correlated with hematuria and albuminuria.

Conclusion. Our data suggest that the urinary level of soluble VCAM-1 significantly correlates with overall disease activity and damage scores, but not with nephritis in SLE.

Introduction

Adhesion molecules play a pivotal role in the dynamic interaction of leukocytes with endothelial cells during inflammatory processes (1). Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) of the immunoglobulin supergene family are weakly expressed on glomerular endothelial and tubular epithelial cells in normal kidneys (2,3). Their expression increases in the presence of proliferative lupus nephritis, in

a manner correlating to disease activity (2-5). Similarly, VCAM-1 and ICAM-1 are markedly expressed in the MRL-lpr murine model of lupus nephritis and correlate with cytokine-induced inflammation in the kidney (6,7). Recently, it has been shown that renal arterial infusion of tumor necrosis factor- (TNF) induced glomerular expression of VCAM-1 and ICAM-1 in a nephritis animal model (8). Indeed, soluble VCAM-1 and ICAM-1 in the serum are regarded as *in vivo* markers of cytokine-induced endothelial activation (9), as occurs in active systemic lupus erythematosus (SLE) (10-12). Previous studies have demonstrated that urinary soluble adhesion molecules are reliable markers of renal allograft rejection (13, 14), suggesting that the urinary level reflects the renal endothelial activation. Overt lupus nephritis usually manifests with proteinuria, hematuria and/or azotemia, although the paucity of urinary sediment findings does not exclude active renal inflammation (15,16). Hence, finding additional reliable urinary markers of lupus nephritis would facilitate the detection of active renal disease in SLE.

We hypothesized that increased urinary levels of soluble endothelial adhesion molecules are associated with active renal disease. Thus we sought to determine the urinary level of soluble VCAM-1 and ICAM-1 in SLE patients and relate it to clinical indices of glomerulonephritis, as well as to scores of disease activity and damage.

Patients and methods

Patients

Twenty-four consecutive patients with SLE who were evaluated at the Rheumatology Unit of Rabin Medical Center, Beilinson Campus, Israel, over a two-year period (November 1996 to November 1998) were recruited for the study. All fulfilled 4 or more of the revised criteria for SLE defined by the American College of Rheumatology (ACR) (17), and all signed an informed consent form before participating in the study.

Seven healthy individuals with no history of systemic or renal disease served as a control group for determining the

normal urinary levels of VCAM-1 and ICAM-1.

Clinical and laboratory evaluation

All participating patients were evaluated for the purposes of the study, as part of their regular clinic visits. Patients were evaluated for signs and symptoms of the disease, and laboratory tests were carried out, including a complete blood count, erythrocyte sedimentation rate (ESR), serum creatinine, and urinalysis. A 24-hour urine collection was analyzed for the quantity of albumin excretion and creatinine clearance. Serum antibodies to ds-DNA were determined by the Farr method (normal level < 20%), as well as C₃, C₄, CH100 levels and anti-Sm, Ro (SS-A), La (SS-B), RNP and cardiolipin antibodies. Disease activity was scored according to the SLE Disease Activity Index (SLEDAI) (18). Lupus nephritis was defined according to the 1982 ACR revised criteria (17) and renal function was assessed by determining serum creatinine and creatinine clearance. Disease-related damage was scored using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI) (19).

The urinary levels of soluble VCAM-1 and ICAM-1 were determined at the first visit of the study. Ten milliliters from each 24-hour urine collection were centrifuged at 200 g for 5 min, and the supernatant was kept at -70°C until assayed. Soluble VCAM-1 and ICAM-1 levels were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems); all assays were performed in duplicate. Before ELISA, the urine was diluted 1: 5 with the provided sample diluent. Urinary levels of the soluble adhesion molecules were corrected with the value of urinary creatinine.

Statistical analysis

Results are given as means \pm standard deviations. Student's t-test was used to analyze statistically significant differences in mean continuous variables (VCAM, SLEDAI, etc.) and categorical variables (prevalence of fever, fatigue, etc.) between cases and controls.

The chi-square test or Fisher's exact test were used where appropriate. Pearson's correlation coefficient (r) and the significance for it (p) were calculated between the variables. Renal outcome (defined by serum creatinine) and disease damage (defined by the SLICC/ACR damage index) were predicted by fitting multivariate linear regression models to the data. P values 0.05 were considered statistically significant.

Results

The 24 patients included 23 females and 1 male; the mean age was 40.2 ± 9.5 years (range 19-54) at entry into the study. The disease manifestations and laboratory findings at the entry visit are detailed in Tables I and II, respectively. The mean serum creatinine level of the patients was 0.91 ± 0.23 mg/dl (range 0.6-1.7 mg/dl) and the mean creatinine clearance was 82.06 ± 24.49 ml/min (range 45-152 ml/min); the control group was comprised of 7 healthy individuals (5 females, 2 males, mean age 46 years) whose mean serum creatinine and creatinine clearance were 0.8 ± 0.2 mg/dl (p = NS) and 105 ± 9 ml/min (p = 0.0001), respectively. The mean 24-hour urine albumin excretion in the patients was 391.8 ± 616.6 mg/24 h (range 2-2010 mg/24 h). Hematuria (> 5 RBC/field) was detected in 16%. At the study visit, 38.7% of the patients were receiving prednisone (< 5 mg/day) and 35.5% hydroxychloroquine; one patient each was receiving i.v. cyclophosphamide or p.o. methotrexate. The mean SLEDAI score for the SLE group was 6.7 ± 5.6 (range 0-11). Four (16.7%) of the 24 study patients had hypertension, while no patient had evidence of diabetes mellitus, nephrolithiasis or urinary tract infection.

Urinary soluble VCAM-1 levels

The level of urinary soluble VCAM-1 as determined in a urine collection on the study visit was 32.3 ± 34.3 ng/mg creatinine (range 0-97.00 ng/mg creatinine) in the study group and 4.6 ± 3.8 ng/mg (range 0-10.00 ng/mg creatinine) in the control group; the difference was statistically significant (p = 0.0005). The urinary soluble VCAM-1 level was

Table I. Disease manifestations of the 24 SLE patients at the study visit.

| Manifestations | No. (%) |
|------------------|-----------|
| Fever | 3 (12.5) |
| Fatigue | 13 (54.2) |
| Malar rash | 5 (20.8) |
| Discoid rash | 3 (12.5) |
| Photosensitivity | 3 (12.5) |
| Arthritis | 2 (8.3) |
| Serositis | 1 (4.2) |
| Seizures | 0 |
| Psychosis | 0 |

Table II. Laboratory data on the 24 SLE patients at the study visit.

| Parameters | Mean \pm SD |
|---------------------------------|---------------------------|
| ESR | 42.66 ± 26.8 mm/h |
| Serum creatinine | 0.91 ± 0.23 mg/dl |
| Creatinine clearance | 82.06 ± 24.49 ml/min |
| Urine albumin excretion | 391.8 ± 616.6 mg/24 h |
| Urine erythrocytes | 2.3 ± 4.1 RBC/field |
| Anti-dsDNA antibodies (N < 20%) | $35.9 \pm 28.6\%$ |
| C ₃ (N > 70) | 85.8 ± 21.3 mg/dl |
| C ₄ (N > 16) | 18.9 ± 5.5 mg/dl |

statistically significantly correlated with disease activity, as expressed by an elevated SLEDAI score (p = 0.002, r = 0.6) and low serum C₃ level (p = 0.02, r = -0.5). We also found a significant negative correlation with the glomerular filtration rate (measured as creatinine clearance) (p = 0.0007, r = -0.6). However, the VCAM-1 level did not correlate with indices of lupus nephritis as defined by the 1982 ACR criteria (17) (namely, proteinuria and/ or hematuria), or with the erythrocyte sedimentation rate, serum C₄, CH100 and anti-dsDNA antibody levels, or with the presence of serum Sm, Ro, La and RNP antibodies, except for IgG anticardiolipin antibodies, nor did it correlate with patients' medications.

Urinary soluble ICAM-1 levels

The level of urinary soluble ICAM-1 as determined in a urine collection at the study visit was higher in the SLE patients than the control group, although the difference was not statistically significant (4.50 ± 5.19 vs. 2.72 ± 2.31 ng/mg creatinine, p = 0.2). The urinary soluble ICAM-1 level failed to show an

association with any of the SLE manifestations. However, it correlated with the erythrocyte sedimentation rate ($p = 0.04$, $r = 0.5$), hematuria ($p = 0.002$, $r = 0.6$), and 24-hour urine albumin excretion ($p = 0.04$, $r = 0.5$). It did not correlate with serum creatinine, creatinine clearance, serum complement or specific auto-antibodies, with SLEDAI score, or drug treatment.

Renal and disease outcome

The mean SLICC/ACR damage index (19) was 1.6 ± 1.9 (range 0-7). The damage index correlated with the urinary soluble VCAM-1 level ($p = 0.04$, $r = 0.4$), but not with the urinary soluble ICAM-1 level.

Discussion

Our study suggests that in patients with SLE an increased urinary soluble VCAM-1 level significantly correlates with overall disease activity and damage, but not with active nephritis.

The endothelial adhesion molecules VCAM-1 and ICAM-1 have been shown to actively participate in the pathogenesis of immunocomplex-induced inflammation in SLE. Belmont *et al.* (11) found that the surface expression of VCAM-1, ICAM-1 and E-selectin is upregulated on immunohistochemically-stained non-lesional skin from SLE patients, and more so in the presence of active than inactive disease. Similarly, Jones *et al.* (20) reported increased VCAM-1 expression on endothelial cells from skin lesions in patients with SLE, and Spronk *et al.* (10) noted increased levels of soluble VCAM-1 in the serum of lupus patients in correlation with disease activity and, particularly, with renal involvement. These findings were supported by *in vitro* studies showing that human anti-dsDNA antibodies are capable of inducing the increased expression of VCAM-1 and ICAM-1 on cultured endothelial cells, and that this increased expression correlates with the increase in ICAM-1 mRNA, the increase in neutrophil adherence, and the elevated levels of soluble ICAM-1 and VCAM-1 in the supernatants of the endothelial cells (21). Taken together, these studies indicate that the endothe-

lium actively participates in the pathogenesis of SLE and that the increased serum level of endothelial adhesion molecules is consistent with the activation of these cells.

In normal kidneys, VCAM-1 and ICAM-1 are mildly expressed on the endothelium of glomerular, peritubular and interstitial vessels, and on Bowman's capsule (3, 5, 22, 23). This expression is intensified in patients with active glomerulonephritis, such as lupus nephritis (2-5, 22, 23). Yokoyama *et al.* (24) recently reported that the upregulated glomerular expression of ICAM-1 is associated with high circulating levels of TNF in lupus glomerulonephritis. In a murine model of lupus nephritis using the MRL-lpr mouse, both VCAM-1 and ICAM-1 showed an increased synthesis and surface expression which was induced by pro-inflammatory cytokines (such as TNF) and was associated with increased leukocyte adhesiveness to the glomerular endothelium (6, 7).

In the present study, we show that the soluble VCAM-1 level in the urine is significantly elevated in SLE patients compared to normal controls and positively correlates with both disease activity (as defined by the SLEDAI) and with disease-related damage (as defined by the SLICC/ACR damage index). By contrast, urinary soluble ICAM-1 is not significantly elevated in SLE nor is it associated with disease activity or damage. However, the urinary ICAM-1 level positively correlates with indices of lupus nephritis, i.e. the presence of hematuria and the quantity of albuminuria. However, because the urinary soluble ICAM-1 level was not elevated compared to normal controls, our finding warrants further studies. Our results are in accordance with previous studies showing elevated serum levels of soluble VCAM-1, but not ICAM-1, in patients with active SLE (10, 12, 25). Although we did not determine the serum level of VCAM-1 and ICAM-1 in our patients, other studies support an association of urine with serum levels of soluble adhesion molecules. Tesar *et al.* (12) reported that in lupus nephritis, the urinary soluble VCAM-1 level correlates with urine

TNF and interleukin-8 levels, as well as with plasma levels of soluble VCAM-1 and TNF. In another recent study in lupus patients, the serum level of soluble VCAM-1, but not of soluble E-selectin or ICAM-1, was found to correlate with disease activity and active nephritis (26). We suggest that like serum VCAM-1 in lupus patients, urinary level of soluble VCAM-1 reflects endothelial cell activation in SLE and is not merely a result of renal endothelial activation. In addition, our study suggests that urinary soluble VCAM-1 levels correlate with disease outcome (defined by the SLICC/ACR damage index) and renal function (defined by serum creatinine) which, to the best of our knowledge, was not previously reported. We suggest that determining the urinary level of VCAM-1 can be used in the assessment of disease activity and severity in clinical practice.

In summary, our findings suggest that urinary soluble VCAM-1 level in lupus patients significantly correlates with disease activity and damage, but not with nephritis, similar to results of previous studies on serum level of soluble endothelial adhesion molecules in SLE.

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