Evaluation of endothelial protein C receptor in patients with systemic lupus erythematosus: correlation with disease activity and lupus nephritis

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Introduction

Systemic lupus erythematous (SLE) is a systemic, multifaceted inflammatory disease with clinical manifestations is protean and follows a relapsing and remitting course. Lupus Nephritis (LN) is one of the most frequent and serious manifestation. Endothelial protein C receptor (EPCR) is a transmembrane receptor that is shed into soluble form (sEPCR) in inflammatory status. It is demonstrated as a part of the pathobiology of the SLE disease.

Aim of the work

To assess correlation of sEPCR level in SLE patients to the disease activity in these patients and to relate sEPCR to LN.

Patients and methods

Serum level of sEPCR using enzyme-linked immunosorbent assay (ELISA), chemical and immunological markers of SLE were measured in 30 SLE patients and 30 age and sex matched apparently healthy controls. SLE patients were subgrouped into 20 patients without LN and 10 with LN. Disease activity was assessed using SLE Disease Activity Index (SLEDAI).

Results

A significantly higher sEPCR level was found on comparing SLE patients to controls with statistically highly significant difference (z = 4.8, \(P < 0.001\)). Moreover, there was a significantly higher sEPCR level on comparing SLE patients with LN to those without LN with statistically highly significant difference (z = 3.9, \(P < 0.001\)). Serum sEPCR had a highly significant positive correlation with SLEDAI in SLE patients (\(r = 0.66, P < 0.01\)).

Conclusion

sEPCR has a possible role in the pathogenesis of SLE and particularly LN diseases, reflecting disease activity in SLE patients.

Keywords:
endothelial protein C receptor, lupus nephritis, systemic lupus erythematosus

Endothelial protein C receptor (EPCR) is a transmembrane endothelial receptor with both antithrombotic and anti-inflammatory properties through its regulation of protein C activation [4]. It may serve as an important biomarker as they reflect biologic events occurring at sites where the endothelium has been activated and engaged in inflammation with subsequent loss of functional integrity; it is shed in a pathological state to a soluble form, sEPCR [5].

The shedding of EPCR in SLE patients is promoted by the proinflammatory cytokines thrombin and interleukin (IL)-1 [6]. Also, interferon \(\gamma\) (INF-\(\gamma\)) was reported to participate in the process of its shedding, and this was explained by the recruitment of macrophages to the endothelium by increased expression of adhesion molecules that secrete inflammatory cytokines such as the INF-\(\gamma\) at sites of tissue injury in SLE patients, most notably in those with renal disease [7].
Accordingly, this study was carried out to examine the relation of sEPCR with the SLE disease and particularly in those with LN to explore its role in the disease pattern and activity.

**Patients and methods**
The present study is a cross-sectional one that included 30 patients with SLE who fulfilled the updated American College of Rheumatology (ACR) criteria [8]. They were selected from among inpatients and outpatients in Ain Shams University Hospitals. Thirty age-matched and sex-matched apparently healthy individuals were also included in the study and served as a control group. All the patients were on steroids and cytotoxic drugs. Oral consent was obtained from all patients and controls after a full explanation of the study was provided.

**Exclusion criteria**
Patients with diabetes mellitus, malignancies, sepsis, antiphospholipid syndrome, or those with end-stage renal disease, whether on hemodialysis or not, were excluded from the study.

**Clinical assessment included**
1. Full assessment of history and clinical assessment of the patients.
2. Assessment of the disease activity using the SLE disease activity index (SLEDAI), which is a validated model of experienced clinicians’ global assessments of disease activity in SLE patients with active disease eight or more points [9].

**Laboratory assessment**
1. Complete blood picture.
2. Erythrocyte sedimentation rate using the Westergren method.
3. C-reactive protein using the latex agglutination method.
4. Autoantibodies measurement: antinuclear antibody and Anti-dsDNA.
5. Kidney function tests: creatinine, BUN, complete urine analysis, and protein/creatinine ratio.
6. Serum complement C3 and C4 (assessed using the single radial immune-diffusion method).
7. Measurement of sEPCR was performed using an ELISA for its quantitative determination in human serum [10].

The statistical analysis was carried out by IBM SPSS statistics (V. 19.0, 2010; IBM Corp., USA) used for data analysis. *P* value less than 0.05 was considered to be significant. In addition, Spearman’s rank correlations between sEPCR levels and SLEDAI were calculated.

**Results**

**Clinical and laboratory data of systemic lupus erythematosus patients**
This study included 30 patients with SLE; they were further classified according to whether or not they had LN into two subgroups: group ‘a’ and group ‘b’.

Group ‘Ia’ included 20 (66.7%) patients without LN, 18 females (90%) and two males (10%); their ages ranged from 16.0 to 47.0 years, with a mean age of 28.9 ± 8.4 SD. Their disease duration ranged from 1 to 14.0 years, with a SEM of 4.4 ± 0.8 SEM. The sEPCR level ranged from 5 to 21 ng/ml (mean 9.0 ± 0.9 SEM).

Group ‘Ib’ included 10 (33.3%) patients with LN, eight females (80%) and two males (20%); their ages ranged from 17.0 to 59.0 years, with a mean age of 31.0 ± 13.2 SD. They had been newly diagnosed by renal biopsy. The sEPCR level ranged from 11 to 26 ng/ml (mean 19.9 ± 1.8 SEM).

Evaluation of disease activity by SLEDAI ranged from 2 to 36

The clinical and laboratory data of the patients are shown in (Tables 1 and 2).

Comparison between SLE patients and controls showed a statistically highly significant difference (*z* = 4.8, *P* < 0.001) in serum sEPCR (Table 3).

Comparison between SLE patients with LN and those without LN in sEPCR showed a highly statistically significant difference (*z* = 3.9, *P* < 0.001) in serum sEPCR (Fig. 1).

Correlation between sEPCR and SLEDAI and SLICC/ACR DI in SLE patients with and without LN showed a highly significant positive correlation between sEPCR and SLEDAI (Fig. 2). The Kruskal–Wallis test showed that there were significantly higher values of sEPCR with an increase in the grades of SLEDAI. Post-hoc analysis (Tukey’s HSD) was carried out and showed significance mainly between mild and very high grades (a) (*P* < 0.05) and high significance between moderate and very high grades (b) (*P* < 0.001) as shown in Fig. 3.
Discussion
Quantitative and qualitative modifications of EPCR have been implicated in human SLE, a potentially fatal autoimmune disease affecting multiple organ systems. Immune complexes are believed to induce microvasculature injury, associated with thrombotic manifestations, inflammation, and widespread activation of vascular endothelium [11]. The exposure of healthy endothelial cells to potential stimuli such as circulating INF-α, tumor necrosis factor α, or immune complexes present in patients who have active SLE results in the expression of nitric oxide synthase 2 and the generation of nitric oxide and adhesion molecules. This activated endothelium now has lost its ability to serve as a physiologic brake, which normally prevents the infiltration of inflammatory cells that produce IL-18, a potent chemoattractant for plasmacytoid dendritic cells. Endothelial cells may also be activated by IL-18. Plasmacytoid dendritic cells release INFs,

Table 1 Frequency of the clinical data in systemic lupus erythematosus patients

| Clinical manifestations          | Group Ia (n = 20) | Group Ib (n = 10) |
|--------------------------------|-------------------|-------------------|
|                               | [n (%)]           | [n (%)]           |
| Constitutional symptoms        |                   |                   |
| Fever                          | 16 (80.0)         | 7 (70.0)          |
| Fatigue                        | 19 (95.0)         | 10 (100.0)        |
| Skin manifestations             |                   |                   |
| Loss of hair                   | 6 (30.0)          | 6 (60.0)          |
| Malar rash                     | 20 (100.0)        | 9 (90.0)          |
| Photosensitivity               | 30 (100.0)        | 10 (100.0)        |
| Discoid rash                   | 3 (15.0)          | 1 (10.0)          |
| Oral ulcers                    | 15 (75.0)         | 6 (60.0)          |
| Raynaud’s                      | 1 (5.0)           | 2 (20.0)          |
| Vasculitis                     | 3 (15.0)          | 6 (60.0)          |
| Musculoskeletal manifestations |                   |                   |
| Muscle affection               | 0 (0.0)           | 0 (0.0)           |
| Joint affection                | 11 (55.0)         | 6 (60.0)          |
| Other systems                  |                   |                   |
| CNS                            | 6 (30.0)          | 4 (40.0)          |
| Lung                           | 1 (5.0)           | 2 (20.0)          |
| Heart                          | 3 (15.0)          | 1 (10.0)          |
| Ophthalmic                     | 6 (30.0)          | 2 (20.0)          |
| Serositis                      | 3 (15.0)          | 3 (30.0)          |
| Renal manifestations           |                   |                   |
| Lower limb edema               | 1 (5.0)           | 9 (90.0)          |
| Puffy eyelids                  | 1 (5.0)           | 6 (60.0)          |
| Loin pain                      | 1 (5.0)           | 7 (70.0)          |
| Hypertension                   | 4 (20.0)          | 5 (50.0)          |

Figure 1
Mean value of sEPCR in both SLE patients without LN group 'Ia' and SLE patients with LN group. EPCR, soluble endothelial protein C receptor; SLE, systemic lupus erythematosus.

Table 2 Laboratory data in systemic lupus erythematosus patients

| Laboratory manifestations   | Group Ia | Group Ib |
|-----------------------------|----------|----------|
|                             | Range    | Range    |
| WBC count (thousand/cm³)    | 2.6–11.4 | 1.8–9.5  |
| Hb concentration (g/dl)     | 7.6–14.5 | 8.6–14.1 |
| Platelets count (thousand/cm³) | 132.0–456.0 | 130.0–256.0 |
| ESR (mm/h)                  | 9.0–120.0| 11.0–60.0|
| CRP (mg/l)                  | 4.0–22.0 | 4.0–16.0 |
| Anti-dsDNA (IU/ml)          | 20.0–640 | 160.0–1280|
| C3 (mg/dl)                  | 37.0–185.0| 40.0–102.0|
| C4 (mg/dl)                  | 6.0–45.0 | 6.0–23.0 |
| Creatinine (mg/dl)          | 0.3–1.1 | 0.6–3.7 |
| BUN (mg/dl)                 | 8.0–29.0 | 12.0–38.0|
| Protein/creatinine ratio    | 0.06–0.4 | 0.7–4.9 |
| sEPCR (ng/ml)               | 5.0–21.0 | 11.0–26.0|

BUN, blood urea nitrogen; C3, complement 3; C4, complement 4; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; sEPCR, soluble endothelial protein C receptor; WBC, white blood cells.

Table 3 Comparison between patients and controls in soluble endothelial protein C receptor

| sEPCR | Patient group (mean ± SEM) | Control group (mean ± SEM) | z     | P     | Significance |
|-------|---------------------------|-----------------------------|-------|-------|--------------|
| First visit | 12.6 ± 1.3               | 5.3 ± 0.5                    | 4.791 | <0.001** | HS           |

**HS, highly significant.
which exert a paracrine effect on other cell types to express nitric oxide synthase 2 [12]. In addition to the local inflammatory consequences of activation, endothelial cells are shed into the circulation and membrane EPCR is lost so that EPCR is now circulating as a soluble form (sEPCR) [13].

This study aimed to assess sEPCR in SLE patients and its correlation with disease activity and lupus nephritis in these patients.

In this study, SLE patients showed higher sEPCR levels than the controls and the levels were higher in SLE patients with LN than in those without LN. This is in agreement with Kurosawa et al. [11], who reported that serum EPCR was higher in patients with SLE and sepsis patients than in controls. Also, Sesin et al. [10] reported the same findings in their 81 SLE patients. They reported increased levels of sEPCR in their patients with SLE, particularly those with renal disease. They reported the increased sEPCR levels as being a reflection of endothelial dysfunction, and potentially also contributing to a procoagulant phenotype and thus to the thrombosis observed in SLE.

Other studies were carried out relating EPCR shedding to inflammatory status as in the study of Faioni et al. [14] on inflammatory bowel syndrome. sEPCR was higher in the serum of patients than in controls with an impairment in the activating protein C (APC) pathway and enhancement of microthrombi.

Soluble EPCR has the ability to trap free APC, thereby depriving the latter of its anticoagulant function by blocking its binding to phospholipids and by abrogating its ability to inactivate factor Va.

Also, sEPCR blocks the inhibitory effect of APC of leukocyte adhesion to vascular endothelial cells and reduces neutrophil accumulation; thus, sEPCR inhibits release of proinflammatory cytokines in monocytes and shows proinflammatory properties [15].

In this study, the positive correlation between sEPCR and SLEDAI supports the role of sEPCR as an inflammatory mediator. This is also in agreement with Sesin et al.’s [10] study that reported this positive correlation. They concluded that the increased concentrations of sEPCR could reflect SLE disease activity.

In conclusion, sEPCR may represent a biological marker of vasculopathy in SLE and association with active disease and renal affection.

Acknowledgements
Conflicts of interest
There are no conflicts of interest.

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