J Microbiol Immunol Infect.
2007;40:50-55

Original Article

Elevated serum anti-endothelial cell autoantibodies titer is associated with lupus nephritis in patients with systemic lupus erythematosus

Jui-Cheng Tseng[1], Ling-Ying Lu[1], Rieh-Jieh Hu[1], Chien-Kai Kau[1], He-Hsiung Cheng[1], Pey-Ru Lin[2], Chien-Wen Sun[2], Hui-Ting Liang[2], Hing-Chung Lam[2,3], Ming-Hong Tai[2]

[1]Division of Allergy, Immunology, and Rheumatology, [2]Department of Medical Education and Research, and [3]Division of Metabolism, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

Received: September 13, 2005 Revised: March 26, 2006 Accepted: April 29, 2006

Background and Purpose: Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disease associated with endothelial dysfunction and the existence of multiple species of autoantibodies. However, the association between endothelial dysfunction and renal manifestations remains unclear in Taiwanese SLE patients.

Methods: Serum samples were collected from SLE patients with biopsy-proven lupus nephritis (n = 32), stable SLE patients (n = 32) and healthy controls (n = 32). The SLE Disease Activity Index (SLEDAI) of SLE patients was scored, and levels of anti-endothelial cell antibodies (AECA) and anti-endothelial activities in serum samples were measured by cell-enzyme-linked immunosorbent assay and crystal violet assay, respectively, using cultured human endothelial EA.hy926 cells.

Results: Significantly higher AECA ($p<0.001$) and anti-endothelial activities ($p<0.001$) were found in sera from patients with lupus nephritis compared with that from stable SLE patients or controls. Moreover, AECA titers ($p<0.001$) and anti-endothelial activities ($p<0.001$) were strongly correlated with SLEDAI scores in these patients.

Conclusion: The strong correlations of AECA and anti-endothelial activity with lupus nephritis activity support an endothelial origin for renal complications in Taiwanese SLE patients.

Key words: Anti-endothelial cell antibody; Endothelium; Lupus nephritis; Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by involvement with a broad spectrum of clinical manifestations and the existence of multiple species of autoantibodies. The loss of immune tolerance, increased antigenic load, excess T cell help, defective B cell suppression, and the shifting of T helper 1 (Th1) to Th2 immune responses result in B cell hyperactivity and the production of pathogenic autoantibodies [1]. However, despite intensive investigation, the exact pathogenesis of SLE remains poorly understood [1]. Endothelial dysfunction is the predominant manifestation of SLE. Anti-endothelial cell antibodies (AECA) are a heterogeneous group of autoantibodies directed against different antigens in endothelial cells and have been identified in a variety of diseases including SLE, systemic vasculitis, systemic sclerosis and Kawasaki disease [2]. The proportion of AECA-positive sera ranges from 15% to 80% in SLE cases worldwide. However, the association of AECA levels with SLE disease activity, particularly lupus nephritis, remains to be delineated. In this study, we determined the AECA titer and the anti-endothelial activities in serum from SLE patients with active nephritis or stable disease, in order to evaluate possible correlation with clinical parameters of SLE. The strong correlation between AECA titer and disease activities of lupus nephritis suggests that AECA-mediated endothelial dysfunction may be involved in the pathogenesis of renal disorders in SLE.

Corresponding author: Ming-Hong Tai, Ph.D, Department of Medical Education and Research, Room 3654, Kaohsiung Veterans General Hospital, 386 Ta-Chung 1st Road, Kaohsiung 813, Taiwan. E-mail: mhtai@isca.vghks.gov.tw

Methods

SLE patients

Sixty four SLE patients, who fulfilled the SLE criteria of the American College of Rheumatology, were enrolled at the Rheumatology Clinic at Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan. The clinical and laboratory parameters of SLE patients were recorded. The disease activities were scored according to SLE Disease Activity Index (SLEDAI). Lupus nephritis was verified by light microscopy, immunofluorescence, and electronic microscopy analysis. SLE patients were divided into two groups: active nephritis (32 SLE patients [29 females and 3 males] with biopsy-proven lupus nephritis including 24 cases of class IV, 6 cases of class V, 1 case of class III+V, and 1 case of class IV+V; SLEDAI score >6; age [mean ± standard deviation (SD)], 31.93 ± 10.6 years) and stable SLE (32 stable SLE patients [29 females and 3 males] with prednisolone dosage ≤5 mg/day; SLEDAI score ≤6, age [mean ± SD], 34.94 ± 9.3 years). The sera were collected from SLE patients and 32 controls (29 females and 3 males; mean age, 34.13 ± 6.8 years), then aliquoted and stored at –70°C until use. For SLE patients with active nephritis, sera were obtained prior to renal biopsy.

Assay for AECA titer

The AECA titers in sera of SLE patients were determined by cell-enzyme-linked immunosorbent assay (cell-ELISA) as previously described with slight modification [3]. EA.hy926 endothelial cells [4] were seeded onto 96-well microtiter plates (density, 10^4 cells per well) and cultured with Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 mg/mL streptomycin and 100 U/mL penicillin, 1% hypoxanthine, aminopterin, and thymidine at 37°C and 5% carbon dioxide. After growth to near confluence, cells were washed with phosphate-buffered saline (PBS) and fixed with ethanol (100 μL per well) on ice for 5 min. After washing with PBS three times, plates were blocked with 2% bovine serum albumin for 1 h at 37°C. After washing with PBS three times, 100 μL of serum (1:100 diluted with PBS) was added into each well. After overnight incubation, cells were washed then incubated with horseradish peroxidase-conjugated anti-human immunoglobulin G (IgG; diluted 1:4000; Molecular Probe, OR, USA) for 1 h. The detection was performed by incubation with tetramethylbenzidine chromogen/substrate solution (Wampole Laboratories, Cranbury, NJ, USA) for 30 min then terminated by adding 50 μL stop solution to each well. For each serum sample, the absorbance at 492 nm in wells with EA.hy926 cells was subtracted from that in wells without cells and expressed as mean ± SD percentage increment over control from triplicate data. The absorbance at 492 nm of sera from three healthy volunteers was averaged and used as the control value (100%).

Assay for anti-endothelial activity

The anti-endothelial activities in serum of SLE patients were determined using crystal violet assay. Briefly, cultured endothelial EA.hy926 cells were harvested by trypsin-ethylenedamine tetra-acetic acid and seeded onto 96-well microtiter plates (at a density of 10^4 cells per well), and 5 μL sera from SLE patients or controls was added into each well. After 24 h, cells were stained with 0.05% crystal violet solution (in 3.7% formalin in PBS; 30 μL per well) for 1 h, washed with distilled water three times and solubilized with solution containing 50% ethanol and 0.1% acetic acid. The optical density of viable cells was measured by reading absorbance at 595 nm using scanning multi-well spectrophotometer, and then expressed as mean ± SD percentages of inhibition from triplicate data. The absorbance at 595 nm of sera from three additional healthy volunteers was used as control (100%).

Statistical analysis

The experimental data were expressed as mean ± SD from indicated number of patients or experiments. Comparisons between groups of independent samples were assessed by Mann-Whitney *U* test. The correlation between variables was determined by Pearson's correlation test. A p value <0.05 was considered statistically significant.

Results

We measured the clinical parameters, AECA levels and anti-endothelial activities in sera from 32 SLE patients with biopsy-proven lupus nephritis, 32 stable SLE patients, and 32 healthy controls. All of the active SLE patients with lupus nephritis had proteinuria, white blood cell/red blood cell sediments and variable granular and/or hyaline casts in urinalysis. In addition, thirteen patients had nephrotic syndrome. The mean ± SD renal biopsy activity index from the 32 active SLE patients was 8.81 ± 4.84, and the chronicity index was 1.22 ± 1.93.

Table 1. Serologic and clinical parameters of systemic lupus erythematosus (SLE) patients with lupus nephritis or in remission

Serologic parameter	SLE with active nephritis (n = 32) Mean ± SD	Stable SLE (n = 32) Mean ± SD	*p*
Complement 3 (mg/dL)	45 ± 20	84 ± 19	<0.001
Complement 4 (mg/dL)	10 ± 6	22 ± 33	NS
Anti-double-stranded DNA (IU/mL)	180 ± 150	38 ± 41	<0.001
DPL (g/day)	5.9 ± 5.2		ND
Albumin (g/dL)	2.4 ± 0.6		ND
Creatinine (mg/dL)	1.6 ± 1.4	0.76 ± 0.14	0.003
Creatinine clearance (mL/min)	73.8 ± 40.9		ND
White blood cells (/mm^3)	5100 ± 2300	5100 ± 1800	NS
Platelet (1000/mm^3)	212 ± 72	208 ± 62	NS
Hemoglobin	10 ± 2	12.6 ± 1.6	<0.001
SLEDAI	16 ± 3.8	2.5 ± 1.8	<0.001

Abbreviations: SD = standard deviation; DPL = daily protein loss; SLEDAI = SLE Disease Activity Index; ND = not determined; NS = not significant

Analysis of serological factors revealed that patients with lupus nephritis had significantly lower levels of complement 3 (C3; $p<0.001$) and hemoglobin (Hb; $p<0.001$), but higher values of anti-double-stranded DNA (anti-dsDNA) titer ($p<0.001$), SLEDAI score ($p<0.001$) and creatinine level ($p=0.03$) than stable SLE patients (Table 1).

The AECA titer was significantly increased in patients with lupus nephritis (mean increment, 192 ± 163%) compared with that in stable SLE (68 ± 54%; $p<0.001$) or controls (–5.5 ± 26%; $p<0.001$) [Fig. 1].

Sera from patients with lupus nephritis exhibited higher inhibitory activities (mean inhibition, 16 ± 7%) compared with that from stable SLE (mean inhibition, 6.7 ± 7.9%; $p<0.001$) or controls (mean inhibition, 0.35 ± 6.12%; $p<0.001$) [Fig. 2].

The association of serum AECA titer or anti-endothelial activity with clinical parameters of SLE was analyzed by Pearson's correlation. It was found that AECA titer was strongly correlated with the levels of C3 ($p<0.001$), C4 ($p<0.05$), anti-dsDNA ($p<0.001$), white blood cells ($p<0.05$), and Hb ($p<0.01$) and SLEDAI score ($p<0.001$) [Table 2]. Also, the anti-endothelial activities were significantly associated with the level of C3 ($p<0.003$), Hb ($p<0.001$) and SLEDAI score ($p<0.001$) in SLE patients. Thus, both AECA and anti-endothelial activities are elevated during the progression of lupus nephritis.

To investigate the link between anti-endothelial activities and the titer of anti-endothelial IgG auto-antibodies, the relationship between AECA level and anti-endothelial activity was examined in different groups of SLE patients and controls (Fig. 3). Interestingly, in normal individuals, AECA titer was significantly correlated with the anti-endothelial activity ($p=0.021$; r = 0.42), suggesting that AECA might be the predominant inhibitory factor to endothelial proliferation in normal physiological conditions (Fig. 3C). In stable SLE patients, AECA titer remained marginally associated with anti-endothelial activity despite prior SLE onset or medication ($p=0.074$; r = 0.321) [Fig. 3B]. However, such correlation no longer existed in SLE patients with lupus nephritis ($p>0.05$; r = –0.02; Fig. 3A).

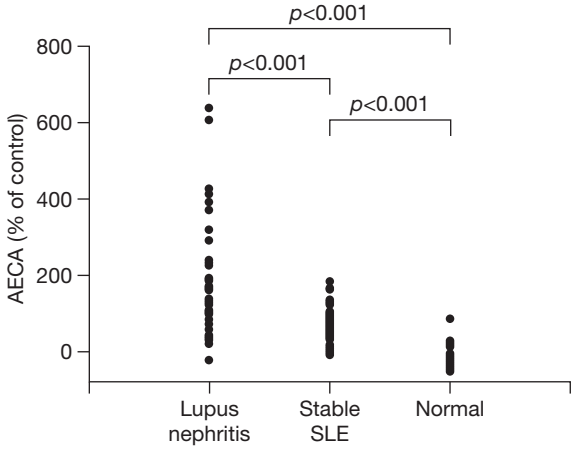

Fig. 1. Anti-endothelial cell antibodies (AECA) titer in systemic lupus erythematosus (SLE) patients with active nephritis. Sera were collected from SLE patients with nephritis (n = 32), stable SLE patients (n = 32) and healthy controls (n = 32). AECA titer was determined by cell-enzyme-linked immunosorbent assay and expressed as mean percentage of control from triplicate determinations.

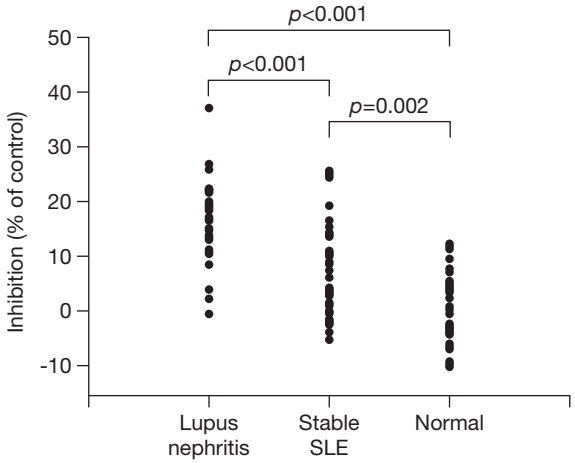

Fig. 2. Anti-endothelial activity in sera from systemic lupus erythematosus (SLE) patients with lupus nephritis. Sera were collected from patients with lupus nephritis (n = 32), stable SLE patients (n = 32) and healthy controls (n = 32). Inhibition of endothelial proliferation was determined by crystal violet assay and expressed as mean percentage increase over control from triplicate determinations.

Discussion

The present study provides conclusive evidence for an association between serum AECA and nephritis in SLE patients in Taiwan. These findings are consistent with previous studies on SLE patients in Taiwan and in groups from other countries, including the UK, Korea and France [5-9]. In previous studies of SLE patients in Taiwan, Liu and Lei found that patients with cutaneous vasculitis, Raynaud's phenomenon, or lupus nephropathy tended to have higher mean values of AECA than patients without such manifestations [6]. However, such correlation was not statistically significant in their study. In another report on AECA titer in SLE patients, Li et al identified a 66-kDa membrane antigen recognized by IgG-AECA from patients with lupus nephritis, vasculitis and hypocomplementemia [7]. Although data were not shown, they also mentioned that the highest AECA levels were observed in SLE patients with diffuse proliferative glomerulonephritis, and in patients with proteinuria and nephrotic syndrome, which is consistent with results of the present study and other reports [7]. In addition to predicting the involvement of nephritis and vasculitis, recent evidence indicates that AECA levels are correlated with psychiatric manifestations in SLE patients [10]. Therefore, AECA plays a pivotal role in the pathogenesis of systemic complications of SLE.

Instead of relying only on cell-ELISA, the present study employed both cell-ELISA and cell viability assays to delineate the role of endothelial dysfunction in patients with lupus nephritis. Overwhelmingly, differential AECA titer and anti-endothelial activity in SLE patients with or without nephritis support a role for AECA and anti-endothelial activity as diagnostic markers to detect nephritic states of SLE patients (Fig. 1 and Fig. 2). Moreover, AECA titers and anti-endothelial activities are not only higher in patients with lupus nephritis (Fig. 1 and Fig. 2), but also strongly associated with the SLEDAI (Table 2). Together, these findings strongly support the involvement of endothelial dysfunction during disease progression of SLE.

The pathogenic mechanism of AECA in SLE has been under active investigation. AECA may exert their pathological effects by direct induction of apoptosis in endothelial cells directly by targeting heat-shock protein 60 (Hsp60) [11,12], activation of endothelial cells with increased expression of adhesion molecules [13], and increased secretion of proinflammatory cytokines [13, 14], thereby facilitating the recruitment and trafficking of leucocytes into the inflamed vessels. The role of AECA in promoting inflammation is further strengthened by the recent observation that AECA recognize and bind apoptotic endothelial cells, thereby enhancing the phagocytosis and release of proinflammatory cytokines by macrophages [15].

Table 2. Correlation of anti-endothelial cell antibodies (AECA) and anti-endothelial activities with clinical parameters of systemic lupus erythematosus (SLE) patients

Clinical parameter	AECA	Anti-endothelial proliferation
Complement 3 (mg/dL)	$p<0.001$	$p<0.003$
Complement 4 (mg/dL)	$p<0.05$	NS
Anti-double-stranded DNA (IU/mL)	$p< 0.001$	NS
White blood cells (/mm^3)	$p<0.05$	NS
Platelet (1000/mm^3)	NS	NS
Hemoglobin	$p<0.01$	$p<0.001$
SLEDAI	$p<0.001$	$p<0.001$

Abbreviations: SLEDAI = SLE Disease Activity Index; NS = not significant

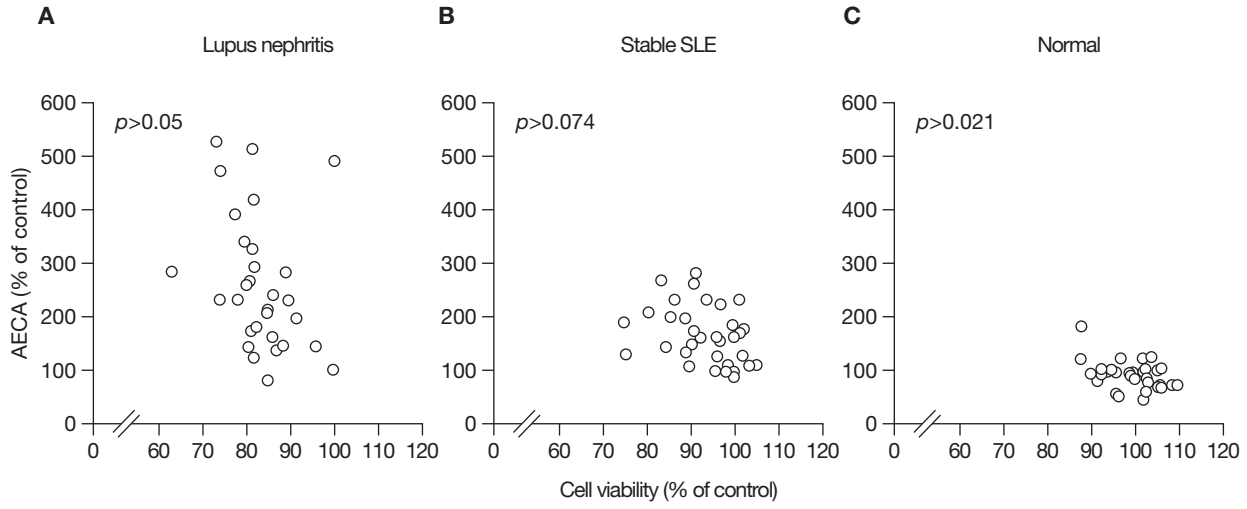

Fig. 3. Correlation between anti-endothelial cell antibodies (AECA) titer and anti-endothelial activity in: (A) systemic lupus erythematosus (SLE) patients with active nephritis (n =32; $p>0.05$, r = –0.02); (B) stable SLE patients (n = 32; $p=0.074$, r = 0.321); and (C) age-matched healthy controls (n = 32; $p=0.021$, r = 0.42).

In SLE patients with active nephritis, serum AECA titers are not correlated with potency in inhibiting endothelial viability, unlike the case in stable SLE patients or normal controls. This could be attributed to the dramatic changes of circulating cytokines/chemokines levels during nephritic disease, which might stimulate the proliferation or other functions of endothelial cells. Recent studies indicate that serum concentration of angiogenic factors such as hepatocyte growth factor [16] and vascular endothelial growth factor (VEGF) [16-19] were significantly increased in active SLE patients. Besides, a positive correlation between VEGF serum concentration and SLE activity was observed in SLE patients [19]. Finally, serum levels of soluble VEGF receptor-1 are increased, while levels of soluble VEGF receptor-2 are reduced in patients with active SLE [17]. Together with the AECA overproduction, the imbalance between pro- and anti-angiogenic factors may contribute to the vascular deficits in SLE patients.

In conclusion, the present study demonstrates that elevated AECA titer is correlated with SLEDAI disease activity in SLE patients with lupus nephritis. Such correlation sheds light on endothelial dysfunction in the pathogenesis of lupus nephritis. Serum AECA titer may serve as a diagnostic or prognostic marker for SLE. By serial determination of AECA or anti-endothelial activities, future longitudinal prospective studies are warranted to evaluate the potential of these variables for use in monitoring disease activity in SLE patients.

Acknowledgments

The authors thank Dr. C.J.S. Edgell at the University of North Carolina for kindly providing EA.hy926 cells. This work was supported in part by grants from the National Science Council, Taiwan (NSC-90-2320-B-075B-005) and Kaohsiung Veterans General Hospital (VGHKS-90-24 and VGHKS-92-30).

References

1. Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol. 2003;56:481-90.
2. Renaudineau Y, Dugue C, Dueymes M, Youinou P. Antiendothelial cell antibodies in systemic lupus erythematosus. Autoimmun Rev. 2002;1:365-72.
3. Revelen R, Bordron A, Dueymes M, Youinou P, Arvieux J. False positivity in a cyto-ELISA for anti-endothelial cell antibodies caused by heterophile antibodies to bovine serum proteins. Clin Chem. 2000;46:273-8.
4. Edgell CJ, McDonald CC, Graham JB. Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc Natl Acad Sci U S A. 1983;80:3734-7.
5. D'Cruz DP, Houssiau FA, Ramirez G, Baguley E, McCutcheon J, Vianna J, et al. Antibodies to endothelial cells in systemic lupus erythematosus: a potential marker for nephritis and vasculitis. Clin Exp Immunol. 1991;85:254-61.
6. Liu MF, Lei HY. Anti-endothelial cell antibodies in patients with systemic lupus erythematosus. J Formos Med Assoc. 1991; 90:221-4.
7. Li JS, Liu MF, Lei HY. Characterization of anti-endothelial cell antibodies in the patients with systemic lupus

erythematosus: a potential marker for disease activity. Clin Immunol Immunopathol. 1996;79:211-6.
8. Song J, Park YB, Lee WK, Lee KH, Lee SK. Clinical associations of anti-endothelial cell antibodies in patients with systemic lupus erythematosus. Rheumatol Int. 2000;20:1-7.
9. Constans J, Dupuy R, Blann AD, Resplandy F, Seigneur M, Renard M, et al. Anti-endothelial cell autoantibodies and soluble markers of endothelial cell dysfunction in systemic lupus erythematosus. J Rheumatol. 2003;30:1963-6.
10. Conti F, Alessandri C, Bompane D, Bombardieri M, Spinelli FR, Rusconi AC, et al. Autoantibody profile in systemic lupus erythematosus with psychiatric manifestations: a role for anti-endothelial-cell antibodies. Arthritis Res Ther. 2004;6: R366-72.
11. Bordron A, Revelen R, D'Arbonneau F, Dueymes M, Renaudineau Y, Jamin C, et al. Functional heterogeneity of anti-endothelial cell antibodies. Clin Exp Immunol. 2001;124: 492-501.
12. Dieude M, Senecal JL, Raymond Y. Induction of endothelial cell apoptosis by heat-shock protein 60-reactive antibodies from anti-endothelial cell autoantibody-positive systemic lupus erythematosus patients. Arthritis Rheum. 2004;50:3221-31.
13. Carvalho D, Savage CO, Isenberg D, Pearson JD. IgG anti-endothelial cell autoantibodies from patients with systemic lupus erythematosus or systemic vasculitis stimulate the release of two endothelial cell-derived mediators, which enhance adhesion molecule expression and leukocyte adhesion in an autocrine manner. Arthritis Rheum. 1999;42:631-40.
14. Papa ND, Raschi E, Moroni G, Panzeri P, Borghi MO, Ponticelli C, et al. Anti-endothelial cell IgG fractions from systemic lupus erythematosus patients bind to human endothelial cells and induce a pro-adhesive and a pro-inflammatory phenotype in vitro. Lupus. 1999;8:423-9.
15. Williams JM, Colman R, Brookes CJ, Savage CO, Harper L. Anti-endothelial cell antibodies from lupus patients bind to apoptotic endothelial cells promoting macrophage phagocytosis but do not induce apoptosis. Rheumatology (Oxford). 2005; 44:879-84.
16. Navarro C, Candia-Zuniga L, Silveira LH, Ruiz V, Gaxiola M, Avila MC, et al. Vascular endothelial growth factor plasma levels in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. Lupus. 2002;11:21-4.
17. Robak E, Sysa-Jedrzejewska A, Robak T. Vascular endothelial growth factor and its soluble receptors VEGFR-1 and VEGFR-2 in the serum of patients with systemic lupus erythematosus. Mediators Inflamm. 2003;12:293-8.
18. Robak E, Wozniacka A, Sysa-Jedrzejowska A, Stepien H, Robak T. Serum levels of angiogenic cytokines in systemic lupus erythematosus and their correlation with disease activity. Eur Cytokine Netw. 2001;12:445-52.
19. Robak E, Wozniacka A, Sysa-Jedrzejowska A, Stepien H, Robak T. Circulating angiogenesis inhibitor endostatin and positive endothelial growth regulators in patients with systemic lupus erythematosus. Lupus. 2002;11:348-55.

