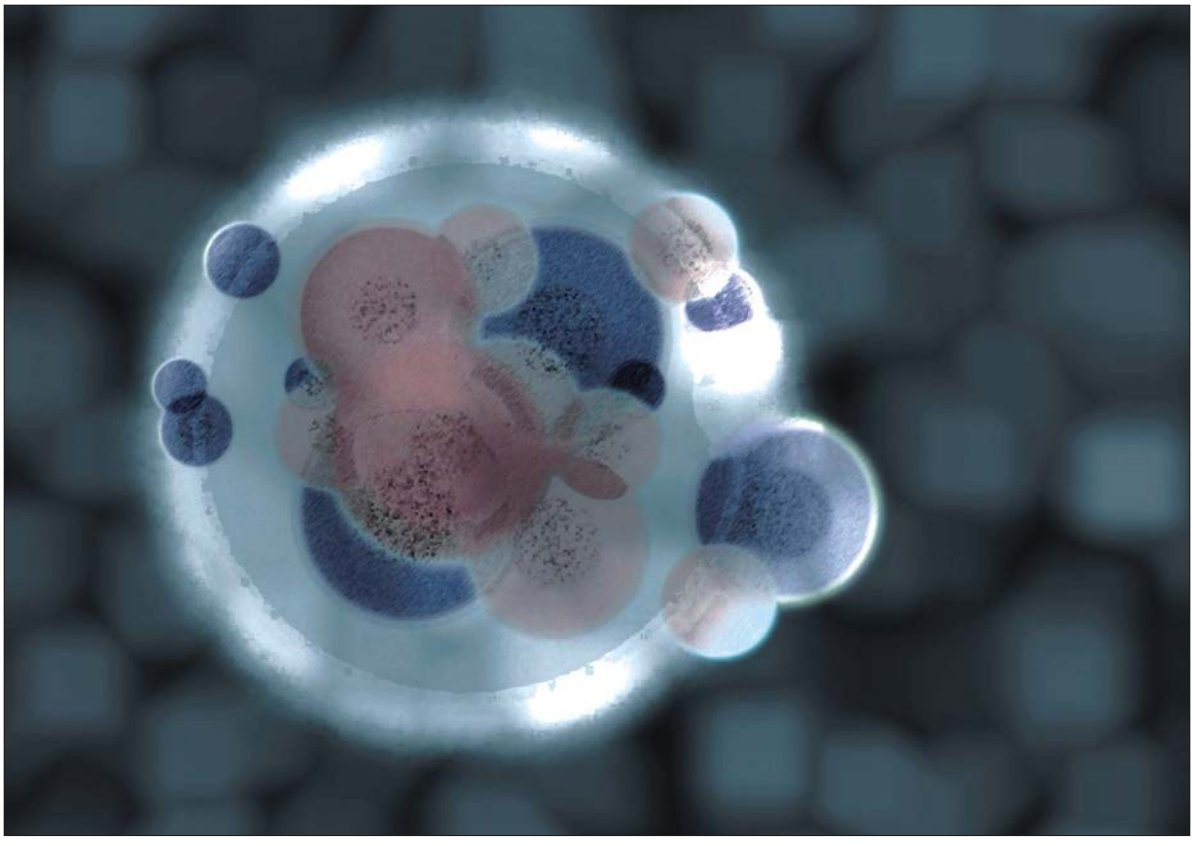

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 9 (6): 549-554, 2009
ISSN 1727-3048

The Clinical Utility of Vascular Endothelial Growth Factor as Predictive Marker for Systemic Lupus Erythematosus Activity in Children and Adolescents

Nevine S. ELhelaly, Ismail M. Elhawary,
Iman A. Abd Alaziz, Mona I. Abd Alsalam, Hussien M. Elfishawy and Mai M. Sherif
Departments of Internal Medicine and Clinical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

Abstract: This study conducted to investigate the serum concentration of Vascular Endothelial Growth Factor (VEGF) using an Enzyme-linked immunosorbent assay (ELISA) in a group of 23 children and adolescents with Systemic Lupus Erythematosus (SLE) and 25 healthy controls to asses for changes of VEGF in lupus patients and its relation to lupus activity. Vascular Endothelial Growth Factor (VEGF) was detectable in all patients with SLE and in all normal individuals. The level of serum VEGF in lupus patients was higher than control, also its level in active SLE was higher than inactive disease or in controls. We found that serum levels of VEGF was significantly higher in patients with renal involvement, patients with moderate to sever skin disease, neurological and joint involvement but the differences were statistically insignificant. A positive correlation was detected between higher VEGF serum levels and ESR and SLAM score ($p<0.01$ and <0.04, respectively). In conclusion, VEGF serum levels are higher in children and adolescents with SLE patients especially active lupus. Also, its level is correlated to many of clinical and laboratory parameter of lupus. So, it may be a useful marker of disease activity.

Key words: Vascular endothelial growth factor, predictive marker, systemic lupus erythrematosus

INTRODUCTION

The angiogenic process plays a major role in the development of vascular supply in some pathological diseases including neoplastic and collagen diseases (Sezer *et al*., 2001). A family of pro-and anti-angiogenic factors tightly regulates angiogenesis. A large number of cytokines have been shown to stimulate angiogenesis, including Vascular Endothelial Growth Factor (VEGF), transforming growth factor-beta (TGF-beta), Hepatocyte Growth Factor (HGF) and basic fibroblast growth factor (bFGF) (Talks and Harris, 2000).

Vascular Endothelial Growth Factor (VEGF,) formerly called vasculotropin or Vascular Permeability Factor (VPF) is a chimeric glycoprotein with a molecular weight of 34-35 Kds and generated by alternative splicing of single mRNA. The VEGF receptors are high affinity transmembrane tyrosine kinase receptors which are designated as VEGFR-1 and VEGFR-2 (Neufeld *et al*., 1999). The VEGF stimulates endothelial cell proliferation and differentiation, increases vascular permeability, mediates endothelium-dependent vasodilatation via upregulation of the expression of endothelial nitric oxide synthase (NO33) in endothelial cells and increases the production of nitric oxide (Hood *et al*., 1998). Moreover, VEGF supports vascular survival by preventing vascular apoptosi, induces plasminogen activator, plasminogen activator inhibitor-1 and interstitial collagenase, factors important in matrix remodling. Also, it promotes monocytes chemotaxis and expression of adhesion molecules (Ferrara and Gerber, 2001).

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by immune dysregulation resulting in the production of Anti-Nuclear Antibodies (ANA), generation of immune complexes and activation of the complement system and a predilection for clinical involvement of the Joints, skin, kidneys, brain, lungs, serosa, heart and gastrointestinal tract. The pathological hallmark of the disease is recurrent, widespread and diverse vascular lesions (Rahman and Isenberg, 2008).

Systemic Lupus Erythematosus (SLE) is primarily a disease of young adult women; however, in 10-15% of patients, the diagnosis is first established during childhood (Vilá *et al*., 2004).

According to earlier reports (JSLE) is rarely seen in children under 5 years of age and the peak incidence of childhood SLE occurs around puberty (Moradinejad *et al*., 2008).

The clinical manifestations of the disease, which are remarkably diverse, include fever, erythematous rash, polyarthralgia and arthritis, polyserositis, anemia,

Corresponding Author: Nevine Elsaid Elhelaly, Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt

thrombocytopenia, renal, neurological and cardiac abnormalities such as pericarditis, myocarditis and endocarditis (Bader Menuier *et al*., 2005).

The importance of JSLE derives from the fact that it is a life-threatening, long-term illness associated with significant complications (Brunner *et al*., 2008).

Moreover, the atypical presentation, common in this age group, is often responsible for major diagnostic delay. In patients with childhood-onset SLE, the initial symptoms have been reported to be more severe than in adults (Font *et al*., 1998).

There has long been a need for biomarkers of disease activity in lupus. Such markers ideally would be capable of detecting early sub-clinical disease and could be used to gauge response to therapy (Li *et al*., 2006).

The aim of the current study is to estimate the changes of serum VEGF level in a group of children and adolescents with SLE with assessment of its relation to lupus activity. Also, correlation of its level with selected clinical laboratory parameters and disease activity score.

MATERIALS AND METHODS

Study population: The current study was conducted in the pediatric and internal medicine departments faculty of medicine Cairo university in the period of January till August 2008 and it included 48 subjects, classified into two main groups. Group I consisted of 23 patients (21 females and 2 males) with SLE, with the age ranged from 8 to 18 years. The diagnosis of SLE was based on the revised criteria of the American Rheumatism Association (ARA) (Tan *et al*., 1982). Group II included 25 sex-and age-matched healthy controls.

Neither the patients with SLE nor the controls showed any clinical signs of infections or neoplastic disease and were not given antibiotics or any other antibacterial or antiviral medications for at least 4 weeks prior to blood collection. The selected patients and controls were subjected to proper history taking and physical examination.

Clinical assessment: Disease activity was evaluated by Systemic Lupus Activity Measure (SLAM) system. This system uses disease manifestations derived from the literature and refined in 1983 by members of the ARA Council on SLE and by clinical judgment. The items chosen for the scale represent those manifestations that occur more frequently, those that can be graded and those that can be operationally defined and reliably rated. (Liang *et al*., 1989). The SLAM covers symptoms that occurred during the previous month and includes 24 clinical manifestations and 8 laboratory parameters to evaluate organs which cannot be assessed otherwise. Parameters of immune function are not included. Since disease activity is always considered with disease severity, both dimensions are incorporated in the scales. Severity is then used to expand a scale's gradations and is judged by the need to treat with immunosuppressive agents, the need to follow the patient more closely, or the functional or prognostic consequences of the manifestation (Liang *et al*., 1989). Selected clinical and laboratory items of SLAM system are demonstrated in Table 1.

In this study, the score of the patients ranged from 9 to 25 points, so a score of 0-15 was considered as inactive disease and a score more than 15 points as active disease. Accordingly SLE patients in group (I) were subdivided to two subgroups, group (Ia) included 16 patients with active SLE and group (Ib) contained 7 patients with inactive disease.

In the view of SLAM score, assessment of patients for serum creatinine, creatinine clearance, proteinurea, hematuria, pyuria and urinary casts. Six patients were considered to have active renal disease and subjected to renal biopsy to verify the type of nephritis. Two patients had WHO class III nephritis (focal segmental lupus nephritis), 2 patients had WHO class IV nephritis (diffuse proliferative lupus nephritis) and 2 patients had WHO class V nephritis (membranous nephritis).

Neurological evaluation of SLE patients showed that only 4 patients had symptoms more specific for SLE, including CVA, seizures, depression and psychosis.

Mucocutanous involvement was found in 18 SLE patients, of them only 12 patients had mild skin disease (Score of skin affection up to 3), while 6 had moderate to sever skin disease (Score of skin affection 4-9). In addition to renal neurological and mucocutanous involvement, SLE patients showed activity mixed manifestations as constitutional, serosal, hematological and musculoskeletal affection. None of the study patients had clinical and/or laboratory findings fulfilled the international criteria of secondary antiphospholipid syndrome.

The history of drug therapy in SLE patients showed that 20 patients had been treated with prednisone in a dose of 0.5-2 mg/kg/day or every other day with a maximum dose of 60 mg day^{-1} during the course of the disease of them only 16 patients were receiving he drug during blood sampling and 4 patients were receiving combined therapy with prednisone and cyclophosphamide. The remaining 3 SLE patients were treated only by non-steroidal anti-inflammatory drugs.

Table 1: Selected items of SLAM scoring system

	Severity/Score			
Clinical/Lab item	Zero	Mild 1	Moderate 2	Sever 3
Mucocutanous				
Oral/nasal ulcers, periugual erythema, malar rash, nail fold infarcts, photosensitive rash	-ve	Present	-	-
Alopeci	-ve	Hair loss with Trauma	Spontaneous hair loss	-
Erythematous, maculopapular rash, or discoid lupus, or lupus profundus, or bullous 1esions	-ve	<20% TBA*	20-50% of TBA	>50% of TBA
Vasculitis (urticaria, palpable purpura, livedo reticularis and ulcers)	-ve	<20% TBA	20-50% of TBA	>50% of TBA
Neuromotor				
Strocke syndrome		Single TIA	Mltiple TIA/RIND, mononeuritis multiplex, cranial neuropathy or chorea	CVA, Myelitis or retinal vascular occlusion
Seizure		1-2/month	>12/month	Status epiliepticum
Myalgia/Myositis		Complaint	Limits same activity	Incapitating
Cortical dysfunction		Mild depression Personality disorder or cognitive deficit	Altered sensorium, sever depression or impaired cognation	Psychosis or servere severe dementia or coma
Joint pain		Arthralgia only	Objective inflammation	Limited function
Laboratory				
ESR	<25	25-50	51-70	>75
Serum creatinine (mg dL^{-1})	0.5-1.3	1.4-2	2.1-4	>4
Or creatinine clearance	80-100%	76-60%	30-60%	<30%
Urine sediment				
RBCs and or WBCs/HPF		>5	>10	>25
casts/HPF		0 to 1-3	>3	Red cell casts
Proteins and or 24 h proteins		1-2+ and or <0.5 g L^{-1}	3-4+ and or 3-5 g L^{-1}	>4+ and or >3.5 g L^{-1}

Liang *et al.* (1989); *TBA: Total body area

Laboratory assessment: In SLE patients, the following laboratory parameters were done: complete Blood Cell Count (CBC), Erythrocyte Sedimentation Rate (ESR), urine analysis, 24 h urinary proteins, serum creatinine, alamine aminotransferase (ALT), asparate aminotransferase (AST), bilirubin, Partial Thromboplastin Time (PTT), complement level (C3), Anti-Nuclear Antibodies (ANA), anti-cardiolipin antibodies and Lupus anticoagulant assay (Kikuchi *et al*., 1998).

VEGF determination: Venous blood sample (5 mL) was obtained from all SLE patients and controls and collected in pyrogen-free tubes and allowed to clot at 4°C for one hour then centrifuged for 10 min. The obtained sera were allocated into separate vials and stored at -25°C until assayed for VEGF. The measurement of VEGF levels was performed using ELISA sandwich kits employing human anti-VEGF antibodies (R and D system Inc, Minneapolis, USA), using horseradish peroxidase detection in accordance with the manufacturer's instructions. The absorption was read at 492 nanometer. The appropriate recombinant human cytokine was used in each assay to generate the standard curve. Standards as well as samples were assayed as duplicates and the interassay variations were shown to be within the range given by the manufacture. Assay sensitivity was 9. 0 pg mL^{-1} for VEGF (Dirix *et al*., 1997).

Statistical analysis: Statistical analysis was performed using software (SPSS 10.0; SPSS: Chicago, IL). All results are expressed in Means±SD. The mean values were compared among study groups using student's t test or Mann-Whitney test, according to whether the corresponding values followed a normal distribution or not, as tested by kolmogorov-Smirnov test. Linear regression analysis was used to investigate the potential relationships between variables. $p<0.05$ was considered statistically significant.

RESULTS

Group (I) comprised of 23 patients with SLE, 16 of them were diagnosed as having active and 7 with inactive disease. The mean duration of the disease was 47±6.5 months. The VEGF was detectable in all SLE patients and in all controls. The serum VEGF level was significantly high in group I (mean 295.4±167 pg mL^{-1}) compared to control group (mean = 118.2±46 pg mL^{-1}) with the $p<0.004$. Again, patients with active systemic lupus (group Ia) showed significantly high serum VEGF (Mean = 319.8±190 pg mL^{-1}), while patients with inactive systemic lupus (group Ib) showed serum VEGF values near that of control group (mean = 132±117.4 pg mL^{-1}) with statistically significant difference between group Ia and Ib ($p<0.001$). However, there was no statistically

Table 2: VEGF in different groups

Group	No. of cases	Serum VEGF pg mL^{-1}	p-value
I	23	295.4±167	<0.004 (groupI and II)
II	25	118.2± 46	
Ia	16	319.8±190	<0.001 (group Ia and Ib)
IIb	7	132±117.4	NS (group Ib and II)

NS: Not significant

Table 3: VEGF in various clinical catigogries

		Serum VEGF (pg mL^{-1})		
Variablea	No. of cases (+ve/-ve for the variable)	+ve cases	-ve cases	p-value
Renal involvement	6/17	286.3±144	201.8±122.5	<0.003
Mucocutanous involvement	Moderate-sever: 6/no-mild:17	221.0±159	192.0±134	NS
Joint involvement	11/12	254.0±127	236.0±153	NS
Neurological involvement	4/19	278.0±196	243.0±164	NS

NS: Not significant

Table 4: Significant correlation of VEGF with laboratory parameters

	VEGF	
Variables	r	p-value
Platlet count	-0.51	<0.05
Complement (C3)	-0.54	<0.10
ESR	0.49	<0.01
SLAM score	0.67	<0.04

significant difference between VEGF level in group Ib and controls (p>0.05) (Table 2).

The correlations between VEGF serum level and some selected clinical and laboratory parameters were investigated. Patients with active renal disease had a significantly higher VEGF levels (mean = 286.3±144 pg mL^{-1}) compared to patients with no renal involvement (mean = 201. 8±122.5 pg mL^{-1}) with p<0.03. Concerning mucocutanous involvement, the VEGF mean serum level was higher in patients with moderate to sever skin disease (mean 221±156 pg mL^{-1}) compared to its serum levels in patients with no or mild skin disease (mean 192±134 pg mL^{-1}) but this difference was statistically insignificant (p>0.05). Also, there were no statistically significant differences in the VEGF mean levels in SLE patients with and without arthritis (mean 254±127 verses 236±153 pg mL), patients with or without neurological affection (mean 278±196 verses 243±164 pg mL) and patients receiving prednisone alone or with cyclophosphamide (mean 209±128 vs. 237±107 pg mL^{-1}) with p>0.05. Table 3 demonstrates the relation of VEGF to some clinical features of SLE.

A negative correlation was detected between VEGF mean level and platelet count (r = -0.51, p<0.05), also VEGF mean level was negatively correlated with C3 (r = -0.54, p<0.01). On the other hand, a positive correlation was found between VEGF and ESR (r = 0.49, p<0.01) and with lupus severity as expressed by (SLAM) scoring system (r = 0.67, p<0.04). However, there was no significant correlation between VEGF level and WBCs (r = 0.23, p>0.05), ANA (r = 0.19, p>0.05). Also, there was no

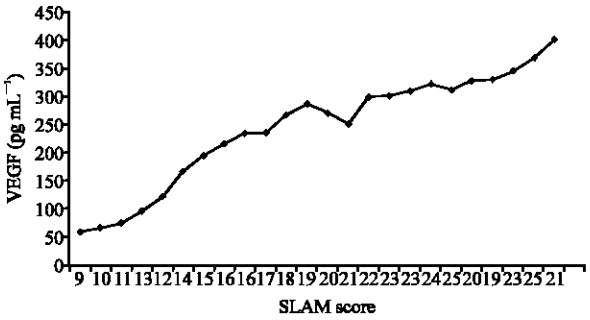

Fig. 1: The possitive correlation between VEGF and SLAM score

significant correlation with the age of the patients or the mean disease duration (r = 0.9, p>0.05 and r = 0.15, p>0.05, respectively). Significant correlation are demonstrated in Table 4. Figure 1 shows possitive correlation between VEGF and SLAM score.

DISCUSSION

The reported levels of VEGF were found in the serum of all examined SLE patients and healthy controls. VEGF was previously evaluated in patients with Rheumatoid Arthritis (RA) and connective tissue diseases. Kikuchi *et al.* (1998) found a significantly higher VEGF levels in patients with polymyositis/ dermatomyositis and RA compared to control group, however in SLE patients, the level was similar to that in healthy volunteers. On the other hand, Harada *et al.* (1998) reported higher levels of VEGF in SLE patients in comparison to healthy persons.

The results of this study showed a higher serum levels of this cytokine in children and adolescents with SLE compared to control group. Further more, a significantly higher VEGF was found in active lupus compared with inactive lupus. This profoundly higher

concentration of VEGF in active SLE patients detected here, is supported by the outcome of a study conducted on 47 SLE patients and 30 controls, which concluded that VEGF may be a useful marker of disease activity and internal organs involvement in SLE patients (Kuryliszyn-Moskal *et al*., 2007).

Also, in this study, the VEGF level was significantly higher in SLE patients with active renal disease compared to those without renal involvement. This finding is supported by another study conducted on 25 SLE patients in the same age group, which concluded that these higher levels of VEGF in active renal SLE patients were also positively correlated with the severity of renal involvement (Heshmat and El-Kerdany, 2007). Normally VEGF mRNA and/or protein were detected predominantly in glomerular podocytes, distal tubules, collecting ducts and to a lesser extent in proximal tubules (Kang *et al*., 2001). Moreover, VEGFR-1 and VEGFR-2 are predominantly expressed on pre-glomerular, glomerular and peri-tubular endothelial cells (Thomas *et al*., 2000). Given the role of VEGF in promoting micro vascular permeability, it has been speculated that the strongly expressed VEGF by visceral epithelial cells may regulate glomerular permeability, also it acts as an autocrine factor on calcium homeostasis and cell survival in human podocytes (Eremina *et al*., 2003). The pathological reduction of VEGF-expressing cells due to epithelial damage or destruction in diffuse endocapillary proliferative glomerulonephritis associated with SLE, will leads to local release of large amounts of VEGF, resulting in increase glomerular permeability (Honkanen *et al*., 2000).

It has been shown that angiogenesis is also involved in the skin lesions of SLE. Serum levels of VEGF are higher in patients with vasculitis and other skin lesions in SLE especially with immunoglobulin deposits at the dermal-epidermal junction (Robak *et al*., 2003). In this study, the mean VEGF serum levels were higher in SLE patients with moderate to sever skin lesions, however, when compared to patients without skin involvement or patients with mild disease, the correlation was statistically insignificant. Also, no correlation was reported between VEGF level and neurological manifestations in our SLE patients.

It has been postulated that VEGF is involved in the pathogenesis of synovitis in RA, being the hyperplastic synovial pannus behaves like a solid tumour with high vascularity.

In this study as earlier reported, there was no statistically significant difference in VEGF levels in SLE patients with and without active arthritis. This finding could be explained by the non-erosive patteren of Joints affection in SLE (Edworthly, 2001).

The statistical analysis of data showed a negative correlation between VEGF levels in SLE patients and the blood platelet count. A similar correlation was found by Robak *et al*. (2003) in SLE patients and by Di Raimondo *et al*. (2000) in patients with multiple myeloma. It seems that this correlation is connected with VEGF accumulation in platelets and megakaryocytes.

Also, a negative correlation between VEGF and C3 was reported in this study. Furthermore, there was a positive correlation between VEGF level and ESR in SLE patients. Similar observation was made by Harada *et al*. (1998) in patients with RA. Finally, we observed a strong positive correlation between VEGF levels and SLE activity system (SLAM), this was also reported by Carvalho *et al*. (2007), who concluded that serum levels of VEGF correlate with disease activity in a large number of autoimmune diseases and fall with the use of standard therapy.

CONCLUSION AND RECOMMENDATIOS

VEGF serum levels are increased in children and adolescents with SLE with positive correlation with disease activity, suggesting that it may be used as a useful marker for SLE activity. Further studies are needed for better understanding its role in pathogenesis of the disease and so can provide a novel approach for SLE managements.

REFERENCES

Bader-Meunier, B., J.B. Armengaud, E. Hadad, R. Salomon and G. Deschenes *et al*., 2005. Initial presentation of childhoodonset systemic lupus erythematosus: A French Multicenter study. J. Pediatr., 146: 648-653.

Brunner, H.I., D.D. Gladman, D. Ibañez, M.D. Urowitz and E.D. Silverman, 2008. Difference in disease features between childhood onset and adult - onset Systemic lupus Erythematosus. Arthritis Rheum, 58: 556-562.

Carvalho, J. F., M. Blank and Y. Shoenfeld, 2007. Vascular endothelial growth factor in autoimmune diseases. J. clini. Immunol., 27: 246-256.

Di Raimondo, F., M.P. Azzaro and G.A. Palumbo *et al*., 2000. Angiogenic factors in multiple myeloma. Higher levels in bone marrow than in peripheral blood. Haematologica, 85: 800-805.

Dirix, L.Y., P.B. Vermeulen and A. Pawinski *et al*., 1997. Elevated levels of the angiogenic cytokines, basic fibroblast growth factor and vascular endothelial growth factor in sera of cancer patients. Br. J. Cancer, 76: 238-243.

Edworthly, S.M., 2001. Clinical Manifestations of Systemic Lupus Erythematosus. In: Kelley's Textbook of Rheumatology, Ruddy, S., E.D. Harris, C.B. Sledge, R.C. Budd and J.S. Sergent (Eds.), 6th Edn., WB Saunders., Philadelphia, pp: 1105-1123.

Eremina, V., M. Sood, J. Haigh, A. Nagy and G. Lajoie *et al*., 2003. Glomerular specific alterations of VEGF. A expression lead to distinct congenital and acquired renal diseases. J. Clin. Invest., 111: 707-716.

Ferrara, N. and H.P. Gerber, 2001. The role of vascular endothelial growth factor in angiogenesis. Acta Haematol., 106: 148-156.

Font, J., R. Cervera, G. Espinosa, L. Pallarés, M. Ramos-Casals, S. Jiménez, M. García-Carrasco, L. Seisdedos and M. Ingelmo, 1998. Systemic lupus erythematosus (SLE) in childhood: Analysis of clinical and immunological findings in 34 patients and comparison with SLE characteristics in adults. Ann. Rheum. Dis., 57: 456-459.

Harada, M., K. Mitsuyama, H. Yoshida, S. Sakisaka and E. Taniguchi *et al*., 1998. Vascular endothelial growth factor in patients with rheumatoid arthritis. Scand. J. Rheumatol., 27: 377-380.

Heshmat, N.M. and T.H. El-Kerdany, 2007. Serum levels of vascular endothelial growth factor in children and adolescents with systemic lupus erythematosus. Pediatr Allergy Immunol., 18: 346-353.

Honkanen, E. O., A.M. Teppo, C. Grönhagen-Riska, 2000. Decreased uminary excretion of vascular endothelial growth factor in idiopathic membranous glomerulonephritis. Kidney Int., 57: 2243-2249.

Hood, J.D., M. Ziche, C.J. Meininger, Z. Marina and J.G. Harris, 1998. VEGF upregulation of ec Nos message, protein and NO production in human endothelial cells. Am. J. Physiol., 274: H1054-H1058.

Kang, D.H., S. Anderson, Y.G. Kim, M. Mazzalli and S. Suga *et al*., 2001. Impaired angiogenesis in the aging kidney. Vascular endothelial growth factor and thrombospondin-1 in renal disease. Am. J. kidney Dis., 37: 601-611.

Kikuchi, K., M. Kubo, T. Kadono, N. Yazawa, H. IHN and K. Tamaki, 1998. Serum concentration of vascular endothelial growth factor in collagen disease. Br. J. Dermatol., 139: 1049-1051.

Kuryliszyn-Moskal, A., P.A. Klimiuk, S. Sierakowski and M. Cio³kiewicz, 2007. Vascular endothelial growth factor in systemic lupus erythematosus: Relationship to disease activity, systemic organ manifestations and nailfold capillaroscopic abnormalities. Archivum Immunolo. et Therapiae Experimentalis, 55: 179-185.

Li, Y., T. Marco, N. Sonali, V.B. Elena, S.S. Eric, S.S. Mark and B.R. Hanno, 2006. Urinary biomarkers in lupus nephritis. Autoimmun Rev., 5: 383-388.

Liang, M.H., S.A. Socher, M.G. Larson and P.H. Schur, 1989. Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. Arthritis Rheum., 32: 1107-1118.

Moradinejad, M.H., G.R. Zamani, A.R. Kiani and T. Esfahani, 2008. Clinical features of Juvenile lupus erythematosus in Iranian children. Acta Reumatol. Port, 33: 63-67.

Neufeld, G., T. Cohn and Z. Poltorak, 1999. Vascular endothelial growth factor (VEGF) and its receptors. FASEB. J., 13: 9-22.

Rahman, A. and D.A. Isenberg, 2008. Review article: Systemic lupus erythematosus. N Engl. J. Med., 358: 929-939.

Robak, E., A. Sysa-Jedrzejewska and T. Robak, 2003. Vascular endothelial growth factor and its soluble receptors VEGFR-1 and VEGFR-2 in the serum of patients with systemic lupus erythematosus. Mediators Inflamm., 12: 293-298.

Sezer, O., C. Jakob, J. Eucker, K. Niemöller, F. Gatz, K. Wernecke and K. Possinger, 2001. Serum levels of the angiogenic cytokines basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in multiple myeloma. Eur. J. Haematol., 66: 83-88.

Talks, K.L. and A.I. Harris, 2000. Current status of angiogenic factors (Review). Br. J. Haematol., 109: 477-489.

Tan, E.M., A.S. Cohen, J.F. Fries, A.T. Masi, D.J. Mcshane, N.F. Rothfield, J.G. Schaller, N. Talal and R.J. Winchester, 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum., 25: 1271-1277.

Thomas, S., J. Vanuystel, G. Gruden, V. Rodriguez, D. Burt, L. Gnudi, B. Hartley and G. Viberti, 2000. Vascular endothelial growth factor receptors in human mesangium in vitro and in glomerular disease. J. Am. Soc. Nephrol., 11: 1236-1243.

Vilá, L.M., G.S. Alarcón, G. McGwin Jr, A.W. Friedman and B.A. Baethge *et al*., 2004. Early clinical manifestations, disease activity and damage of systemic lupus erythematosus among two distinct US Hispanic subpopulations. Rheumatology, 43: 358-363.