Serum levels of vascular endothelial growth factor in children and adolescents with systemic lupus erythematosus


The angiogenic cytokine vascular endothelial growth factor (VEGF) may have a role in the pathogenesis of collagen diseases. We aimed to assess its serum levels in children and adolescents with systemic lupus erythematosus (SLE) and to elucidate its correlation with clinical features, laboratory parameters, and the overall disease activity. This study comprised 25 children and adolescents with SLE and 30 healthy controls. Disease activity was evaluated by SLE disease activity index (SLEDAI) score. Laboratory investigations included complete blood count, erythrocyte sedimentation rate (ESR), urine analysis, 24-h total urinary protein, assay of serum creatinine, ANA, anti-DNA, complement component C3, lupus anticoagulant, and VEGF. Serum levels of VEGF were significantly increased in SLE patients (579.5 ± 184.7 pg/ml) when compared with controls (113.2 ± 30.8 pg/ml) (p < 0.0001). VEGF serum levels were significantly increased in patients having renal involvement and neurologic symptoms than those who did not have them (p < 0.0001, p < 0.005, respectively). Serum levels of VEGF were higher in patients with antiphospholipid syndrome, vasculitis, and skin symptoms than those without, but the difference did not reach statistical significance. Meanwhile, they were similar in patients with and without arthritis (p > 0.05). VEGF serum levels were not correlated to age; inversely correlated to platelet count, serum C3 level; and positively correlated to ESR. SLEDAI score was positively correlated to VEGF serum level (r = 0.86, p < 0.0001). VEGF may be relevant to SLE pathogenesis. Its concentration seems to be a marker of SLE activity, which could help in disease monitoring and planning of treatment.

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is both a potent enhancer of microvascular permeability and a selective endothelial cell growth factor (1–4). It is a key regulator of vasculogenesis and angiogenesis both in normal and pathological conditions. It is produced by endothelial cells, macrophages, fibroblasts, and smooth muscle cells (5, 6). It is a chimeric glycoprotein with a molecular weight of 34–45 kDa, consisting of two subunits. Five isoforms of human VEGF have been described, each generated by alternative splicing of a single mRNA and resulting in proteins of varying amino acid lengths (VEGF)\textsuperscript{121,145,165,189,206}. VEGF interacts with at least two specific tyrosine kinase receptor proteins found in endothelial cells termed VEGF receptor 1 (VEGFR-1) and VEGFR-2 (7). VEGF plays an important role in the increased permeability and angiogenesis associated with
malignancy (8–11), wound healing (12), and certain inflammatory conditions (13–15).

Dysregulated VEGF expression has been implicated in a number of pathological situations, including tumor growth and metastasis (16), diabetic retinopathy (17), glomerular disease (18, 19), and primary antiphospholipid syndrome (APS) (20).

Angiogenic cytokines and angiogenesis inhibitors play an important role in the pathogenesis of several diseases, especially in neoplastic tumor growth (21, 22). Their importance in the pathogenesis and influence on the course of connective tissue disease has been less intensively investigated than in neoplastic diseases. Detectable levels of the angiogenic basic fibroblast growth factor have been found in the serum of patients with systemic lupus erythematosus (SLE) and dermatomyositis (23).

VEGF has been found elevated in serum and synovial fluid of patients with rheumatoid arthritis (RA) (24–26). Very few studies had investigated the role of VEGF in adult patients with SLE and they gave inconsistent results (27, 28). To the best of our knowledge, the serum levels of VEGF in children and adolescents with SLE have not been investigated to date.

The objective of this study was to investigate serum levels of VEGF in children and adolescents having SLE. In addition, we aimed to investigate its association with SLE activity as well as the clinical and laboratory characteristics of SLE patients particularly lupus nephritis and APS.

Methods

Patients and controls

This study was conducted on 55 children and adolescents, of whom 25 had SLE and 30 were healthy subjects included as a control group. The SLE patients were enrolled consecutively from the Pediatric Allergy and Immunology Unit, Children’s Hospital, Ain Shams University. All of them were diagnosed according to the 1982 American Rheumatism Association Revised Criteria for diagnosis of SLE (29). They were 24 females and one male, and their ages ranged between 9 and 18 yr with a mean age (s.d.) of 14.1 (2.6) yr. The control group comprised 29 females and one male, and their ages ranged between 8 and 18 yr with a mean age (s.d.) of 14.0 (2.5) yr. Informed consent was obtained from the parents or caregivers of each patient or control before enrollment in the study.

All SLE patients were receiving oral corticosteroids in a dose ranging between 0.5 and 2 mg/kg/day or every other day with a maximum dose of 60 mg/day. Eight patients (32%) were receiving non-steroidal anti-inflammatory drugs in the form of ibuprofen. Seven patients (28%) were receiving pulsed intravenous cyclophosphamide in a standard protocol of 600 mg/m² monthly for 7 months followed by every 3 months for an additional 30 months.

Disease activity was determined in SLE patients by SLE disease activity index (SLEDAI) score. This index takes into consideration 24 variables representing nine organ systems. Each variable is rated as (present or absent) over 10 days before, and including the day of evaluation. The maximal theoretical score is 105, but in practice few patients have scores greater than 45 (30).

Renal biopsy was undertaken in 16 patients. Eleven patients had WHO class IV nephritis (diffuse proliferative lupus nephritis), two patients had WHO class III nephritis (focal segmental lupus nephritis), two patients had class II-B nephritis, and one patient had class V nephritis (membranous lupus nephritis). According to the clinical and laboratory manifestations of renal disease, patients were categorized into two groups: renal and non-renal SLE groups. Non-renal SLE group included six patients (24%) who had no clinical symptoms of renal disease. All had negative protein dipstick tests, protein in urine <0.2 gm/24 h, and no evidence of microscopic or macroscopic hematuria, pyuria, or urinary casts, and normal serum creatinine concentration. Renal SLE group included 19 patients (76%). They had one or more of the following: protein in urine >0.5 gm/24 h, hematuria, pyuria, urinary casts (red cell, hemoglobin, granular, tubular, or mixed casts), and/or abnormal serum creatinine concentrations. In calculation of SLEDAI score, the following four variables representing renal affection are considered:

1. Urinary casts (heme-granular or red blood cell casts).
2. Hematuria (>5 red blood cells/high power field after exclusion of stones, infection, or other cause).
3. Proteinuria (>0.5 gm/24 h).
4. Pyuria (>5 white blood cells/high power field after exclusion of infection).

The presence of each of the aforementioned variables is given a weight of 4. To assess the degree of renal affection in each patient, we calculated the sum of the weight of those four
variables and we considered it as a renal score. The range of the renal score in our series was 0–16 with a mean (s.d.) of 7.0 (5.3).

Nine of the studied SLE patients (36%) had neurologic manifestations in the form of cognitive dysfunction, severe anxiety, psychosis, organic brain syndrome, or optic neuropathy.

Seven of the studied SLE patients (28%) had secondary APS. They were fulfilling the international criteria of APS, i.e., they had at least one proved venous or arterial thrombosis and two positive anticardiolipin antibody assays or lupus anticoagulant (LA) taken at least 6 wk apart (31). All of them were free of clinical arterial and venous thrombosis at the time of the study, and had positive LA assay.

Blood sampling
Venous blood (5 ml) was taken from each patient and separated into three tubes: 2 ml were added to EDTA for complete blood count (CBC) and erythrocyte sedimentation rate (ESR) measurement, the remaining 3 ml were left to clot at room temperature for 30 min and the tube was centrifuged at 1500 rpm for 15 min and serum was separated into two sterile aliquots: one was stored at −20°C till time of assay of VEGF and the other was used for serum creatinine, ANA, anti-DNA, and C3 assay. Venous blood (1.5 ml) was taken from each control subject for VEGF assay.

Study measurements

1. **Complete blood count** was performed with the Coulter counter (Coulter Microdiff 18, Beckman Coulter Inc., Miami, FL, USA).
2. **Erythrocyte sedimentation rate** by Westergren method.
3. **Complete microscopic urine analysis** for WBCs, RBCs, and casts.
4. **Twenty-four-hour urinary total protein** (TP) by the turbidimetric method using TP Kit supplied by Stanbio (Stanbio Laboratory Inc., San Antonio, TX, USA).
5. **Serum creatinine** was carried out on Synchron CX7 autoanalyzer (Beckman Instruments, Brea, CA, USA) by modified rate Jaffe method (32).
6. **Serum ANA and anti-DNA** by indirect immunofluorescence supplied by IMMCO Diagnostics (Buffalo, NY, USA) (33).
7. **Serum complement component C3** by quantitative determination using the turbidimetry (Behringwerke Diagnostics, Marburg, Germany) (34).
8. **Lupus anticoagulant** by diluted Russell’s viper venom test and confirm using kits supplied by American Diagnostica Inc., Greenwich, CT, USA. Test results were reported as positive/negative for LA.
9. **Serum levels of VEGF** in patients and controls by enzyme immunoassay (EIA) using ACCUCYTE® human VEGF EIA kits (Cytimmune Sciences Inc., Rockville, MD, USA). ACCUCYTE human VEGF is a competitive enzyme immunoassay which measures the total (bound and unbound) amount of VEGF. With the ACCUCYTE assay system, pre-coated goat anti-rabbit antibodies are used to capture a specific VEGF complex in which sample consisting of VEGF antibody, biotinylated VEGF, and sample/standard. Biotinylated VEGF conjugate (competitive ligand) and sample or standard from a competition reaction for VEGF specific antibody binding sites. Therefore, as the concentration of VEGF in the sample increases, the amount of biotinylated VEGF captured by the antibody decreases. With the addition of streptavidin-conjugated alkaline phosphatase (which binds only to the biotinylated VEGF) followed by the addition of the color reagent solution, the amount of biotinylated VEGF is detected. The standard curve was plotted on semi-log graph paper. It had a sigmoid shape that showed an inverse relationship between VEGF concentrations and the corresponding optical density (OD) (absorbances). The higher the OD, the less is the VEGF in the sample.

Statistical analysis
The results were analyzed by a commercially available computer software package (StatView, Abacus Concepts Inc, Berkeley, CA, USA). The data are presented as mean and standard deviation. The student’s *t*-test was used to compare between the two groups, as regards parametric data. The correlation between the various numerical parameters was studied by the Pearson correlation coefficient (r) test. Statistical significance was defined as p-value < 0.05. The receiver operating characteristic (ROC) curve analysis was used to get the optimal cut-off point.

Results
The results of this study revealed elevated serum levels of VEGF in SLE patients [range = 335–910 pg/ml, mean (s.d.) = 579.5 ± 184.7 pg/ml]
compared with healthy controls [range = 89–189 pg/ml, mean (s.d.) = 113.2 ± 30.8 pg/ml] \( t = 12.48, p < 0.0001 \) as shown in Fig. 1. The cut-off value of serum VEGF (obtained from the ROC curve) was 189 pg/ml. All the studied SLE patients had VEGF serum levels above this cut-off value.

We investigated the correlation between serum levels of VEGF with selected clinical and laboratory findings of SLE patients. We found that SLE patients having clinical or laboratory evidence of renal involvement (renal SLE patients) had significantly higher serum levels of VEGF [mean (s.d.) = 637.9 (173.0) pg/ml] than non-renal SLE patients [mean (s.d.) = 394.7 (45.6) pg/ml] \( t = 5.55, p < 0.0001 \) as shown in Fig. 2. Moreover, a positive correlation was observed between serum levels of VEGF and the renal score \( r = 0.74, p < 0.0001 \). The mean value of serum VEGF was higher in patients having APS [mean (s.d.) = 664.9 (167.1) pg/ml] than those who did not have it [mean (s.d.) = 546.3 (184.8) pg/ml], but this finding was statistically insignificant \( t = 1.48, p > 0.05 \) as shown in Fig. 3.

Table 1 shows VEGF serum levels of SLE patients according to their clinical and laboratory characteristics. Patients having neurologic manifestations, thrombocytopenia, leucopenia, hypocomplementemia, and positive anti-DNA had significantly higher levels than those who did not have them \( t = 3.28, p < 0.005; t = 3.01, p < 0.05; t = 2.11, p < 0.05; t = 3.53, p < 0.005; t = 2.37, p < 0.05, \) respectively. Mean values of VEGF seemed higher in patients with APS, vasculitis, and skin symptoms than those without, but this finding was statistically insignificant. Meanwhile, they were similar in patients with and without arthritis \( p > 0.05 \). The serum levels of VEGF were comparable in patients receiving cyclophosphamide and those who were not receiving it \( p > 0.05 \).

VEGF serum levels were not correlated to age neither in patients nor in controls \( r = 0.00, p > 0.05; r = 0.20, p > 0.05, \) respectively. Serum levels of VEGF were not correlated to disease duration or WBC count \( r = 0.04, p > 0.05; r = -0.39, p = 0.054, \) respectively; inversely correlated to platelet count and serum levels of C3 \( r = -0.48, p < 0.05; r = -0.62, p < 0.001, \) respectively; and positively correlated to ESR \( r = 0.63, p < 0.001 \). A strong positive correlation was observed between the SLEDAI score and serum levels of VEGF \( r = 0.86, p < 0.0001 \) (Fig. 4).
Discussion

Vascular conditions such as those occurring in SLE, which include inflammation, vessel occlusion or thickening of the vascular wall, might be a strong stimulus for angiogenic factor production (5, 35, 36). So, we analyzed serum levels of VEGF in children and adolescents with SLE. We found elevated levels of VEGF in SLE patients when compared with healthy controls. Very few studies were conducted on adults to assess serum VEGF in SLE, and they gave inconsistent results. Some investigators reported results similar to ours (28, 37, 38), whereas others observed comparable serum VEGF values in SLE patients and controls (27). In addition, we tried to investigate the association between VEGF and clinical and laboratory findings, as well as SLE activity.

Our results revealed increased levels of serum VEGF in SLE patients with renal involvement than those without and it was positively correlated to severity of this involvement. Avihingsanon et al. reported that urinary VEGF mRNA levels are increased in active class IV nephritis and are decreased with the response to treatment (39). In normal human kidney, VEGF mRNA, and protein are predominantly strongly expressed by visceral glomerular epithelial cells (podocytes), where its physiological function and role in development of renal disease are obscure and uncertain (40–42). In many glomerular diseases, VEGF expressing cells were decreased in number or absent in areas of focal or global glomerular sclerosis. Decreased numbers of VEGF-expressing cells in glomeruli were also noted in amyloidosis, diabetes, crescentic glomerulonephritis, and diffuse endocapillary proliferative glomerulonephritis associated with SLE. The decrease in the number of VEGF-expressing cells noted in glomerular diseases is likely a result of epithelial damage or destruction. Normally, release of VEGF must be under strict control because it is some 50,000 times more potent than histamine as an inducer of microvascular per-

### Table 1. Serum levels of VEGF in SLE patients according to their clinical and laboratory characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%) of patients having the variable</th>
<th>In SLE patients having the variable (mean ± s.d.)</th>
<th>In SLE patients not having the variable (mean ± s.d.)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurologic symptoms</td>
<td>9 (36)</td>
<td>715.9 ± 197.9</td>
<td>502.8 ± 127.6</td>
<td>3.28</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>4 (16)</td>
<td>690.5 ± 22.1</td>
<td>558.4 ± 175.2</td>
<td>1.33</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Arthritis</td>
<td>13 (52)</td>
<td>590.1 ± 180.6</td>
<td>568.1 ± 196.5</td>
<td>0.29</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Myositis</td>
<td>4 (16)</td>
<td>671.0 ± 254.7</td>
<td>562.1 ± 171.0</td>
<td>1.08</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>19 (76)</td>
<td>637.9 ± 173.0</td>
<td>394.7 ± 45.6</td>
<td>5.55</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Skin symptoms</td>
<td>18 (72)</td>
<td>670.3 ± 165.9</td>
<td>544.2 ± 183.7</td>
<td>1.58</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Serositis</td>
<td>5 (20)</td>
<td>634.8 ± 260.6</td>
<td>565.7 ± 168.2</td>
<td>0.75</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RES involvement</td>
<td>7 (28)</td>
<td>692.1 ± 138.6</td>
<td>535.7 ± 194.7</td>
<td>2.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Anemia</td>
<td>15 (60)</td>
<td>625.5 ± 187.1</td>
<td>510.5 ± 166.5</td>
<td>1.57</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>7 (28)</td>
<td>733.9 ± 148.3</td>
<td>519.5 ± 163.7</td>
<td>3.01</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>7 (28)</td>
<td>696.4 ± 197.1</td>
<td>534.1 ± 163.2</td>
<td>2.11</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Low C3</td>
<td>11 (44)</td>
<td>706.2 ± 193.1</td>
<td>480.0 ± 100.5</td>
<td>3.53</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>Positive anti-DNA</td>
<td>18 (72)</td>
<td>617.9 ± 199.5</td>
<td>480.7 ± 88.9</td>
<td>2.37</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Secondary APS</td>
<td>7 (28)</td>
<td>684.9 ± 167.1</td>
<td>546.3 ± 184.8</td>
<td>1.48</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Treatment with CYC</td>
<td>7 (28)</td>
<td>573.9 ± 206.1</td>
<td>581.7 ± 182.1</td>
<td>0.09</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Anemia, hemoglobin level <11 gm/dl; thrombocytopenia, platelet count <150 × 10^9/mm³; leucopenia, white blood cell count <3.5 × 10³/mm³; low C3, low serum complement component C3 <55 mg/dl; RES, reticuloendothelial system; APS, antiphospholipid syndrome; CYC, cyclophosphamide.

*Highly significant difference.
†Significant difference.
meability. Damage of visceral epithelial cells in a variety of glomerular diseases has the potential for releasing relatively large amounts of VEGF locally, leading to increased glomerular permeability and alterations. In addition, loss of normal controlled secretion of VEGF after damage to visceral epithelial cells could lead to important alteration in glomerular endothelial cell function (41).

VEGF was found to mediate glomerular endothelial repair promoting healing from glomerular injury and it was suggested as a novel therapeutic approach to glomerular diseases characterized by endothelial damage, such as various glomerulonephritides and renal transplant rejection (43–45). In SLE renal tissue samples, immunostaining showed a strong expression of VEGF (37). This observation, in addition to the increased serum VEGF detected in our renal SLE patients, makes it possible that this angiogenic factor is related to endothelial repair in SLE. However, the role of VEGF in pathogenesis of lupus nephritis or in its healing is in need of further studies to be elucidated.

We observed increased VEGF serum levels in SLE patients with APS when compared with those without; however, this finding was statistically insignificant. The mechanism underlying the prothrombotic tendency of APS has not been elucidated. Numerous procoagulant mechanisms have been tested including soluble tissue factor (sTF). Raised plasma levels of sTF have been shown in primary APS (46, 47), although its origin remains unclear. VEGF has been demonstrated to cause up-regulation of TF expression (48). It has been suggested that circulating antiphospholipid antibodies (aPL) promote VEGF release leading to TF expression and the initiation of coagulation (20). The absence of evidence of arterial and venous thrombosis at the time of the study in our SLE patients with secondary APS could explain that the increase in their VEGF did not reach statistical significance.

Four patients in our series had evidence of vasculitis in the form of ulceration, gangrene, tender finger nodules, livedo reticularis, periangual infarction, and/or splinter hemorrhage. Eighteen SLE patients had other skin symptoms in the form of malar rash or discoid rash. Serum levels of VEGF were higher in patients with vasculitis and other skin symptoms than those without. This was in line with what was previously observed concerning increased VEGF serum levels in SLE patients with immunoglobulin deposit at the dermal–epidermal junction (positive lupus band test) (28, 38). VEGF serum levels were increased in SLE patients with reticuloendothelial system (RES) involvement, especially lymphadenopathy and/or hepatosplenomegaly than those without; however, the difference did not reach statistical significance.

Several investigators reported involvement of VEGF in the pathogenesis of synovitis in RA. The hyperplastic synovial pannus in RA behaves like a solid tumor, because it is rich in blood vessels, invades the joint, and destroys cartilage and bone (49). VEGF was demonstrated to be increased in serum and synovial fluid of RA patients and it was correlated to disease activity. VEGF-positive cells existed in synovial tissue in patients with RA (50–52). Our results revealed similar values of VEGF in SLE patients with and without arthritis similar to what was previously reported (28, 38). This could be explained by the fact that arthritis in SLE is usually non-erosive (53).

The current study showed increased VEGF serum levels in patients having thrombocytopenia, leucopenia, and hypocomplementenemia which occur with SLE activity.

In our series, we observed a strong positive correlation between VEGF serum levels and the SLEDAI score. Similar results were reported by a previous study in which systemic lupus activity measure (54) was used instead of SLEDAI to assess SLE activity.

In conclusion, VEGF serum levels were increased in SLE patients. It was significantly higher in patients with neurologic symptoms, renal involvement, thrombocytopenia, and hypocomplementenemia than those without. Serum levels of VEGF were higher in patients having APS, vasculitis, skin symptoms, and RES involvement than those without, but the difference did not reach statistical significance, and they were comparable in patients with and without arthritis. As each patient could have more than one of the aforementioned findings, it is difficult to assess the effect of each finding separately on VEGF. However, a very strong positive correlation was demonstrated between serum levels of VEGF and the SLEDAI score, suggesting it as a serologic marker to monitor SLE disease activity.

More studies to explore the role of angiogenic and antiangiogenic factors in the pathogenesis of SLE, particularly renal disease, are recommended. Better understanding of the pathogenesis may provide novel approach for treating SLE patients, especially cases refractory to conventional therapy.
References


