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

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Abstract

Background: Angiogenesis plays an important role in the pathogenesis and progression of collagen diseases. Vascular endothelial growth factor (VEGF) is a potent stimulating factor for angiogenesis and vascular permeability.

Aim of Work: To assess for changes of VEGF in lupus patients in childhood and adolescents and its relation to lupus activity and to demonstrate the possible correlation between the serum level of VEGF and certain clinical and laboratory parameters and disease activity score.

Subjects and Methods: We investigated the serum concentration of VEGF using an enzyme-linked immunosorbent assay (ELISA) in a group of 35 children and adolescents with systemic lupus erythematosus (SLE) and 25 healthy controls. All cases were recruited from Kasr El Aini Hospital.

Results: 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 than those with no renal involvement (p<0.003). It was also higher in SLE patients with moderate to severe skin disease neurological and joint involvement compared with patients with no or mild skin disease, no neurological and no joint involvement respectively 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), on the other hand VEGF was negatively correlated to platelets count and complement level (C3) (p<0.05 and <0.01 respectively).

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 – Systemic lupus erythematosus.

Introduction

THE angiogenic process plays a major role in the development of vascular supply in some pathological diseases including neoplastic and collagen diseases [1]. A family of pro-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) [2].

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 [3]. 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 [4]. Moreover, VEGF supports vascular survival by preventing vascular apoptosis [8], induces plasminogen activator, plasminogen activator inhibitor-1 and interstitial collagenase, factors important in matrix remodelling. Also, it promotes monocytes chemotaxis and expression of adhesion molecules [5].

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by immune dysregulation resulting in the production of antinuclear 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 [6].

**Aim of the work:**

The importance of angiogenic cytokines in the pathogenesis of collagen diseases and their clinical significance in these diseases, has been less intensively investigated than in neoplastic diseases. So, 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.

**Methodology**

**Study population:**

The current study included 60 subjects, classified into two main groups. Group I consisted of 35 patients (31 females and 4 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) [7]. Group II included 25 sex-and age-matched healthy controls. The patients with SLE and controls showed no 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 detailed physical examination. Verbal consent was taken from all participants.

**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 [8]. 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. A manifestation or symptom is determined to be either active or not active. 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 [8].

In our 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 26 patients with active SLE and group (Ib) contained 9 patients with inactive disease.

In the view of SLAM score, assessment of patients for serum creatinine, creatinine clearance, proteinurea, hematuria (after exclusion of calculi or other causes), pyuria (after exclusion of infection) urinary casts (red cells, tubular, granular or mixed) 10 patients were considered to have active renal disease and subjected to renal biopsy to verify the type of nephritis. 3 patients had WHO class III nephritis (focal segmental lupus nephritis), 4 patients had WHO class IV nephritis (diffuse proliferative lupus nephritis) and 3 patients had WHO class V nephritis (membranous nephritis).

Neurological evaluation of SLE patients showed that only 6 patients had symptoms more specific for SLE, including CVA, seizures, depression and psychosis. However, other minor symptoms elicited in other patients such as headache, poor concentration and mood swings, were considered to be difficult to be differentiated from other coexistent conditions. Mucocutanous involvement was affecting 22 SLE patients, of them only 15 patients had mild skin disease (Score of skin affection up to 3), while 7 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 0.5-2mg/Kg/day or every other day with a maximum dose of 60mg/day during the course of the disease. Of them only 23 patients had receiving he drug during blood sampling and 7 dt patients were receiving combined therapy with prednisone and cyclophosphamide. The remaining 5 SLE patients had treated only
with non-steroidal anti-inflammatory drugs, with no history of therapy with steroids or other immunosuppressive agents.

**Laboratory assessment:**

In SLE patients, the following laboratory parameters were analyzed: Complete blood cell count (CBC), erythrocyte sedimentation rate (ESR), urine analysis, 24hs urinary proteins, serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, partial thromboplastin time (PTT), complement level (C3), antinuclear antibodies (ANA), anti-cardiolipin antibodies and Lupus anticoagulant assay.

**VEGF determination:**

Venous blood sample (5ml) was obtained from all SLE patients and controls and collected in pyrogen-free tubes and allowed to clot at 4ºC for one hour then centrifuged for 10 minutes. The obtained sera were allocated into separate vials and stored at –25ºC until assayed for VEGF. The measurement of VEGF levels was performed using ELISA sandwich kits employing human anti-VEGF antibodies (R & 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 9.0pg/ml for VEGF.

**Statistical analysis:**

Statistical analysis was performed using software (SPSS 10.0; SPSS: Chicago, IL). All results are expressed in means ± standard deviation (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 value <0.05 was considered statistically significant.

**Results**

In group (I) 35 patients with SLE, 26 were diagnosed as having active Vs 9 with inactive disease. The mean duration of the disease was 47±6.5 months. VEGF was detectable in all SLE patients and in all controls. The serum VEGF level was significantly high in group I (mean 295.4±167pg/ml) compared to control group (mean=118.2±46pg/ml) with the p value <0.004. Again, patients with active systemic lupus (group Ia) showed significantly high serum VEGF (mean=319.8±190pg/ml), while patients with inactive systemic lupus (group Ib) showed serum VEGF values near that of control group (mean=132±117.4pg/ml) with statistically significant difference between group Ia and Ib (p value <0.001). However, there was no statistically significant difference between VEGF level in group Ib and controls (p value >0.05). The results are presented in Table (1) & Fig. (1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of cases</th>
<th>Serum VEGF pg/ml</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (I)</td>
<td>35</td>
<td>295.4±167</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Group (II)</td>
<td>25</td>
<td>118.2±46</td>
<td></td>
</tr>
<tr>
<td>Group (Ia)</td>
<td>26</td>
<td>319.8±190</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group (Ib)</td>
<td>9</td>
<td>132±117.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

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±144pg/ml) compared to patients with no renal involvement (mean=201.8±122.5pg/ml) with p value <0.03. Concerning mucocutaneous involvement, the VEGF mean serum level was higher in patients with moderate to sever skin disease (mean 221±156pg/ml) compared to its serum levels in patients with no or mild skin disease (mean=192±134pg/ml) but this difference was statistically insignificant (p values >0.05). Also, there were no statistically significant differences in the VEGF mean levels in SLE patients with and without arthritis (mean 254±127 Vs 236±153pg/ml), patients with or without neurological affection (mean=278±196 Vs 243±164pg/ml) and
patients receiving prednisone alone or with cyclophosphamide (mean = 209±128 Vs 237±107 pg/ml) with $p$ values >0.05. Table (2) demonstrates the relation of VEGF to some clinical features of SLE.

Table (2): VEGF in various clinical categories.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of cases (+ve/-ve for the variable)</th>
<th>Serum VEGF in pg/ml</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal involvement</td>
<td>10/25</td>
<td>286.3±144</td>
<td>201.8±122.5</td>
</tr>
<tr>
<td>Mucocutaneous involvement</td>
<td>Moderate-sever: 7/no-mild:28</td>
<td>221±159</td>
<td>192±134</td>
</tr>
<tr>
<td>Joint involvement</td>
<td>21/14</td>
<td>254±127</td>
<td>236±153</td>
</tr>
<tr>
<td>Neurological involvement</td>
<td>6/29</td>
<td>278±196</td>
<td>243±164</td>
</tr>
</tbody>
</table>

A negative correlation was detected between VEGF mean level and platelet count ($r$ = –0.51, $p$ value <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, $p$ value <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 significant correlation with the age of the patients or the mean disease duration ($r$ = 0.9, $p$ >0.05 & $r$ = 0.15, $p$ >0.05 respectively). Significant correlation are demonstrated in Table (3).

Table (3): Significant correlation of VEGF with laboratory parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>VEGF</th>
<th>$r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet</td>
<td></td>
<td>–0.51</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Complement C3</td>
<td></td>
<td>–0.54</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>ESR</td>
<td></td>
<td>0.49</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SLAM score</td>
<td></td>
<td>0.67</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

Fig. (2): VEGF in various clinical categories.

Fig. (3): The positive correlation between VEGF and SLAM score.
Discussion

Angiogenic cytokines and angiogenesis inhibitors play an important role in the pathogenesis of several diseases especially in neoplastic tumour growth \[2\]. Some of these angiogenic cytokines may have a prognostic value in cancer patients. For example, raised serum level of VEGF is related to a poor prognosis for patients with non-Hodgkin’s lymphoma, chronic lymphatic leukemia and multiple myeloma \[9\]. In contrast to neoplastic diseases, the significance of angiogenesis, as well as angiogenic cytokines, in the pathogenesis of connective tissue diseases have not been very well investigated.

The aim of our study was to evaluate the concentration of VEGF in the serum of children and adolescents with SLE and assess its correlation to disease activity, selected clinical and laboratory findings. 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. \[10\] 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. \[11\] reported higher levels of VEGF in SLE patients in comparison to healthy persons.

The results of our 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 in our study, 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 \[12\].

In our 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 \[13\]. Normally VEGF mRNA and/or protein were detected predominantly in glomerular podocytes, distal tubules, collecting ducts and to a lesser extent in proximal tubules \[14\]. Moreover, VEGFR-1 and VEGFR-2 are predominantly expressed on pre-glomerular, glomerular and peri-tubular endothelial cells \[15\]. Given the role of VEGF in promoting microvascular 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 \[16\]. 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 \[17\].

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 \[18\]. In this study, the mean VEGF serum levels were higher in SLE patients with moderate to severe 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. VEGF was detected in high levels in the serum and synovial fluid of RA patients and it was correlated with disease activity \[19\]. In our study as previously 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 pattern of Joints affection in SLE \[20\].

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. \[18\] in SLE patients and by Di Raimondo et al. \[21\] 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 our study. Furthermore, there was a positive correlation between VEGF level and ESR in SLE patients. Similar observation was made by Harada et al. \[11\] 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. \[22\], 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 recommendations:

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 management.

References