IMPORTANCE OF MEASURING THE LEVEL OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE SERUM OF PATIENTS WITH RHEUMATOID ARTHRITIS, SCLERODERMA AND SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Background: It is suggested that angiogenic factors such as vascular endothelial growth factor (VEGF) play a role in the pathogenesis of collagen diseases through endothelial cell modulation.

Objective: It was to assess the serum VEGF level in rheumatoid arthritis (RA), systemic sclerosis (SSc) and systemic lupus erythematosus (SLE) patients to elucidate the potential involvement of VEGF in the pathogenesis of these diseases; and determine the correlation between the serum level of VEGF and disease activity or the functional impairment in these patients.

Materials & Methods: The serum level of VEGF was assessed in 10 RA patients, 10 SSc patients, 10 SLE patients and 10 healthy volunteers. Its level was correlated to the different clinical and laboratory parameters of disease activity and functional impairment in the patients.

Results: When compared with the control group, each group of patients showed a significantly higher concentration (p < 0.001) of serum VEGF. A statistically significant correlation was found between this higher concentration and disease activity in RA and SLE patients as well as the development of lung fibrosis in SSc patients.

Conclusion: The results obtained suggested that angiogenesis caused by VEGF may play an important role in the pathogenesis of collagen diseases. Measurement of serum VEGF reflected the disease activity in RA and SLE patients as well as increased frequency of lung fibrosis in SSc patients. Additionally, inhibition of VEGF either by drugs or receptor antagonism may improve the clinical manifestations or decrease the progress of these diseases. However, further studies are needed to elucidate the exact role of VEGF in relation to other cytokines involved in the pathogenesis of collagen diseases.

Keywords: Endothelial growth factor, connective tissue disease etiopathogenesis

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known to up-regulate VEGF expression. These include tumor necrosis factor alpha (TNF-α), transforming growth factor–beta (TGF-β), interleukin–1 (IL-1) as well as hypoxia4–7. On the other hand, VEGF secretion is down-regulated by (IL-4), (IL-10), (anti-TNF-α) and drugs such as dexamethasone, penicillamine and gold sodium thiomolate8–10. Many studies have suggested that VEGF is an angiogenic factor in RA, leading to increased VEGF in the synovial fluids of these patients11, 12. Moreover, a significant increase in basic fibroblast growth factor was observed in the serum of patients with SSc and dermatomyositis. This is another great stimulator of angiogenesis13. These studies have led to the measurement of VEGF in the serum of patients with collagen diseases and evaluation of its clinical importance and correlation with disease activity in these patients.

MATERIALS & METHODS
The study was carried out on 30 patients with different collagen diseases, recruited from the Rheumatology, Dermatology and Internal Medicine outpatient clinics of Ain Shams University hospitals. There were 10 RA patients, 10 SSc patients and 10 SLE patients.

Among RA patients, nine were females and one was a male. Their ages ranged from 20–54 years. They were diagnosed according to the American College of Rheumatology (ACR) 1987 criteria14. SSc patients comprised eight females and two males, with ages ranging from 20–62 years. All fulfilled the preliminary criteria for SSc as proposed by the ACR15. SLE patients included nine females and one male, with ages ranging from 16–54 years. All met ACR’s criteria for diagnosis of SLE16. None of the patients had received corticosteroids or immunosuppressive drugs at the time of sampling.

Ten completely normal individuals (20–52 years old) were also included in the study; these included seven females and three males.

All participants in the study:

1. Underwent a thorough medical history assessment and clinical examination with emphasis on the specific clinical criteria for each disease activity. For RA patients, the specific clinical criteria were scoring for the duration of morning stiffness, visual pain scale, number of affected joints, number of tender joints and number of swollen joints. For SSc patients, the specific clinical criteria were esophageal, heart or joint involvement and lung fibrosis determination via chest X-ray. SLE patients were screened for organ involvement of the disease and relevant data was collected;

2. Were subjected to routine investigation (complete urine analysis, CBC, ESR and X-ray of the chest);

3. Had to take the Rose Waller test to determine rheumatoid factor; and

4. Had to take the C-reactive protein (CRP) test.

Patients with SLE were made to take the Complement 3 (C3) test.

Serum level of VEGF was assessed through specific ELISA kits (Amersham, Bucks, UK, R&D Systems Inc.). This test employs the quantitative enzyme immunoassay technique entailing the following steps.

An antibody specific for VEGF is precoated onto a microplate. Standard, patients’ samples, control and conjugate are pipetted into the wells and any VEGF present is sandwiched by immobilized antibodies and the enzyme-linked antibody specific for VEGF. This is followed by a wash to remove any unbound substrate and/or antibody enzyme reagent. Thereafter, a substrate is added to the wells and color develops in proportion to the quantity of VEGF bound. Development of color is stopped and intensity of the color is measured.

Statistical Analysis
The data was analyzed using the student t-test and ANOVA test.
RESULTS
The study was carried out on 40 subjects divided into four groups:

Group I included 10 patients (nine females and one male) diagnosed with RA based on ACR’s criteria. Their ages ranged from 20–54 years, with a mean ± standard deviation (SD) of 34.2 ± 1. The duration of the disease ranged between 1 year and 15 years, with a mean ± SD of 3.9 ± 4.

Group II comprised 10 patients diagnosed with SSc according to the criteria prescribed by ACR. There were eight females and two males, with ages ranging from 20–62 years; the mean ± SD was 41.3 ± 5. The disease duration ranged between 1.5 years and 18 years, with a mean ± SD of 6.73 ± 8.1.

Group III had 10 patients diagnosed with SLE according to the criteria listed by ACR. There were nine females and one male. Their ages ranged from 16–54 years, with a mean ± SD of 38.7 ± 3.2. The duration of disease ranged from 1 year to 13 years, with a mean ± SD of 8.21 ± 6.4.

Group IV comprised 10 healthy volunteers from the hospital staff. There were seven females and three males, with ages ranging from 20–52 years and a mean ± SD of 29.1 ± 7.

The serum level of VEGF in healthy individuals (group IV) was in the range of 2.75–6.5 pg/ml, with a mean ± SD of 4.22 ± 1.12 pg/ml. For RA patients (group I), the range was 8–24 pg/ml, with a mean ± SD of 15.17 ± 1.7 pg/ml. For SSc patients, it was 10–29.8 pg/ml, with a mean ± SD of 17.57± 3.6 pg/ml. For SLE patients, it was 9.75–23 pg/ml, with a mean ± SD of 17.1 ± 1.05 pg/ml.

Comparison of results indicated that the serum level of VEGF of each group (I, II and III) was significantly higher than that for the control group (IV); p value was <0.001. However, no statistically significant differences were observed when the VEGF serum levels of the first three groups were compared with each other (refer to Table 1).

Table 1. Serum level of VEGF in pg/ml in all groups

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Group I (RA)</th>
<th>Group II (SSc)</th>
<th>Group III (SLE)</th>
<th>Group IV (Controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>20.25</td>
<td>15</td>
<td>4.25</td>
</tr>
<tr>
<td>2</td>
<td>13.5</td>
<td>15</td>
<td>12.2</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>17.75</td>
<td>12.75</td>
<td>4.75</td>
</tr>
<tr>
<td>4</td>
<td>12.25</td>
<td>12.25</td>
<td>15.25</td>
<td>3.25</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>10</td>
<td>23</td>
<td>2.75</td>
</tr>
<tr>
<td>6</td>
<td>13.5</td>
<td>14.25</td>
<td>17.75</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>15.25</td>
<td>9.75</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>15.25</td>
<td>22</td>
<td>24.2</td>
<td>5.25</td>
</tr>
<tr>
<td>9</td>
<td>18.15</td>
<td>19.1</td>
<td>23</td>
<td>6.5</td>
</tr>
<tr>
<td>10</td>
<td>20.2</td>
<td>29.8</td>
<td>18.25</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td>15.17</td>
<td>17.57</td>
<td>17.1</td>
<td>4.57</td>
</tr>
<tr>
<td>SD</td>
<td>1.7</td>
<td>3.6</td>
<td>3.6</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Clinical and laboratory findings were compared with VEGF serum levels. With regard to RA patients, positive correlations were detected between the VEGF serum value and the duration of morning stiffness score, visual pain score, number of affected joints and elevated ESR. However, no correlation was found between VEGF level and age, sex, disease duration, seropositivity and CRP. Also, a positive correlation between serum levels and disease activity was observed. Active RA was defined as the simultaneous presence of at least three of the following conditions:

1. Nine or more tender joints
2. Six or more swollen joints
3. Morning stiffness > 45 minutes
4. ESR >28 minutes in the first hour

The serum level of VEGF in patients with active RA was significantly higher than those with inactive RA [(p < 0.01); refer Table 2].

Table 2. Comparison of serum VEGF levels in active and inactive RA patients

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Active RA patients</th>
<th>Inactive RA patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean serum VEGF level in pg/ml</td>
<td>17.63 ± 7.81</td>
<td>9.41 ± 3.2</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>
In SSc patients, the VEGF serum level showed no correlation with age, sex, disease duration or involvement of associated organs such as esophagus, heart or joints. However, a significant difference (p < 0.05) was observed in the VEGF serum levels of patients with and without lung fibrosis; it was higher for the first group (Table 3).

**Table 3.** Comparison of serum VEGF level in SSc patients with and without lung fibrosis

<table>
<thead>
<tr>
<th></th>
<th>SSc patients with lung fibrosis</th>
<th>SSc patients without lung fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mean serum VEGF level in pg/ml</td>
<td>21.78 ± 3.1</td>
<td>13.35 ± 6.4</td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In SLE patients, the serum level of VEGF showed no correlation with age, sex, disease duration or involvement of organs. However, it was found to be significantly higher in patients with decreased C3 level (<70 mg/dl) and elevated ESR (>28/hour) than in those with normal C3 and ESR values (the p value was <0.05; refer to Table 4).

**Table 4.** Comparison of serum VEGF level between active and inactive SLE patients

<table>
<thead>
<tr>
<th></th>
<th>Active SLE patients</th>
<th>Inactive SLE patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Mean serum VEGF level in pg/ml</td>
<td>18.51 ± 3.72</td>
<td>14.48 ± 1.76</td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td></td>
<td></td>
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</table>

**DISCUSSION**

Angiogenesis, which refers to the formation of new micro vessels from a pre-existing vasculature, is a complex and highly regulated physiological process. In normal adults, angiogenesis is restricted to the female reproductive cycle and wound healing17. Pathological angiogenesis is now recognized as a fundamental component of pannus development in RA18. Abundant endothelial cell proliferation is characteristic in pulmonary hypertension associated with SSc and SLE19, 20.

The initiation of pathological angiogenesis is associated with the expression of a number of angiogenic growth factors. These include acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), platelet derived endothelial cell growth factor and vascular endothelial growth factor (VEGF), which is the most potent one. These growth factors stimulate vascular endothelial cells in autocrine and paracrine manners21.

Based on the hypothesis that endothelial cell modulation is indirectly produced by effector cytokines such as VEGF in the pathogenesis of collagen diseases, VEGF serum levels were assessed in RA, SSc and SLE patients and thereafter correlated with different clinical and laboratory parameters of disease activity and functional impairment2.

The study was carried out on 40 individuals divided into four groups: group I including 10 RA patients; group II including 10 patients with SSc; group III including 10 patients with SLE; and group IV including 10 healthy volunteers as a control group.

The results of the study showed that serum VEGF concentrations were significantly high in each group of patients compared to that for the control group (p < 0.001). These results are in line with those of many previous studies that reported increased serum level of VEGF in RA patients2, 10, 22. They are also in line with the results of a study by Kikuchi et al. that demonstrated high levels of serum VEGF in SSc patients compared to the control group, but not in patients with SLE when compared to the control group2.

In this study, the serum level of VEGF was significantly higher in active RA patients (p < 0.01), active SLE patients (p < 0.05) and those with lung fibrosis associated with SSc (p < 0.05) than in patients with inactive RA, inactive SLE and SSc patients without lung fibrosis, respectively. These results are in line with those of studies conducted by Ballara et al., Roback and Kikuchi et al. that reported a positive correlation between elevated serum level of...
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VEGF and active RA, active SLE and lung fibrosis in SSc patients, respectively2,23–25.

Previous studies suggested that VEGF plays a major role in joint inflammation in RA patients by increasing vascular permeability, resulting in the leakage of vascular fluid into the surrounding tissues10,18,19,21–23. This is clinically apparent in rheumatoid joints in the form of joint effusion. In Arthritis & Rheumatology 2003, it was mentioned that the pivotal cytokine that induces VEGF production in synergy with IL-1beta or TNF alpha is IL-626. Therefore, this can be used to explain the hypothesis behind the mechanism by which IL-6 receptor blockade, in combination with the corresponding monoclonal antibodies of IL-1beta and TNF alpha, can effectively suppress VEGF production in the synovial fluid of rheumatoid arthritis patients. It was also proved that anti-angiogenic treatment, such as anti-TNFα, results in the reduction of joint fluid content, as determined by MRI27. Koch speculated that increase in levels of serum VEGF in RA patients is directly a result of inflammation and local hypoxia in the affected joints, rather than a result of circulating pro-inflammatory cytokines18. The polycarticular nature of RA and highly vascular synovial fluid facilitate the permeability of VEGF from the synovial fluid into the circulation of patients.

With regard to SSc, a disease characterized by vascular abnormalities, altered extracellular matrix and variable degrees of hypoxia, Kikuchi et al. indirectly linked the endothelial cell modulation to VEGF and β-FGF, which are responsible for the fibrotic process occurring in SSc2. The correlation between increased serum VEGF level and occurrence of lung fibrosis in SSc patients can be attributed to the abundant presence of VEGF in lungs28. It is possible that VEGF serum levels increase during lung vessel remodeling associated with the development of lung fibrosis2.

It was previously reported that serum level of angiogenic VEGF may be relevant in SLE pathogenesis and its concentration seems to be a marker of SLE activity24,29.

CONCLUSION

Our results, like those of other studies, suggest that measuring serum VEGF level in RA, SLE and SSc patients can be useful in evaluating the disease activity of RA and SLE as well as the development of lung fibrosis in SSc. Consequently, inhibiting VEGF, either by decreasing its production with the help of drugs (such as anti-TNF-α) or by receptor antagonism, may improve the clinical manifestations and decrease the progress of these diseases. This data serves as evidence for the role of VEGF in the development and persistence of these diseases. Nevertheless, more researches on angiogenesis in collagen diseases are required for determining the mechanisms of these diseases.

REFERENCES


