

## **SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR IN COLLAGEN DISEASES: CORRELATION WITH DISEASE ACTIVITY**

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**KEY WORDS:** *ENDOTHELIAL GROWTH FACTOR, CONNECTIVE TISSUE DISEASE ETIOPATHOGENESIS.*

### **ABSTRACT**

**Background:** *A role for angiogenic factors such as vascular endothelial growth factor (VEGF) in the pathogenesis of collagen diseases through endothelial cell modulation has been suggested.*

**Aim of the work:** *Assessment of 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. Also, to find out a relation between its serum level and the disease activity or the functional impairment in those patients.*

**Study design:** *The serum level of VEGF was assessed in 10 RA patients; 10 SSc patients; 10 SLE patients as well as 10 healthy volunteers. Its level was correlated to the different clinical & 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 & SLE patients as well as the development of lung fibrosis in SSc patients.*

**Conclusion:** *The results of this study suggest that angiogenesis produced by VEGF may play an important role in the pathogenesis of collagen diseases. Measurement of serum VEGF reflects the disease activity in RA & SLE patients as well as increased frequency of lung fibrosis in SSc patients. In addition, 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.*

## INTRODUCTION

Vascular endothelial growth factor (*VEGF*) is one of the major stimulators of angiogenesis involved in physiological as well as pathological processes <sup>1</sup>. It specially targets vascular endothelial cells <sup>2</sup>. VEGF mRNA has been shown to be produced by activated macrophages, keratinocytes, synovial fibroblasts and vascular smooth muscle cells but not vascular endothelial cells <sup>2,3</sup>.

A number of cytokines and growth factors are known to up-regulate VEGF expression. They include tumor necrosis factor alpha (*TNF- $\alpha$* ) <sup>4</sup> transforming growth factor-beta (*TGF- $\beta$* ) <sup>5</sup>, interleukin-1 (*IL-1*) <sup>6</sup> as well as hypoxia <sup>7</sup>. On The other hand, VEGF secretion is down-regulated by (*IL-4*), (*IL-10*) <sup>8</sup>, (*anti-TNF- $\alpha$* ) <sup>9</sup> and drugs like dexamethasone, penicillamine and gold sodium thiomalate <sup>10</sup>.

Many studies suggested that VEGF acts as an angiogenic mediator in rheumatoid arthritis (*RA*) <sup>11, 12</sup> and elevated levels of VEGF have been reported in synovial fluids of RA patients <sup>12</sup>. In addition, it has been shown that systemic sclerosis (*SSc*) and dermatomyositis (*DM*) patients have marked elevated serum basic fibroblast growth factor (*bFGF*), which is another major stimulator of angiogenesis <sup>13</sup>. These studies motivated us to measure serum VEGF level in patients with collagen diseases and to investigate its relationship with the clinical and laboratory features of those diseases.

## PATIENTS & METHODS

This study was carried out on 30 patients with different collagen diseases recruited from the Rheumatology, Dermatology & Internal Medicine Outpatient Clinics of Ain Shams University Hospitals. They were 10 RA patients, 10 SSc patients and 10 SLE patients.

The RA patients were 9 females and 1 male. Their ages ranged from 20-54 years. They were diagnosed according to the American College of Rheumatology (ACR) 1987 criteria<sup>14</sup>.

The SSc patients were 8 females and 2 males with an age range from 20 to 62 years. All of them fulfilled the preliminary criteria for SSc proposed by the American College of Rheumatology (ACR)<sup>15</sup>.

The SLE patients were 9 females and 1 male with age range from 16 to 54 years. They all fulfilled the criteria of ACR for diagnosis of SLE<sup>16</sup>.

None of the patients had received corticosteroids or immunosuppressive drugs at the time of sampling.

Ten completely normal individuals were also included in the study. They were 7 females and 3 males. Their ages ranged between 20 & 52 years.

### **All individuals included in the study were subjected to:**

- Thorough history taking & clinical examination with stress on the specific clinical criteria for each disease activity. In RA, the specific clinical criteria asked for were scoring for the duration of morning stiffness, visual pain scale, number of affected joints, number of tender joints and number of swollen joints. In SSc patients clinical specific criteria searched for were oesophageal, heart or joint involvement and lung fibrosis on chest X ray. In SLE patients we searched for data of organ involvement of the disease.
- Routine investigation: complete urine analysis, CBC, ESR and Chest X ray.
- Rheumatoid factor with Rose Waller test.
- C-reactive protein (CRP).
- Complement – 3 (C<sub>3</sub>) only for patients with SLE.
- **Assessment of serum level of VEGF with specific ELISA kits (Amersham, Bucks, UK, R & D system Inc.).** This assay employs the quantitative enzyme immunoassay technique according to the following steps:

An antibody specific for VEGF has been precoated onto a microplate.

Standard, patients samples, control and conjugate were pipetted into the wells and any VEGF present was sandwiched by immobilized antibodies & the enzyme linked antibody specific for VEGF.

Following a wash to remove any unbound substrate and/or antibody enzyme reagent, substrate was added to the wells and colour develops in proportion to the amount of VEGF bound.

The color development was stopped and the intensity of the colour was measured.

### Statistical analysis:

Was performed using the student t-test and ANOVA test.

## RESULTS

### This study was carried out on 40 subjects divided into 4 groups:

**Group I:** Included 10 patients who presented with RA diagnosed according to ACR criteria. They were 9 females & 1 male. Their ages ranged from 20-54 years with a mean  $\pm$  standard deviation (*SD*) of  $34.2 \pm 1$ . The duration of the disease showed a range between 1-15 years with a mean  $\pm$  SD of  $3.9 \pm 4$ .

**Group II:** Included 10 patients who presented with SSc diagnosed according to the criteria of ACR. They were 8 females & 2 males with an age range from 20 to 62 years and a mean  $\pm$  SD of  $41.3 \pm 5$ . The disease duration ranged between 1.5 and 18 years with a mean  $\pm$  SD of  $6.73 \pm 8.1$ .

**Group III:** Included 10 patients fulfilling the criteria of ACR for the diagnosis of SLE. They were 9 females and 1 male. Their ages ranged from 16 to 54 years with a mean  $\pm$  SD of  $38.7 \pm 3.2$ . The duration of their disease ranged from 1 to 13 years with a mean  $\pm$  SD of  $8.21 \pm 6.4$ .

**Group IV:** Included 10 healthy volunteers from the hospital staff. They were 7 females & 3 males with age range from 20 to 52 years and a mean  $\pm$  SD of  $29.1 \pm 7$ .

The serum level of VEGF in healthy individuals (*group IV*) ranged between 2.75 & 6.5 pg / ml with a mean  $\pm$  SD of  $4.22 \pm 1.12$  pg / ml. The range in RA patients (*group I*) was 8-24 pg / ml with a mean  $\pm$  SD of  $15.17 \pm 1.7$  pg/ml. In SSc patients it was 10 – 29.8 pg / ml with a mean  $\pm$  SD of  $17.57 \pm 3.6$  pg/ml. Lastly, in SLE patients it was 9.75 – 23 pg /ml with a mean  $\pm$  SD of  $17.1 \pm 1.05$  pg/ml.

On comparing the results of the serum level of VEGF of each group (*I, II & III*) to the control group (*IV*) a highly statistical significant increase

( $p < 0.001$ ) was found. However, no statistical significant differences appeared when we compared the results of the first 3 groups to each other (Table 1).

Table (1): Shows the serum level of VEGF in pg / ml in all groups.

Patient No.	Group I (RA)	Group II (SSc)	Group III (SLE)	Group IV (Controls)
1	14	20.25	15	4.25
2	13.5	15	12.2	5
3	8	17.75	12.75	4.75
4	12.25	12.25	15.25	3.25
5	8	10	23	2.75
6	13.5	14.25	17.75	4
7	24	15.25	9.75	6
8	15.25	22	24.2	5.25
9	18.15	19.1	23	6.5
10	20.2	29.8	18.25	4
Mean	15.17	17.57	17.1	4.57
SD	1.7	3.6	1.05	1.12

We compared the clinical & laboratory findings in patients to their serum VEGF level. As regard RA patients, there were positive correlations between serum VEGF value and the duration of morning stiffness score, visual pain score, number of affected joints and elevated ESR. No correlation was found between VEGF level and age, sex, disease duration, seropositivity & C-RP. The serum levels showed a positive correlation with disease activity in RA patients as active RA was defined by the simultaneous presence of at least 3 of the following criteria:

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

On comparing the serum level of VEGF in patients with active RA to those with inactive RA we found a statistical significant ( $p < 0.01$ ) increase in the first group (Table 2).

Table (2): Comparison of serum VEGF level in active &amp; inactive RA patients.

	Active RA patients	Inactive RA patients
Number of patients	7	3
Mean serum VEGF level in pg/ml	17.63 ± 7.81	9.41 ± 3.2

p<0.01

In SSc patients, the serum level of VEGF showed no correlation with age, sex, disease duration or prevalence of oesophageal, heart or joint involvement. However, a statistical significant difference (p<0.01) emerged on comparing patients with and without lung fibrosis being higher in the first group (Table 3) indicating a positive correlation between the serum level of VEGF and development of lung fibrosis in SSc patients.

Table (3): Comparison of serum VEGF level in SSc patients with and without Lung fibrosis.

	SSc patients with Lung fibrosis	SSc patients without Lung fibrosis
Number of patients	5	5
Mean serum VEGF level in pg/ml	21.78 ± 3.1	13.35 ± 6.4

p<0.01

In SLE patients, the serum level of VEGF showed no correlation with age, sex, disease duration or frequency of organ involvement. However, significantly higher levels of VEGF (p<0.05) were found in patients with decreased C<sub>3</sub> levels (< 70 mg / dl) and elevated ESR (> 28/ hour) than in those with normal C<sub>3</sub> and ESR values (Table 4) indicating a positive correlation between the VEGF levels and disease activity in SLE patients.

Table (4): Comparison of serum VEGF level between active &amp; inactive SLE patients

	Active SLE patients	Inactive SLE patients
Number of patients	6	4
Mean serum VEGF level in pg/ml	18.51 ± 3.72	14.48 ± 1.76

p<0.05

## DISCUSSION

Angiogenesis which means the formation of new microvessels from a pre-existing vasculature is a complex highly regulated physiological process. In normal adults, angiogenesis is restricted to the female reproductive cycle and wound healing<sup>17</sup>. Pathological angiogenesis is now recognized as a fundamental component of pannus development in RA<sup>18</sup>. It has also been reported that an exuberant endothelial cell proliferation is observed in patients with pulmonary hypertension associated with SSc<sup>19</sup> and SLE<sup>20</sup>.

The initiation of pathological angiogenesis is associated with 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 manners<sup>21</sup>.

Owing to the hypothesis that effector cytokines such as VEGF are indirectly involved in the pathogenesis of collagen diseases through endothelial cell modulation<sup>2</sup>, we have assessed the serum VEGF levels in RA patients, SSc & SLE and correlated their levels with different clinical and laboratory parameters of disease activity and functional impairment.

This study was carried out on 40 individuals divided into 4 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 our study showed that serum VEGF concentrations were highly significantly elevated ( $p < 0.001$ ) in each group of patients compared to the control group. These results agree with many previous studies that reported increased serum levels of VEGF in RA patients<sup>2, 22, 23</sup>. They also coincide with those of *Kikuchi et al.*<sup>2</sup> who demonstrated high levels of serum VEGF in SSc patients. However they reported no significant difference in the serum levels of VEGF in patients with SLE when compared to their control group.

Furthermore, in our study the serum levels of VEGF were significantly higher in active RA patients ( $p < 0.01$ ), active SLE ( $p < 0.01$ ) and those with lung fibrosis associated with SSc ( $p < 0.01$ ) than in patients with inactive RA, inactive SLE and SSc patients without lung fibrosis respectively. These results coincide with those of Ballara et al.<sup>24</sup>, *Roback*<sup>25</sup> and *Kikuchi et al.*<sup>2</sup> reported a positive correlation between elevated serum

levels of VEGF and active RA active SLE & lung fibrosis in SSc patients respectively.

Previous studies<sup>18, 19, 21–24</sup> suggested that VEGF plays a major role in joint inflammation in RA patients through the induction of increased vascular permeability and leakage of vascular fluid into the surrounding tissues. This is clinically apparent in the rheumatoid joints as joint effusion. It was also proved that anti-angiogenic treatment as anti-TNF $\alpha$  results in reduction of joint fluid content as determined with MRI<sup>26</sup>. Koch<sup>18</sup> speculated that increased levels of serum VEGF in RA patients are directly derived from inflammation & local hypoxia in the affected joints rather than from circulating pro-inflammatory cytokines. The polyarticular nature of RA & highly vascular synovial fluid allows immediate access of VEGF from synovial fluid into the circulation.

As regards SSc, it is a disease characterized by vascular abnormalities, altered extracellular matrix and variable degrees of hypoxia. Kikuchi *et al.*<sup>2</sup> hypothesized that effector cytokines such as VEGF and  $\beta$ -FGF are indirectly involved in the fibrotic process occurring in SSc through endothelial cell modulation. The correlation between increased serum VEGF level & the frequency of lung fibrosis in patients with SSc can be attributed to the abundant presence of VEGF in the lungs<sup>27</sup> and it is possible that VEGF participates in the lung vessel remodeling that accompanies the development of lung fibrosis<sup>2</sup>.

It was previously reported<sup>25</sup> that serum level of the angiogenic VEGF may be relevant in SLE pathogenesis and its concentration seem to be a marker of SLE activity.

### **Conclusion:**

Our results like those of other studies, suggest that the measurement of serum VEGF level in RA patients, SLE & SSc can be useful in the evaluation of disease activity of RA & SLE as well as the development of lung fibrosis in SSc patients. Consequently, the inhibition of VEGF either by decreasing its production by drugs as anti-TNF- $\alpha$  or by receptor antagonism may improve the clinical manifestations and decrease the progress of these diseases. All these data represent evidences for the role of VEGF in the development and persistence of these diseases. However, further studies of VEGF and other angiogenic factors in the affected tissues of collagen diseases are needed to delineate the exact role of these factors in the pathogenesis of collagen diseases.



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