

# The relation of serum vascular endothelial growth factor level with disease duration and activity in patients with rheumatoid arthritis

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Received: 19 July 2009 / Revised: 1 December 2009 / Accepted: 10 December 2009 / Published online: 13 January 2010  
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**Abstract** Vascular endothelial growth factor (VEGF) is known to be involved in the pathogenesis of rheumatoid arthritis (RA). In order to elucidate the association between VEGF levels and RA disease activity, VEGF concentrations were measured in RA patients at different phases and severity levels. Thirty-eight healthy subjects and 40 patients with RA were prospectively included in the study. Subjects were further categorized into four subgroups (high, moderate, low, or remission) using the disease activity score-28 (DAS28) scoring system. VEGF levels were significantly higher in patients than controls ( $p < 0.001$ ). VEGF levels differed significantly in controls, early and late-phase RA patients ( $p = 0.002$ ). A significant difference was found between controls and patients with high RA disease activity scores ( $p < 0.0001$ ). VEGF levels were not correlated with age ( $r = -0.016$ ;  $p = 0.921$ ) or sex ( $r = 0.209$ ;  $p = 0.921$ ). VEGF values were correlated with erythrocyte sedimentation rate ( $r = 0.445$ ;  $p = 0.004$ ), but was not correlated with serum rheumatoid factor levels ( $r = -0.130$ ;  $p = 0.424$ ) in the patient group. In conclusion, higher VEGF levels are associated with late phase and high disease activity in RA, independent of age and sex.

**Keywords** Angiogenesis · Rheumatoid arthritis (RA) · Vascular endothelial growth factor (VEGF)

## Introduction

Rheumatoid arthritis (RA) is characterized by persistent synovial inflammation and joint damage. Cartilage and joint damage occurs with the invasion of hypertrophic synovial pannus into the cartilage tissue [1–4]. Synovial angiogenesis, the formation of new blood vessels in the synovium from preexisting vessels, plays a key role in the development of rheumatoid pannus. Various growth factors and inhibitors play roles during this angiogenic phase. Vascular endothelial growth factor (VEGF), the most potent angiogenic factor, is predominantly produced by synoviocytes in the pannus [5]. VEGF increases vascular permeability, and this is responsible for the joint swelling in RA. Additionally, it leads to the proliferation and migration of endothelial cells in order to form new blood vessels. VEGF also has a direct proinflammatory role in the pathogenesis of RA, increasing production of tumor necrosis factor and interleukin 6 [6]. VEGF therefore promotes pannus formation, and as the pannus grows, more VEGF is produced, forming a vicious circle.

In the literature, VEGF elevation in patients with RA has been demonstrated in numerous studies [1–14]. High levels of VEGF have been observed in late-phase RA patients [8, 15]. There seems to be an association between VEGF levels and disease activity scores and erythrocyte sedimentation rate (ESR) [7–9, 11, 15–17]. In the present study, based on the hypothesis of a close relationship between VEGF and the pathophysiology of RA, serum VEGF levels were measured in patients with RA at various phases and different disease activity scores and correlated with clinical and laboratory variables.

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**Table 1** Descriptive information for the control group and RA patient group

	Control group	Patient group	Significance
Age (years)	44 (36–51)	46 (38.0–55)	$p=0.112$
VEGF (pg/dL)	93 (70–132)	127 (86.5–243.0)	$p<0.001^*$
Disease duration (year)	N/A	5 (1.3–10.0)	N/A
RF positive	N/A	77.5%	N/A
DAS28	N/A	4.77 (4.1–5.5)	N/A
Male, $n$ (%)	20 (52.6)	8 (20)	$p=0.003^{**}$
Female, $n$ (%)	18 (47.4)	32 (80)	$p=0.003^{**}$

Values are given as the median with 25th–75th percentiles in parentheses

*Med* median, *DAS28* disease activity score-28, *VEGF* vascular endothelial growth factor, *N/A* not applicable

\* $p<0.001$ , Mann–Whitney *U* test; \*\* $p<0.01$ , chi-square test

## Materials and methods

The study was conducted between August 2005 and February 2006 with the Physical Medicine and Rehabilitation Clinic of the Ministry of Health, Istanbul Research and Training Hospital. Patients with RA, meeting the American College of Rheumatology diagnostic criteria, were recruited to the study. Subjects were surveyed about demographic data, current medications, duration of the disease, smoking, and comorbidities associated with neovascularization, such as hypertension, malignancy, diabetes mellitus, or pulmonary disease. Subjects with these comorbidities, smokers and pregnant subjects were excluded from the study. Age-matched healthy volunteers from hospital staff and their relatives were enrolled as the control group. They were also evaluated with regard to the aforementioned pathologies, and the exclusion criteria were also applied to the control group. All individuals gave informed consent in accordance with the Helsinki Declaration of 1975.

In order to analyze their VEGF values, the patients were grouped in two different ways. For the first grouping, patients were divided into two groups according to the

duration of their disease. If their duration of disease (the time period since the first symptom) was  $<2$  years, they were classified as early phase RA patients. If their duration of disease was  $\geq 2$  years, they were classified as late-phase RA patients.

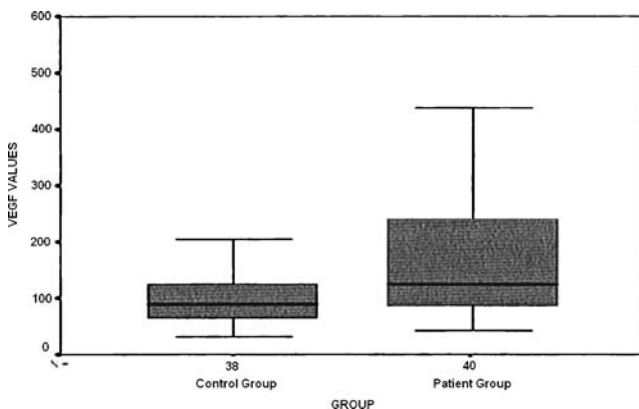
Subjects were further categorized into four subgroups using the disease activity score-28 (DAS28) scoring system [16]. Scoring was as follows:  $>5.1$  high disease activity, 3.2–5.1 moderate disease activity, 2.6–3.2 low disease activity, and  $<2.6$  remission.

### Measurement of serum VEGF concentration

Fasting serum VEGF levels were obtained from the patients. All tests were done on the day of sampling. VEGF levels were measured using a solid phase sandwich enzyme linked-immunosorbent assay (ELISA; BioSource International, Inc., California, USA). VEGF165 isoform analyses were performed with this kit. Study procedure was performed according to the protocol of manufacturer. All analyses were performed in duplicate.

### Statistical methods

Data obtained in the study were evaluated by using the Number Cruncher Statistical System (NCSS) 2007 (Kaysville-Utah) package program. In addition to the descriptive statistical methods (mean, standard deviation, median, minimum, and maximum values) used during the evaluation of the study data, a Kruskal–Wallis test was conducted for the intergroup comparisons of quantitative data, and a Dunn's multiple comparison test was used for the determination of the groups responsible for the difference. A Mann–Whitney *U* test was used for the comparison of the continuous variables between the patient and the control groups. Significance levels of  $p<0.05$  and 95% confidence intervals were used to evaluate outcomes. A chi-square test was used for the comparison of expected



**Fig. 1** Box plot graph of the distribution of VEGF values for control and RA patient groups

**Table 2** VEGF values for the control group and early and late-phase subgroups of RA patients

VEGF (pg/dL)	Control group ( $n=38$ )	Early phase ( $n=15$ )	Late phase ( $n=25$ )	Significance
Mean	93 (36–350)	86 (44–390)	208 (44–506)	$p=0.002^*$

VEGF vascular endothelial growth factor, *Min* minimum, *Max* maximum

\* $p<0.01$ , Kruskal–Wallis test

and observed values. Spearman and Pearson correlation tests were used for the correlation between parameters. Receiver operating characteristics curves were used for the determination of diagnostic competency for VEGF.

## Results

Thirty-eight healthy subjects and 40 RA patients were prospectively included in the study. There were more female subjects in the patient group than in the control group ( $p=0.003$ ; Table 1); mean age was similar between the groups ( $p=0.112$ ; Table 1).

Fifteen of the patients had early phase disease and 25 had late-phase disease. According to DAS28 values, one patient was in remission, three had low, 22 had moderate, and 14 had high disease activity. The first two subgroups were excluded from activity evaluation due to the limited number of cases. Ninety percent of patients ( $n=36$ ) were taking disease-modifying antirheumatic drugs (DMARDs). This included all late-phase patients ( $n=25$ ) and 73% ( $n=11$ ) of early phase patients.

VEGF values for the patient group were significantly higher than those of the control group ( $p<0.001$ ; Table 1). The group distribution of VEGF values is presented in Fig. 1. There were significant differences between the VEGF values of the control group and patient groups with early- and late-phase RA ( $p=0.002$ ; Table 2, Fig. 2). In Dunn's multiple comparison test, significant differences

were found between the groups with early- and late-phase RA ( $p=0.032$ ) and between the control group and the patient group with late-phase RA ( $p<0.0001$ ). However, no significant difference was observed between the control group and the patient group with early phase RA ( $p=0.811$ ).

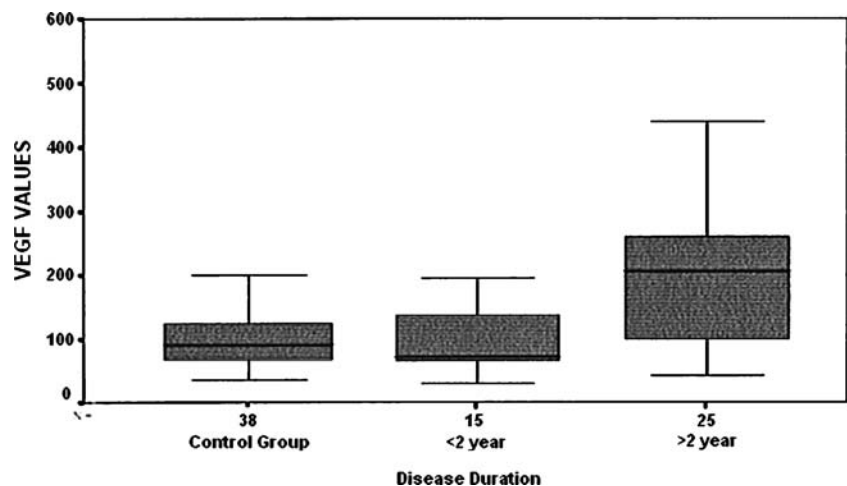
VEGF levels in patients with moderate activity scores was not different from the control group ( $p=0.723$ ). VEGF levels were significantly higher in patients with high disease activity than those in the control group and the patients with moderate disease activity ( $p<0.0001$ ; Table 3, Fig. 3).

VEGF values were correlated neither with age ( $r=-0.016$ ;  $p=0.921$ ), nor with sex ( $r=0.209$ ;  $p=0.921$ ) in RA patients. VEGF values were correlated with erythrocyte sedimentation rate ( $r=0.445$ ;  $p=0.004$ ) in the patient group. There was no correlation between VEGF values and serum rheumatoid factor levels ( $r=-0.130$ ;  $p=0.424$ ; Fig. 4).

## Discussion

RA has been defined as an angiogenic process, just as diabetic retinopathy, fibrovascular disease, and tumor development. The most important factor leading to chronic RA as well as deformity development is the pannus formation, an expansion of synovial tissue. Synovial angiogenesis is one of the many factors responsible for pannus formation, and is thought to be driven by tissue

**Fig. 2** Box plot graph of VEGF values for the control group and for early- and late-phase RA patient subgroups



**Table 3** VEGF values for the control group and the moderate and high disease activity subgroups of RA patients

VEGF (pg/dL)	Control group ( <i>n</i> =38)	RA group, moderate activity ( <i>n</i> =22)	RA group, high activity ( <i>n</i> =14)	Significance
Med (min–max)	93 (36–350)	98 (44–280)	225 (100–500)	<i>p</i> =0.0001

RA rheumatoid arthritis, VEGF vascular endothelial growth factor, Min minimum, Max maximum.

\**p*<0.0001, Kruskal–Wallis test

hypoxia [8]. VEGF is one of the most important inducers of angiogenesis [18–20]. Several studies have demonstrated elevated synovial and serum VEGF levels in RA patients when compared with healthy subjects or patients with osteoarthritis, gout arthritis, systemic lupus erythematosus, or scleroderma, collagen tissue diseases [1, 3, 8, 9, 11–14, 21, 22]. VEGF seems to be one of the central players in the RA-related joint destruction [8, 23], as evidenced by the magnitude of radiologic changes and disease activity scores in patients with high VEGF levels. In fact, anti-VEGF antibody has been found to prevent collagen-induced arthritis in animal studies [6, 18].

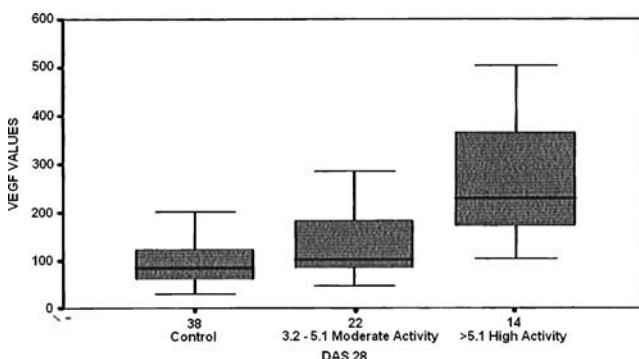
Higher VEGF levels have been associated with higher disease activity, underlining the relationship between VEGF levels and organ damage [7–9, 11, 15–17]. In several studies serum VEGF correlate with disease activity including acute phase markers and swollen and tender joint counts. In our study, serum VEGF levels correlated with disease activity variables such as ESR and number of joints with active arthritis [7, 8, 19, 24]. VEGF increases vascular permeability, which in return leads to joint swelling seen in RA. As VEGF level increases, the number of swelling and sensitive joints, which forms one of the DAS28 calculation parameters, increases as expected, leading to high disease activity scores.

There have also been attempts to locate the VEGF elevations within the time course of RA, with conflicting results. While higher VEGF levels have been correlated with late-phase disease in RA by Pinheiro et al. [15], Ballara et al. [8] found higher levels in early phase RA

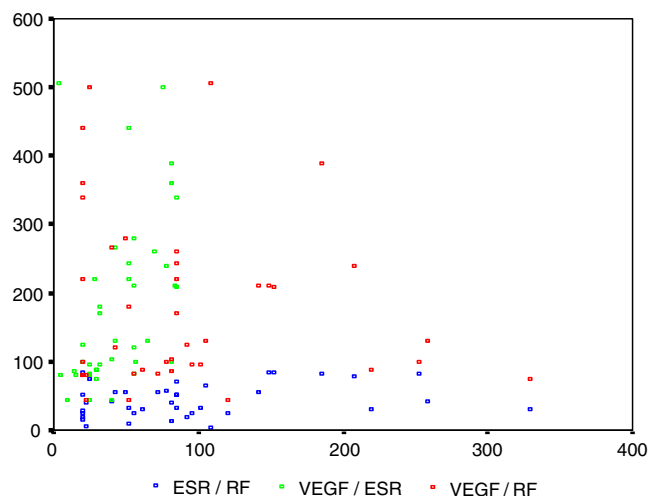
patients. These patients subsequently developed chronic synovitis, and the authors concluded that high VEGF levels could be used to detect the subgroup of patients in whom a chronic and destructive course is anticipated and in whom a more aggressive treatment strategy is indicated. In our study, we found higher VEGF levels in late-phase patients, despite treatment with DMARDs. Poor disease control may be the reason for high VEGF in late-phase RA patients. [25–27]. Perhaps higher serum VEGF levels could be a marker of higher disease activity, regardless of the phase of the disease. Serial VEGF levels in a cohort of RA patients, obtained over a long follow-up period, could help to elucidate the association between angiogenesis and the phase of the disease.

In conclusion, our study corroborates the previous studies and supports the hypothesis that higher serum VEGF levels could be a marker of disease activity. One of the limitations of our study is that the control group was not sex-matched with the patients; however, serum VEGF levels have not been associated with sex or age [7]. Another limitation is the difficulty to investigate the influence of drug combinations on VEGF levels in RA patients.

Preventing structural damage of the joints is mainstay of RA management. The correlation of high disease activity with high VEGF level suggests that VEGF may be a useful biochemical parameter to gauge the effectiveness of



**Fig. 3** Box plot graph of VEGF values for the control group and for moderate and high disease activity RA patient subgroups



**Fig. 4** Correlation graph of VEGF values with erythrocyte sedimentation rate and rheumatoid factor values

treatment and as a prognostic factor. Future studies should investigate the need for a more aggressive treatment in patients with high serum VEGF levels to prevent destructive joint disease.

**Disclosures** None

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