Serum vascular endothelial growth factor in Iranian patients with moderate-severe psoriasis before and after treatment: a PASI-75 response as a practical treatment goal

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Background: Psoriasis is a chronic disease with multiple biochemical and vascular abnormalities. Several studies have evaluated circulating levels of vascular endothelial growth factor (VEGF) in psoriasis, but none of them evaluated it after reaching a PASI-75 response, as a practical treatment goal. The aim of this study was to evaluate serum levels of VEGF in moderate to severe psoriatic patients before and after treatment compared with healthy controls.

Methods: This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. Fifty-eight patients with moderate- severe psoriasis and 60 age-and gender-matched healthy controls were recruited to this study**.** Serum VEGF levels (pg/ml) of both groups were measured. We used Psoriasis Area and Severity Index (PASI) scoring to assess disease activity in patients. According to the disease severity, the patients received proper treatment. When they reached a PASI-75 response, serum VEGF levels were measured once more.

Results: In our study, the median serum VEGF level was significantly higher in psoriatic patients (before and after treatment) as compared to healthy controls. Moreover, patients showed a significant reduction in their serum VEGF levels after reaching PASI-75. The median time of therapeutic effect (reaching a PASI-75 response) was four months. Furthermore, our study showed a significant correlation between the serum VEGF level and age, BMI, PASI, and disease duration (P<0.05).

Conclusion: A PASI-75 response is believed to be a non-invasive assessment tool for evaluation of the treatment response. Using the PASI-75 response for evaluation or prediction of the reduction of VEGF levels could provide further clinical benefits.

Keywords: serum vascular endothelial growth factor, PASI 75 response, psoriasis, treatment

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INTRODUCTION

Psoriasis is a common, chronic inflammatory and immune-mediated disorder with a strong genetic basis defined by complex alterations in epidermal growth and multiple biochemical and vascular abnormalities [1-3]. It is estimated to affect approximately 2% of the population.

The redness of the lesions is caused by vascular changes including increased number of elongated and tortuous capillaries and angiogenesis [1,4,5]. In the pathogenesis of psoriasis, vascular change

has received relatively little attention [6]. The angiogenic molecules are believed to derive from the hyperplastic epidermis [7] including tumor necrosis factor α (TNF-α) [8], angiopoietin, and vascular endothelial growth factor (VEGF) [9-11]. VEGF is a multifunctional cytokine that plays a key role in angiogenesis and also enhances vascular permeability [12,13]. VEGF induces monocyte activation and chemotaxis, thus it may be an important link between inflammation and angiogenesis in the pathogenesis of psoriasis [4,5]. VEGF is identified as a major vessel-specific growth factor that is overexpressed in psoriatic skin lesions and might contribute to the epidermal changes observed in psoriasis [14-15]. Several studies have demonstrated that serum levels of VEGF are associated with disease activity [9,10]. Enhanced skin vascularity and vascular permeability in VEGF transgenic mice also supports this hypothesis [16]. On the other hand, measuring VEGF levels in practice will increase the costs and other inexpensive tools are required to help evaluate or predict the reduction of VEGF levels. The purpose of this study was to evaluate serum levels of VEGF in Iranian patients with moderate to severe psoriasis before and after treatment (reaching a PASI-75 response) compared with age- and sex-matched healthy control subjects, to investigate its association with age, sex, the disease duration, especially PASI score, and also to have a quick overview of the role of VEGF in psoriasis provided by prior studies.

PARTICIPANTS AND METHODS

Patients

This prospective study was done in accordance with the tenets of the Declarations of Helsinki, and was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. Written informed consent was obtained from all participants.

Sixty patients with confirmed moderate to severe chronic plaque psoriasis were recruited to the study consecutively. They had received no systemic therapy or phototherapy in the last 6 months and no topical treatment apart from emollients was applied for at least 4 weeks prior to the study. An age- and sex-frequency matched healthy control group of 60 individuals (33 men and 27 women; age range, 20-84 years) was enrolled. All eligible subjects were recruited from the dermatology clinics of our institute. Exclusion criteria included receiving anti-VEGF therapy for any reason such as bevacizumab; receiving COX-1 or COX-2 nonsteroidal anti-inflammatory drugs that could inhibit angiogenesis [14]; a history of co-morbidity with metabolic syndrome, hepatic, renal or cardiac insufficiency, rheumatoid arthritis and other chronic inflammatory disorders; pregnancy and lactation; smoking; and not achieving a PASI-75 response after 8 months of proper treatment. Baseline demographic data of the patients including sex, age, disease duration, family history, and PASI score were recorded.

Methods

We used the Psoriasis Area and Severity Index (PASI) scoring to assess disease activity in patients before and after treatment [17]. Based on recent guidelines, moderate to severe psoriasis is defined as a PASI score >10 [18]. Venous blood samples (at least 2 hours after breakfast) were taken from all 60 patients before treatment and also from control subjects for VEGF analysis. Samples were taken from premenopausal women outside menstrual bleeding. According to the disease severity and the quality of response to previous managements, the patients received proper treatment (topical, phototherapy or systemic agents). Although the treatment goal is the complete clearance of skin lesions, a PASI-75 response has been regarded as a practical treatment goal which is the percentage of patients who achieve a reduction of at least 75% in their baseline PASI. The latter should be evaluated 10-16 weeks after the initiation of treatment [18]. Thus, our patients were followed after two months and then monthly for up to 8 months for evaluation of PASI-75 response. Some patients received combination therapy or even various treatment modalities within the follow-up period. Two patients could not achieve a PASI-75 response after 8 months treatment and were excluded from the study. When our patients achieved a PASI-75 response, a venous blood sample was taken again for VEGF analysis. The serum was separated by centrifugation (3000 rpm for10 min at room temperature) and stored at −70°C until use. Serum levels of VEGF were measured using

a commercially available quantitative enzyme-linked immunosorbent assay (Human VEGF, ELISA, CUSABIO BIOTECH, Intra assay CV%: 6.4, Sensitivity: 7.8 pg/ml).

Statistical Methods

Continuous variables are presented as mean ± SD or median with total and interquartile (25^{th}- to 75^{th}-percentile) ranges (IQR). Categorical data are reported as frequencies and relative frequencies (%). The normality of continuous variables was examined by the Shapiro-Wilk's *W*-test. The correlation between the serum VEGF level and other continuous variables was assessed using the Spearman's correlation test. The Wilcoxon signed-rank test was used to evaluate the difference in the serum VEGF levels of patients before and after reaching PASI-75. Independent two-sample *t* or Mann-Whitney *U* tests, wherever appropriate, were used to compare the continuous variables between the two groups. Chi-square and Fisher's exact tests, wherever appropriate, were applied for data analysis. All data analyses were performed using the statistical software JMP, Version 7 (SAS Institute Inc., Cary, NC, 1989-2007). All tests were two-sided and $P<0.05$ were considered statistically significant.

RESULTS

In total, 58 patients with moderate to severe psoriasis and 60 age- and sex-matched healthy controls were recruited to this study. Baseline demographics and clinical characteristics of the participants are summarized in Table 1. In this study, 13 patients (22.4%) were treated with topical agents, 18 (31.0%) with phototherapy, and 27 (46.6%) with systemic agents.

The severity of psoriasis was evaluated before and after the treatment using the PASI score. The mean PASI score was 30.9±13.6 on enrolment (median 30.8, range 10.1-62.4). After treatment, a significant decrease was observed in the disease severity (mean PASI: 5.1±3.5, median: 5.1, range: 0-13.6, $P<0.001$). The median percentage improvement of the PASI score was 82.8% (range: 76.7-100%). The median time of therapeutic effect (reaching a PASI-75 score) was 4 months (range: 2-8 months).

Patients showed a significant reduction in their serum VEGF levels after reaching PASI 75 (median: 73.7 pg/ml (range: 12.5-200; IQR 42.2-119.2) before

Table 1. Baseline demographics and clinical characteristics of patients with psoriasis and healthy controls.

Patients' characteristics	Patients with psoriasis (n=58)	Healthy controls (n=60)	*P*
Gender (n) (%)			0.86
Female	27 (46.6%)	27 (45.0%)	
Male	31 (53.4%)	33 (55.0%)	
Age (years)	37.5±14.1; 34.5 (18-72)	39.6±15.2; 36 (20-84)	0.45
BMI (kg/m^2)	26.1±3.5; 26.0 (19.5-37.0)	25.2±2.5; 25.3 (19.9-31.6)	0.12
Positive family history of psoriasis (n) (%)	29 (50%)	7 (11.7%)	<0.001
Duration of disease (years)		-	
Median (range); IQR	6 (0.4-25); (2-11)		
PASI score			
Before treatment	30.9±13.6; 30.8 (10.1-62.4)	-	
After treatment*	5.1±3.5; 5.1 (0-13.6)	-	
Medication (n) (%)			
Topical agents	13 (22.4%)	-	
Phototherapy	18 (31.0%)	-	
Systemic agents	27 (46.6%)	-	
Methotrexate	9 (33.3%)	-	
Cyclosporine	5 (18.5%)	-	
Acitretine	2 (7.4%)	-	
Re-PUVA	8 (29.6%)	-	
Biologic agents	3 (11.1%)	-	

Values are expressed as Mean±SD or median (range), unless otherwise noted.
Abbreviations: BMI: Body Mass Index; IQR: Interquartile range (25^{th} -75^{th} percentiles); PASI: Psoriasis Area and Severity Index
**i.e.* After reaching PASI-75

therapy and 30.1 pg/ml (range: 8.8-103.8, IQR: 16.2-39.8) after therapy, $P<0.001$). The median serum VEGF level of healthy controls was 12.7 pg/ml (range: 8.2-69.0, IQR 10.4-20.8). The median serum VEGF level was significantly higher in psoriatic patients (before and after treatment) than healthy controls (both $P<0.001$) (Figure 1).

There was no significant difference in the median serum VEGF level between males and females (23.8 pg/mL (range: 8.8-200.0) for males *vs.* 28.8 pg/mL (range: 8.2-196.0) for females, $P=0.98$).

The serum VEGF level was positively correlated with age in both groups ($r=0.61$, $P<0.001$ for patients and $r=0.47$, $P<0.001$ for controls) and with BMI in the patient group ($r=0.38$ and $P=0.003$). There were positive correlations between serum levels of VEGF and both PASI score and disease duration (Table 2 and Figure 2).

DISCUSSION

Several pro-angiogenic factors are activated in psoriasis [6]. VEGF plays a key role in angiogenesis [12] and may be an important link between inflammation and angiogenesis in psoriasis [4,5]. The VEGF gene is located on chromosome 6p.21, close to PSORS1 (the most important psoriasis susceptibility region) [19]. The latter may partly explain the correlation between psoriasis and VEGF levels. Several studies have evaluated VEGF levels (in the skin, the serum, and also the synovium) in psoriatic patients [9,10,20,21], but evaluated the VEGF levels after reaching a PASI-75 response, as a practical treatment goal. The aim of this study was not to compare the effect of various treatment modalities on the serum levels of VEGF, but to evaluate serum levels of VEGF in psoriatic patients before and after treatment (a PASI 75

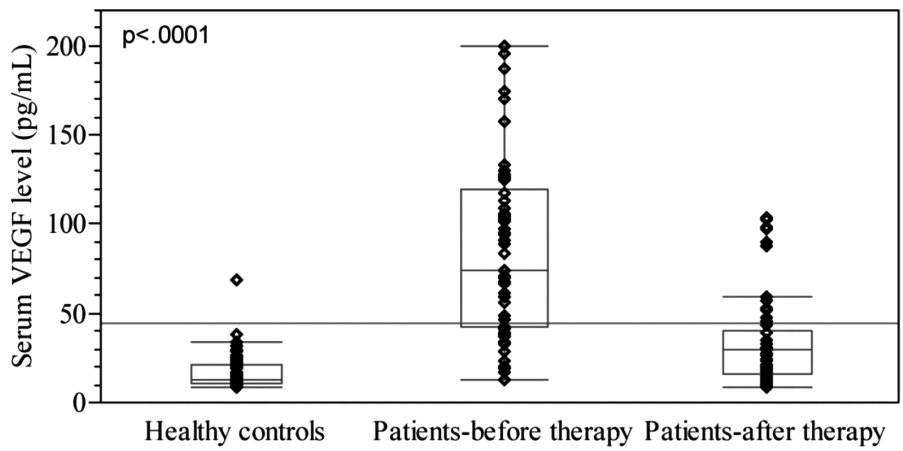

Figure 1. Serum VEGF levels (pg/mL) in patients with psoriasis (before therapy and after reaching a PASI-75 response) and healthy controls. Middle point: median; Box: interquartile range (25-75 percentiles); Whisker: range (excluding outliers).

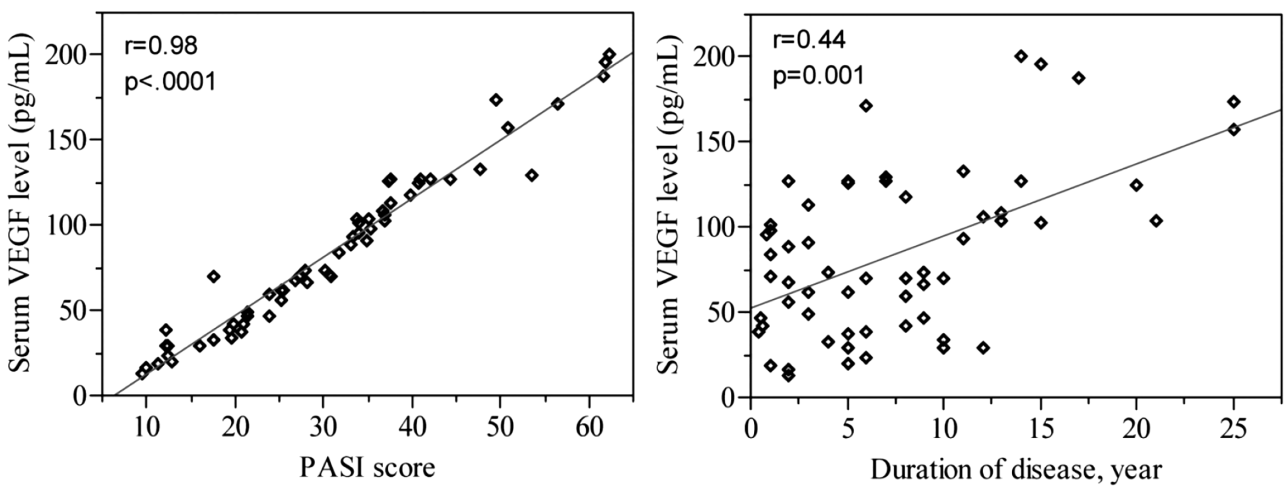

Figure 2. Correlation between serum VEGF levels and both PASI score and disease duration in patients with psoriasis.

Table 2. Correlation between serum VEGF level and all other evaluated variables.

Variables	Serum VEGF levels (pg/mL)			
	Patients with psoriasis (n=58)		Healthy controls (n=60)	
	r	*P*	*r*	*P*
Age (years)	0.61	<0.001	0.47	<0.001
BMI (kg/m^2)	0.38	0.003	0.12	0.34
Duration of disease (years)	0.44	0.001	-	-
PASI score at baseline	0.98	<0.001	-	-
PASI score at endpoint	0.89	<0.001	-	-

Notes *r*: Spearman's correlation coefficient
Abbreviations: VEGF: Vascular Endothelial Growth Factor; BMI: Body Mass Index; PASI: Psoriasis Area and Severity Index

response) compared with healthy control subjects.

In our study, the median serum VEGF level was significantly higher in psoriatic patients (before and after treatment) compared to the healthy controls. Moreover, patients showed a significant reduction in their serum VEGF level after reaching a PASI-75 response. In agreement with our results, Mohammad *et al.* [20] confirmed an increased production of VEGF in psoriatic lesions. Barile *et al.* [22] and Young *et al.* [23] also found that the plasma levels of VEGF and flt-1 were increased in patients with psoriasis. Nofal *et al.* [6] showed that the mean serum levels of VEGF were significantly elevated in psoriatic patients (before and after therapy) than control subjects; they also found no significant difference in the treatment outcome between the three modalities used in their study. In another study, Akman *et al.* [24], found a non-significant difference in the mean serum VEGF levels between patients and controls, as well as between serum VEGF levels and PASI. However, they found a significant decrease in VEGF levels after treatment by PUVA and significant increase in VEGF levels after NBUVB and retinoid-PUVA (Re-PUVA) treatments; therefore, they concluded that VEGF levels cannot be a useful monitor for psoriasis activity and/or treatment response. Different study sample size, the dose of drugs, psoriasis severity, disease duration [6] and the assessment of PASI-75 response might explain the discrepancy between the studies. By considering the reduction of serum VEGF levels after proper therapy, the increase in the serum VEGF level in psoriasis may result from VEGF overproduction and secretion, predominately by keratinocytes and to a lesser degree by fibroblasts [9]. In the present study, there was no significant difference in the median serum VEGF level between males and females. This finding is in agreement with the results of a study conducted by Nofal *et al.* [6]. However, Sandhofer *et al.* [25] found that the plasma levels of VEGF were lower in males than females. Furthermore, the results of our study showed a highly significant correlation between serum VEGF levels and age in both groups and with BMI, PASI score and disease duration in the patient group. Nofal *et al.* [6] and Flisiak *et al.* [26] confirmed the association of VEGF concentration with PASI score but not with age and disease duration. Bhushan *et al.* [9] reported a correlation between VEGF level in skin lesions, PASI, and circulating VEGF levels. Sandhofer *et al.* [25] showed that VEGF correlated positively with age and BMI (similar to our finding).Creamer *et al.* [10] suggested that circulating levels of VEGF reflect the extent of psoriatic skin involvement.

Various treatment modalities have been used to target angiogenesis in psoriasis such as photo(chemo)therapy, retinoids, and cyclosporine-A (the two latter inhibit VEGF-induced angiogenesis). Some recent studies have particularly shown psoriasis improvement through modulation of VEGF production by VEGF receptor tyrosine-kinase inhibitors and biological therapies [20].

One limitation to our study was that we could not analyze our findings based on the treatment modalities because patients with psoriasis frequently experience fluctuations in disease severity and are on various treatment modalities most of the time. Further studies are needed in this regard.

Our findings and the results of other studies support the possible role of VEGF in the pathogenesis of psoriasis. As a result, VEGF can help to evaluate clinical activity and treatment outcomes in patients with moderate to severe psoriasis. On the other hand, we found a significant correlation between serum VEGF level and disease severity; therefore, PASI-75 is believed be a non-invasive assessment tool for the evaluation of treatment response and

generally seems to be a practical treatment goal. Using PASI 75 for evaluation or prediction of the reduction of VEGF levels could provide further clinical benefits.

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