Original Article

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Pathology Section

Expression of VEGF and CD34 in Psoriasis Vulgaris: Correlation with Histological Grading by Trozak Histological Assessment Score

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ABSTRACT

Introduction: Psoriasis is a chronic inflammatory immune-mediated cutaneous disorder. Histologically, it shows epidermal proliferation and neovascularisation in papillary dermis. Histological grading of psoriasis is done using Trozak Histological assessment Score (THS). Angiogenesis drives psoriasis. Vascular Endothelial Growth Factor (VEGF) and Cluster of Differentiation (CD34)/Micro Vessel Density (MVD) are proangiogenic cytokines in psoriasis and are overexpressed in psoriatic skin when compared to normal controls.

Aim: 1) To analyse and compare the IHC expression of VEGF and CD34/MVD in skin biopsies of psoriasis cases and controls; 2) To compare the expression of VEGF, CD34/MVD with histological grade done by THS.

Materials and Methods: A retrospective study was done in the Department of Pathology at Dr. B.R. Ambedkar Medical College and Hospital, Bengaluru, Karnataka, India, between September 2020 to August 2021. Twenty five cases and 25 controls (normal skin) were included in the present study. Histopathology/Immunohistochemistry (IHC) for VEGF and CD34 was performed. Suprabasilar layers of the epidermis were assessed for VEGF expression. Cell showing granular positivity within the cytoplasm was considered positive for VEGF. The mean percentage of positive cells was determined in a minimum of five areas at X400 magnification. CD34/MVD was calculated as the number of vessels in three high density locations. The MVD/High Power Field (HPF) for each individual lesion and graded as <4: negative 1+:4-10 capillaries, 2+:11-20, 3+: >21 per HPF. The THS was done as per standard protocol.

Results: The age range in the present study was 18-68 years with male:female ratio of 1.2:1. An increased VEGF expression (p-value<0.001) in epidermal keratinocytes with accentuated MVD (p-value <0.001) in papillary dermis was seen in all 25 cases which was higher when compared to normal skin. There was positive correlation between VEGF and CD34 (Spearman's rank correlation=0.886). The mean THS was 9.56±3.4 and had positive correlation with VEGF and MVD (r=0.809 and 0.867, respectively) and was statistically significant.

Conclusion: The present study shows that VEGF and CD34 are overexpressed in psoriasis utility for assessing disease severity and further provide insights for targeted therapy against VEGF pathway. Histological grading of psoriasis can give a baseline to evaluate response to treatment.

Keywords: High power field, Immunohistochemistry, Micro vessel density, Vascular endothelial growth factor

INTRODUCTION

Psoriasis is a chronic inflammatory cutaneous disorder with a genetic predilection. Multiple environmental factors also play a role in causation of the disease. The prevalence of psoriasis in various countries ranges from 0.09-11.43% making it a serious global problem with at least 100 million individuals affected worldwide [1]. In India, the prevalence of psoriasis is 0.44-2.8% which commonly affects individuals in 3rd and 4th decade and males being affected two times more than females [2]. Clinically, it is characterised by erythematous papules and plaques with overlying thick silvery scales most commonly in extensor aspect. There are several clinical variants of psoriasis. The chronic plaque type, also known as psoriasis vulgaris and other variants are guttate type of psoriasis, erythrodermic type of psoriasis, pustular type of psoriasis and flexural type psoriasis. Nail involvement and Koebner reactions are seen in psoriasis patients [3]. Histological hallmark of psoriasis is epidermal proliferation, infiltration of leucocytes, elongated rete ridges, hypogranulosis, parakeratosis and Munro's microabscesses. The papillary dermis shows micro vessels which are elongated and dilated capillary proliferations. Histological grading of psoriasis is done using THS [4].

Psoriasis has a complex pathogenesis which is characterised by altered keratinocyte proliferation and differentiation, immune-mediated inflammation, dysregulated angiogenesis and vascular remodelling. The combination between cytokines and growth factors contributes to keratinocyte hyperproliferation, enhanced neovascularisation, and inflammation seen in psoriasis vulgaris. Although the exact trigger for psoriasis is uncertain, it has been postulated that microbial agents or the DNA/RNALL37 complex given by physical factors {Ultraviolet (UV) or skin injury} activate plasmacytoid dendritic cells, which then activate dermal dendritic cells, resulting in Interferon (IFN)-γ production. The immune response can be polarised to Th1 and Th17 in the presence of IFN-γ. This in turn activates γ δT cells to produce Interleukin (IL)-17, which plays a significant role in the pathogenesis of psoriasis since this inflammatory cytokine is a potent stimulator of keratinocyte proliferation [5,6].

Angiogenesis drives psoriasis. New blood vessel formation is seen in early stages of psoriatic lesions and neovascularisation disappears with clearance of disease. Hence, angiogenesis is not only a co-factor but induces development as well. Various proangiogenic cytokines like Tumour Necrosis Factor (TNF), IL-17 and IL-8, Hypoxia Inducible Factor (HIF)-1α are increased in lesional skin of psoriasis [7].

The TNF-α and HIF-1α are proinflammatory cytokines and are of major importance in the pathogenesis of psoriasis. They are known to upregulate the genetic transcription of VEGF and play an important role in the expression of proangiogenic genes implicated in psoriasis. The VEGF, a 40-45-kDa dimeric glycoprotein, regulates neovascularisation during skeletal growth, reproductive functions, embryogenesis, and pathological processes [8].

VEGF, CD31, CD34, Nerve Growth Factor (NGF) and Von Willebrand Factor (vWF) have been established as proangiogenic cytokines which are responsible for inflammatory cycle in psoriasis. Overexpression of VEGF in keratinocytes of epidermis and elevated serum levels of VEGF support the role of VEGF. VEGF is a prognostic marker. It is overexpressed in psoriatic skin when compared to normal controls. VEGF expression is reduced after targeted therapy with TNFα inhibitors [8,9]. CD34 is involved in adhesion and migration of inflammatory cells. Accentuated vascularity is supported by increased expressions of CD34 and higher MVD values in psoriatic skin as compared to normal [10]. The present study aims in comparing the expression of VEGF and CD34 in psoriatic skin lesions and normal skin, which shall provide an insight into the role of angiogenic markers in psoriasis. The aim was also to compare the expression of VEGF, CD34/MVD with histological grade done by THS.

MATERIALS AND METHODS

A retrospective study was done in Department of Pathology at Dr. B.R. Ambedkar Medical College and Hospital, Bengaluru, Karnataka, India, between September 2020 to August 2021. The analysis of the data was done from September 2021 to October 2021. Twenty five cases of skin biopsy of psoriasis patients received at the Department of Pathology were included in the study. Detailed clinical history was retrieved from the request forms of psoriasis patients.

Normal skin samples were used as controls (age and gender matched normal skin present around larger excision biopsy from other specimens sent by Department of Surgery).

Inclusion criteria: All newly diagnosed psoriatic patients, Patients already diagnosed and not under topical or systemic therapy for the last two months prior to the study, confirmed cases of psoriasis by histopathology.

Exclusion criteria: Inadequate biopsy, Patients already diagnosed and under topical or systemic therapy.

Study Procedure

Biopsies were fixed in 10% formalin, grossed as per prescribed standards, embedded in paraffin, 4 to 5 µm thick sections were processed and stained with Haematoxylin and Eosin (H&E) and subjected to histopathological analysis. Additional sections were cut from paraffin blocks and placed on two poly L-lysine coated glass slides for IHC detection of VEGF and CD34 expression.

As part of the normal reporting methodology, the H&E stained slides were examined for psoriatic histology and other characteristics. The THS method was also used to grade the lesions morphologically. The following characteristics were used to determine the score: Hypogranulosis and parakeratosis were each assigned a score of 0-Absent; 1-Focal; 2-Diffuse; regular elongation of rete ridges and perivascular mononuclear infiltrate in the papillary dermis are each given a score of 0-Absent; 1-Present; suprabasal mitoses, "club-shaped" rete ridges, suprapapillary plate thinning, and oedematous dermal papillae are all given a score of 0-Absent; 2-Present; Munro's microabscesses and Kogoj pustules each were given a score of 0-Absent; 3-Present, with a total score of 0-19 for all of the above criteria [4].

The IHC was performed on 4 µm thick section from 10% formalin fixed paraffin embedded tissues using a non biotic polymer-based Horseradish Peroxidase (HRP) detection system for antibodies VEGF165 (Biogenix, USA; catalogue No. AM236-5M ready to use) and CD34 (Biogenix; catalogue No. AR483-5R ready to use).

Suprabasilar layers of the epidermis were assessed for VEGF expression. Cell showing granular positivity within the cytoplasm was considered positive for VEGF. The mean percentage of positive cells for the expression was determined after examining a minimum of five areas at X400 magnification. Biopsies which showed less than 10% of epidermal keratinocytes positivity were considered as negative.

CD34/MVD: The MVD index for the quantification of angiogenesis was calculated using the following method. The slide was screened at low power magnification (X40) to identify the areas with highest vascularisation in papillary dermis. Number of vessels were counted in three such high density areas at higher magnification (X400). Areas with dense haemorrhagic infiltrates and vessels with calibre larger than eight red blood cells were excluded from the count. Single endothelial cells or a cluster of endothelial cells positive for CD34 in membrane/cytoplasm were regarded as a distinct single countable microvessel. The values were finalised after mutual consensus was obtained between two observers and was entered as the MVD/HPF for each individual lesion.

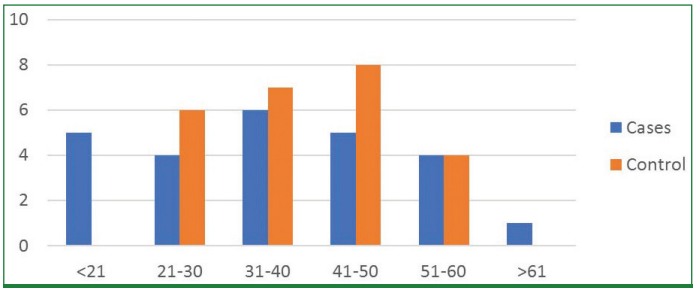
a) 0-3: negative
b) 1+:4-10 capillaries,
c) 2+:11-20,
d) 3+: >21 per HPF.

STATISTICAL ANALYSIS

The Statistical software namely Statistical Package for the Social Sciences (SPSS) 22.0, and R environment version 3.2.2, were used for the analysis of the data and microsoft word and excel used to generate graphs, tables etc. The demographic data were analysed using descriptive statistics, and the VEGF and MVD values were compared using independent t-test. The p-value <0.001 (Significant, Fisher's-Exact test) was considered to be statistically significant. Spearman's rank correlation was used to compare all variables.

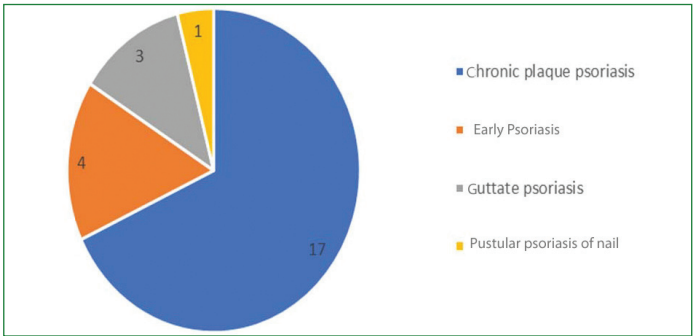
RESULTS

The age range was 18-68 years, with M:F ratio: 1.2:1 and most of cases seen in 3rd and 4th decade [Table/Fig-1].



[Table/Fig-1]: Age distribution of cases and controls.

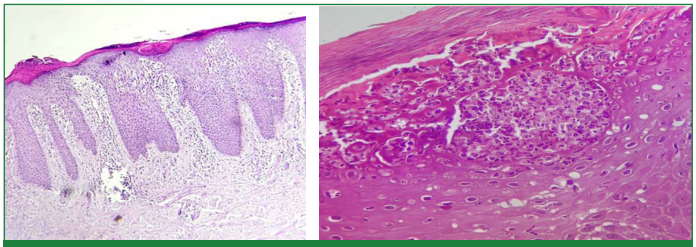
The most common morphological variant was chronic plaque psoriasis and least common was pustular psoriasis [Table/Fig-2]. The mean THS was 9.56±3.4. Histologically chronic plaque psoriasis was characterised by acanthosis, hypogranulosis (focal/diffuse), and parakeratosis (focal/diffuse), regular elongation of rete ridges, Munro's microabscesses and increased number of dermal capillaries with predominant lymphocytic inflammatory cell infiltrate [Table/Fig-3]. Pustular psoriasis showed characteristic spongiform pustule of kogoj [Table/Fig-4].



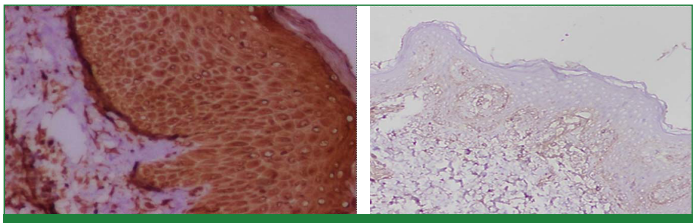
[Table/Fig-2]: Morphological variants of psoriasis.

An increased VEGF [Table/Fig-5] expression in epidermal keratinocytes was seen in cases with a mean of 64.48±10.23 as compared to controls [Table/Fig-6] which showed a mean of 11.12±4.36 [Table/Fig-7]. Accentuated MVD (assessed by grading) [Table/Fig-8] was noted in cases with a mean of 2.68±0.48 (capillary count:18.68±5.20)

as compared to controls which showed a mean of 0.42±0.5 (capillary count: 4.88±2.26) [Table/Fig-9] shows CD34 distribution. There was positive correlation between VEGF and CD34 (Spearman's rank correlation: r=0.886) and was statistically significant [Table/Fig-10].



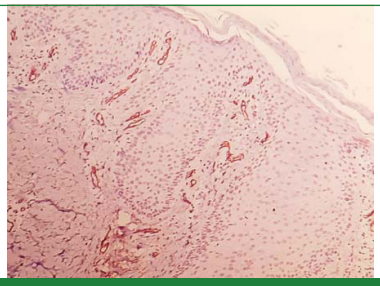
[Table/Fig-3]: Histopathology of psoriasis showing acanthosis, hypogranulosis, parakeratosis and regular elongation of rete ridges (H&E X100).
[Table/Fig-4]: Histopathology of pustular psoriasis showing spongiform pustule of kogoj (H&E X400). (Images from left to right)



[Table/Fig-5]: Strong positive of VEGF expression in epidermal keratinocytes in psoriasis patient (X400).
[Table/Fig-6]: Weak expression of VEGF in controls-limited to basal layer only (x400). (Images from from left to right)

Parameters	Group		p-value**
	Cases	Control	
VEGF-IHC (% positive of epidermal keratinocytes)	64.48±10.23	11.12±4.36	<0.0001
MVD	18.68±5.20	4.88±2.26	<0.0001

[Table/Fig-7]: Comparison of different parameters by group.
**Significant; Fisher-exact test



[Table/Fig-8]: CD34 IHC staining in dermal vessels in psoriasis patients showing increased MVD-3+(x400).

CD34	Cases	Control	Total (N)
Negative	0	14 (56.0)	14 (28.0)
1+	0	11 (44.0)	11 (22.0)
2+	13 (52.0)	0	13 (26.0)
3+	12 (48.0)	0	12 (24.0)
Total	25 (100.0)	25 (100.0)	50 (100.0)

[Table/Fig-9]: CD34 distribution of study participants.

All the morphological variants showed increased VEGF expression and MVD when compared to controls. A positive correlation was noted between VEGF expression, MVD with THS (Spearman's rank correlation r=0.809 and 0.867, respectively) and was statistically significant.

DISCUSSION

Psoriasis was shown to be more frequent in younger age groups, with a slight male preponderance in present study. The majority of the patients were between the ages of 31-40, which was similar to study done by Rajan PT et al., [11].

Variables		VEGF -IHC Expression	CD34-IHC Expression (MVD)	THS
VEGF -IHC Expression	Spearman's rank correlation (r)	1	0.886	0.809
	N	50	50	50
CD34-IHC Expression (MVD)	Spearman's rank correlation (r)	0.886	1	0.867
	N	50	50	50
THS	Spearman's rank correlation (r)	0.809	0.867	1
	N	50	50	50

[Table/Fig-10]: Correlation between angiogenic markers and Trozak Histological Assessment Score (THS).
r value:>0.6 implies significant correlation

The VEGF is thought to be the principal regulator of angiogenesis. VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and Placental Growth Factor (PLGF) are all members of the VEGF superfamily. VEGF normally refers to VEGF-A, the most well-known member of the VEGF superfamily. Endothelial cells respond well to VEGF, which stimulates their proliferation, migration and inhibit apoptosis. It also enhances blood vessel dilation and increases vascular permeability. VEGF is a chemoattractant for leukocytes, as well as a stimulator of Matrix Metalloproteinase (MMP), expression of adhesion molecule and regulates the function of dendritic cells and lymphocytes [11].

Anti-VEGF antibody treatment reduces inflammation by blocking VEGF effects on endothelial cells. VEGF is a well-known regulator of several elements of the inflammatory response, wound healing, and tumour formation in addition to its role in angiogenesis. Furthermore, chronic VEGF overexpression in the skin of K14-VEGF transgenic mice boosted leukocyte rolling and adherence in skin microvessels, most likely as a result of increased expression of adhesion molecules like E- and P-selectin. As a result, systemic treatment with an anti-VEGF antibody inhibited these extra VEGF activities linked to recruiting and activating immune cells [12]. Thus, systemic treatment with an anti-VEGF antibody likely contributed to the decreased inflammatory cell infiltration reported in the skin by inhibiting these extra actions of VEGF linked to recruiting and activating immune cells [13].

In the present study, psoriasis patients had significantly higher VEGF expression throughout the keratinocytes in the epidermis (strong and diffuse positivity) with psoriasis case showing a mean of 64.48±10.23 as compared to controls which had a mean of 11.12±4.36 (p<0.0001). These findings were similar to the study done by Sankar L et al., (2.6±0.5), Rajan PT et al., (49.80%±21 in cases and 3.95%±3.94%) and Kandil A et al., (46.4% in cases, 19.7% in controls) [7,11,14]. Meki A and Al-Shobaili H, study showed elevated serum VEGF in psoriatic patients than in control subjects [13]. Increased intensity of VEGF staining in suprabasilar keratinocytes of psoriasis as compared to psoriasiform lesions was observed in a study done by Chawla N et al., [15].

It has been observed that therapeutic intervention at the level of the vasculature can be adequate to diminish the immunological induced and epidermal components of the disease. VEGF is the most important cytokine marker and it is overexpressed in keratinocytes of lesional psoriatic skin when compared to non lesional skin. Its expressions are reduced after treatment with specific hyphenated inhibitors. TNFα inhibitors are known to modulate angiogenesis [16,17]. In epidermis, the VEGF and Factor VIII immunohistochemical pattern tended to change from diffuse to basal after therapy [10].

The T allele of VEGF +936 (rs3025039) may act as a protective allele in the development of PsA. The following allele loss due to Single-Nucleotide Polymorphism (SNP) was seen in psoriasis patient [18]. This further emphasises the role of VEGF in modulating angiogenesis.

The CD34 is a marker for accentuated microvessels in the papillary dermis. The local release of angiogenic growth factors

is responsible for the uncontrolled endothelial cell proliferation that takes place during tumour neovascularisation and in angiogenesis dependent disease such as diabetic retinopathy, psoriasis, rheumatoid arthritis [19].

Present study showed accentuated MVD with cases showing a mean of 2.68±0.48 when compared to controls: 0.42±0.5 (capillary count: cases: 18.68±5.20, controls: 4.88±2.26: p<0.0001) which was similar to study done by Sankar L et al., (2.3±0.5) and Rajan PT et al., (15.3% in patients as compared to 5.15% in controls) [7,11]. These findings were supported by other studies which showed decrease in new blood vessel formation after anti-TNFα therapy.

El-Aziz Hassan R et al., assessed MVD using CD34 immunostaining and showed that skin of psoriatic cases (involved and uninvolved), had significant higher MVD values in comparison to normal skin of control group (p<0.010) [12].

The THS was significantly reduced from 10.3 before treatment to 5.1 after two weeks and 3.2 after six weeks (p<0.0001) in a study done to correlate histological grade with clinical severity [20]. Histological grading may give baseline value to evaluate response to treatment. In present study, the mean THS was 9.56±3.4. There was positive correlation between trozak histological grading and expression of angiogenic markers and was statistically significant which was discordant to a study done by Rajan PT et al., [11].

Recent study showed VEGF inhibitors (Bevacizumab) used for the treatment of metastatic cancers, showed improvement in psoriatic skin lesions. A Psoriasis Area and Severity Index (PASI) score of 16.8 significantly reduced to 1.4 [21]. Present study further emphasises on the role of VEGF, IHC marker and THS as a prognostic marker in assessing psoriasis severity and response to treatment.

Limitation(s)

The small sample size and being a retrospective study, follow-up of patients was not possible. A wide scale analysis with long term follow-up after targeted therapy against anti-VEGF pathway is a potential area of further research.

CONCLUSION(S)

The involvement of VEGF and its receptors in the pathophysiology of psoriasis is crucial. According to new research, VEGF is involved in not just angiogenesis, but also keratinocyte differentiation. Histological grading may give baseline value prior to initiation of treatment. The present study findings further demonstrate, that, VEGF is overexpressed in all morphological psoriasis subtypes when compared to normal skin controls. As a result, targeting the VEGF pathway could be a novel therapeutic option for psoriasis treatment.

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